THESIS

Muscle Histochemistry and Gliding Posture In California Gulls (*Larus californicus*)

Submitted by:

Ed Mathias

In partial fulfillment of the requirements for the
Thesis in Zoology
Weber State University
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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ED MATHIAS ENTITLED "MUSCLE HISTOCHEMISTRY AND GLIDING POSTURE IN GULLS (*LARUS CALIFORNICUS*)" BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE THESIS IN ZOOLOGY.

Thesis Committee

Sue Fairbanks

Carl D. Marti

Ron Meyer
Advisor

Samuel L. Zeveloff
Department Chair

Abstract

Gliding flight requires the wing to be held in a horizontal position supporting the weight of the body. Slow muscle fibers contracting isometrically could perform this function, thus reducing the amount of energy required to sustain gliding flight. Gliders such as vultures and pelicans possess a deep layer to the pectoralis, composed entirely of slow fibers believed to perform this function. Muscles involved in gliding posture in California gulls (*Larus californicus*) were examined and tested for the presence of slow fibers using myosin ATPase histochemistry and antibodies. Surprisingly small percentages of slow fibers were found in the M. extensor metacarpi radialis and M. coracobrachialis cranialis which function in wrist extension and wing protraction, respectively. The low number of slow fibers in these muscles and the absence of slow fibers in muscles associated with wing extension and body support suggest that slow fibers are not needed for gliding posture in gulls. Gulls also lack the deep belly to the pectoralis found in other gliding birds. Since bird muscle is highly oxidative, fast muscle fibers may function to maintain wing position during gliding flight in California gulls.

Introduction

The ability to glide provides a unique way for birds to maintain flight while reducing the high energy requirements associated with this specialized type of locomotion (Rosser et al. '94). When compared to other types of flight, such as flapping and hovering, gliding requires the least amount of energy (Goldspink '81). According to Goldspink ('81), there are two reasons gliding is more efficient in terms of energy expenditure. First, gliding requires fewer active motor units. Electromyographic study of the pectoralis muscle in herring gulls (*Larus argentatus*; Goldspink et al. '78) and American kestrels (*Falco sparverius*; Meyers '93) showed smaller electrical potentials during gliding flight than flapping flight, indicating less muscle activity. Second, isometric contractions are involved. During an isometric contraction, less ATP is used due to the longer interactions between actin and myosin fibers. ATP is needed to break the actin-myosin interaction. In addition, oxygen consumption decreases. When compared to oxygen consumption at rest there is a large increase during flapping flight whereas a small increase in oxygen consumption occurs during gliding (Tucker '72; Baudinette and Schmidt-Nielsen '74). Flapping flight requires the movement of the wing which increases the amount of energy required to sustain flight.

There are three muscle fiber types found in birds: slow-twitch, fast-twitch and slow tonic, as determined by assaying the muscle for adenosine triphosphatase activity (ATPase) (Hikida '87). Mammalian twitch and tonic fibers differ in the arrangement and number of motor end plates (Goslow '85). Mammalian tonic fibers have multiple motor end plates and lack an action potential whereas twitch fibers have a

single end plate and produce an action potential (Goslow '85). In birds, slow-twitch and tonic fibers are similar in that they are both multiply innervated (Torrella et al. '93). The type of contraction produced is a result of the types of fibers activated within the muscle. Muscles with a greater proportion of fast-twitch fibers are used for activities such as locomotion whereas muscles with higher proportions of slow fibers serve a more postural role (Simpson '79; Meyers '92).

Since gliding requires the wings to be held in a horizontal position, it has been suggested that slow fibers should be present to perform this function (Meyers '93). Studies have shown the presence of slow fibers in the pectoralis muscle of the turkey vulture (*Cathartes aura*; Rosser and George '86a) and the American white pelican (*Pelecanus erythrorhynchos*; Rosser et al. '94). In both these birds, the slow fibers are located in a small, deep, accessory belly of the pectoralis muscle. However, depending on the species, birds in the family Laridae may or may not have this distinct division of the pectoralis into a deep accessory belly and a larger superficial belly (Hudson et al. '69). Unfortunately, Hudson et al. did not indicate which species possessed the deep belly. There are also conflicting reports on the presence of slow fibers in the pectoralis of herring gulls. Talesara and Goldspink ('78) found the pectoralis of the herring gull to contain approximately 6% slow twitch fibers, though the region of the pectoralis was unspecified. Other studies have also analyzed the pectoralis muscle of herring gulls and found an absence of any slow fibers (Rosser and George '86b; Caldow and Furness '93).

The purpose of this study was to examine the muscles associated with maintaining horizontal wing position during gliding flight in the

California gull (*Larus californicus*) and to determine if slow fibers are present which may aid supporting the wing during this type of flight.

Materials and Methods

Four California gulls were collected at local landfills using a shotgun (permits were obtained from state and federal governments). One gull was dissected to access anatomical position of the wing in regard to gliding posture. This permitted the determination of the muscles that were sampled for histochemistry. Tissue samples from three birds were removed mid-belly immediately after death and attached to a piece of cork with 5% gum tragacanth. The tissue was then quick frozen in isopentane cooled to -150°C with liquid nitrogen and stored in an ultracold freezer at -70°C. A cryostat maintained at -20°C was used to cut the tissue into sections 10-12 μm thick. After the tissue was cut, serial sections from each muscle were placed in both acid and alkaline solutions with pH's of 4.2 and 10.4. The histochemistry protocol of Hikida ('87) was used and produces a visible differentiation between the different fiber types found within the muscle. The alkaline preincubation causes avian fast-twitch fibers to stain dark and the slow-twitch fibers to stain light, whereas the acid preincubation causes a light staining of fast-twitch fibers and a darker staining of slow-twitch fibers (Hikida '87; Meyers '92; Rosser et al. '94; see Fig. 1). Tonic fibers stain an intermediate color in both acid and alkaline preincubations (Meyers '92). Rock dove (*Columba livia*) muscle was also prepared to use as a control for the histochemistry procedure. The fiber types in Mm. latissimus

dorsi pars cranialis, biventer cervicis, and pectoralis of the rock dove are well documented (Hikida '87). Each sample was also tested for nicotinamide adenine dinucleotide diaphorase (NADH-D) activity, which determines the oxidative capacity of the individual fibers within the sampled muscles (Novikoff et al. '61).

Antibodies to fast and slow myosin were used to confirm histochemical data. The procedure follows that of Hermanson and Cobb ('92). The antifast antibody, MY32 (Sigma Chemical Co., St. Louis, MO) labels fast-twitch fibers in both mammals and birds, whereas the antislow antibody, ALD58 (Univ. of Iowa Hybridoma Bank) labels slow-twitch fibers in mammals and both slow-twitch and tonic fibers in birds. Samples were incubated against the antibodies in a humidified chamber at 4°C for 16 hours. After rinsing in phosphate-buffered saline, the samples were then incubated in a goat anti-mouse antibody to recognize the primary antibody and then stained with a strepavadin peroxidase system (Zymed Labs, San Francisco, CA).

Black-and-white photographs of muscle samples were taken using an Olympus BH-2 photomicroscope (40x magnification). Overlapping photographs of the various areas within the muscle were taken and the prints taped together forming an enlarged picture of the muscle. Individual fast and slow fibers within the muscle were counted. Fiber percentages were then calculated along with the standard error of the mean for three specimens (Table 1).

Results

Gliding requires the wing to be extended and held in a horizontal position. This requires extension of the wing at the elbow and wrist, and protraction of the wing at the shoulder. In addition, the body must be supported to keep it from falling through the wings (Pennycuick '82). Dissection revealed that four major muscles should play a role in wing posture during gliding: extensor metacarpi radialis dorsalis and ventralis as wrist extensors, triceps brachii as an elbow extensor, coracobrachialis cranialis as a wing protractor, and pectoralis as a body supporter. A description of gross morphology and histochemical data for each muscle follows. Nomenclature follows Meyers ('93) for muscles and *Nomina Anatomica Avium* (Baumel and Witmer '93) for bones.

All muscles sampled possessed fast-twitch and slow-twitch fibers only. No definitive slow-tonic fibers were identified.

All samples stained moderate to intense for oxidative capacity. Slow fibers appeared to stain moderately, whereas fast fiber staining ranged from moderate to intense. Mixed fast-twitch fiber staining was found in all muscles sampled.

M. coracobrachialis cranialis (CBC)

CBC originates on the Processus acrocoracoideus of the coracoid, lateral to the coracoid-furcular articulation. The muscle crosses over the dorso-cranial aspect of the shoulder joint, crossing in front of the coraco-humeral joint, and inserts on the Impressio coracobrachialis of the

cranial surface of the humerus, proximal to the insertion of M. pectoralis.

The CBC functions to protract the humerus which is necessary to keep the wing in proper gliding position. All gulls sampled contained a mixture of fibers with an average of 8.1% slow-twitch fibers (Table 1). The majority of the slow-twitch fibers are concentrated in the caudo-ventral region of the muscle (Fig. 2).

M . extensor metacarpi radialis (EMR)

EMR is comprised of two bellies in *L. californicus*: pars ventralis and pars dorsalis. This muscle lies on the cranial surface of the antebrachium and arises from the Processus supracondylaris dorsalis of the humerus. The ventral belly lies more proximal than the dorsal belly and originates via mixed tendons and fleshy fibers from the underside of the Processus and a small area on the humeral shaft. The muscle extends for approximately one-third the length of the ulna. The dorsal belly originates from a short, thin tendon of origin from the Processus only and becomes fleshy at a point distal to the attachment of the aponeurosis of the propatagial sling to the EMR. Tendons from each belly run along the radius, pass over the cranial aspect of the wrist and jointly insert on the Processus extensorius of the carpometacarpus. The tendon from the ventral belly starts out caudal to the dorsal belly tendon. Midway along the radius the two tendons switch orientation and the ventral belly tendon lays on top of the dorsal belly tendon as they pass over the cranial aspect of the wrist.

EMR functions to extend the wrist and hand as part of the automatic extension-flexion mechanism of the avian wing (Fisher '57; Vazquez '94). This muscle is in a position to maintain wrist extension during gliding flight. Histochemistry shows that both bellies contained slow-twitch fiber populations. The dorsal belly contained an average of 28.6% slow-twitch fibers that were evenly distributed throughout the muscle (Fig. 3; Table 1). The ventral belly contained a substantially lower 5.8% slow-twitch fibers that were concentrated in the dorso-cranial region of the muscle (Fig. 3; Table 1).

M. pectoralis thoracicus (PT)

The pectoralis is the largest muscle found in birds. This muscle originates from the sternal body, ventrolateral edge of the sternal keel, and the lateral surface of the furcula. The majority of its fascicles insert onto the Crista deltopectoralis of the humerus. Some deep fascicles insert onto the tendon of origin of the humeral head of the biceps.

During flapping flight the pectoralis produces the downstroke motion of the wing, but when gliding this muscle is in a position to act as a strut, supporting the body between the wings. Dissection revealed that *L. californicus* lacks the deep layer to the pectoralis found in other gliding species. Muscle samples were taken from the region of the pectoralis analogous to the area where the deep pectoralis layer is located. This region takes origin from the deep furcula and adjacent membranes. Samples were also taken from other areas, including the cranial border of the muscle and fibers attaching onto the biceps tendon

to examine the muscle more completely. Small numbers of slow-twitch fibers, ranging from two to twenty individual fibers, were found only in the area analogous to the deep layer in all gulls sampled (Fig. 4).

M. triceps humeralis (TH)

TH lies on the caudal border of the humerus deep to M. triceps scapularis. This muscle originates from the proximal two-thirds of the caudal border of the humerus. TH inserts via a mixed fleshy and tendinous insertion onto the olecranon of the ulna, distal to the insertion of M. triceps scapularis.

TH, along with triceps scapularis, functions to extend the wing at the elbow. Histochemistry showed the presence of a small number of individual slow fibers (fewer than forty) in only two gulls sampled. The gulls that contained slow-twitch fibers were the same individuals that contained slow fibers in triceps scapularis (Fig. 5a).

M. triceps scapularis (TS)

TS lies along the caudal border of the brachium. It arises by a short tendon from the scapula adjacent to the glenoid fossa. TS inserts onto the olecranon process of the ulna, proximal to M. triceps humeralis.

This muscle along with M. triceps humeralis functions to extend the wing at the elbow. Histochemistry showed this muscle to have almost exclusively fast-twitch fibers. Two out of three gulls sampled had

a very small number of individual slow-twitch fibers (fewer than forty) along the caudal edge of the muscle (Fig. 5b).

Discussion

It has been hypothesized that muscles involved in postural activities such as gliding should contain slow-contracting fibers (Meyers '93). The muscles sampled in this study contained a surprisingly low number of slow-twitch fibers. Only two muscles, EMR and CBC, contained slow-twitch fiber populations that may aid in gliding posture. The highest percentage of slow fibers was found in EMR dorsalis, which comprised 34.6% in one gull. The lack of slow fibers in the pectoralis and triceps was unexpected. These muscles play an important role in gliding wing position, but contained only scattered individual slow fibers. It seems unlikely that these scattered fibers can produce the isometric contraction necessary to maintain gliding posture on their own.

Histochemical analyses of avian slow muscle has primarily focused on the pectoralis muscle, neglecting other muscles of the forelimb (Meyers '92). Slow-tonic fibers located in the pectoralis of flightless emus (*Dromaius novaehollandiae*) and ostriches (*Struthio camelus*) have been hypothesized to function in wing posture (Rosser and George '84,'85a). Tonic fibers have been found in the pectoralis of other avian species, including the domestic chicken (*Gallus gallus*), red-tailed hawk (*Buteo jamaicensis*) and double-crested cormorant (*Phalacrocorax auritus*) (Rosser and George '86b) whereas slow-twitch fibers were found in the

pectoralis of the herring gull (Talesara and Goldspink '78). Slow fibers have also been found in the deep layer to the pectoralis of the American white pelican (Rosser et al. '94) and turkey vulture (Rosser and George '86a), suggesting that slow fibers may be involved in gliding posture in these species. Studies conducted by Maier ('83) and Rosser and George ('85b) examined forearm muscles in rock doves using histochemical analysis, but both of these studies focused on muscle spindles rather than fiber type analysis. Simpson ('79) examined the shoulder muscles of the pigeon and found tonic fibers in Mm. deltoideus minor, coracobrachialis cranialis and latissimus dorsi cranialis. She suggested that these muscles are involved in posture. Slow tonic fibers were also found in the Mm. deltoideus minor, latissimus dorsi cranialis, scapulohumeralis cranialis, and brachialis of the American kestrel, and these fibers are thought to play an important role in folded-wing posture (Meyers '92). The presence of slow-twitch and tonic fibers in the pectoralis of gliding species like the herring gull and vulture indicate the possible role these fibers have in gliding posture.

Wing Extension

Wing extension is the result of extension at the wrist and elbow. EMR functions to extend the wing at the wrist whereas the triceps extends the wing at the elbow. Contraction of the triceps extends the wing via the automatic flexion-extension mechanism (Fisher '57; Vazquez '94). Both the triceps humeralis and triceps scapularis were comprised almost exclusively of fast-twitch fibers. The few slow-twitch

fibers found in two individuals may not be sufficient to maintain wing extension. This means that the gull must use fast-twitch fibers to maintain elbow extension while gliding.

The EMR is divided into two bellies. Nair ('54) suggested that this division is characteristic of gliding species and prevents the muscle from fatiguing. To reduce fatigue one belly can maintain contraction while the other belly is in a relaxed state, allowing the relaxed belly to recover. The dorsal belly contained the highest slow-twitch fiber population, with its fibers distributed throughout the entire muscle. The ventral belly contained a lower percentage of slow-twitch fibers that were concentrated in the dorso-cranial region of the muscle. The number of slow-twitch fibers found in EMR may be sufficient to maintain wrist extension during gliding flight. The automatic flexion-extension mechanism of the avian wing can also help maintain wrist extension (Fisher '57; Vazquez '94).

Wing Protraction

The CBC is in a position to protract the wing and may be involved in moving the wing into gliding position. Histochemical studies of the CBC in the rock dove (Simpson '79) found the presence of tonic fibers, but the kestrel CBC lacked any tonic or slow-twitch fibers (Meyers '92). The CBC in vultures is enlarged and is believed to be an adaptation for soaring (Fisher '46). In *L. californicus* this muscle contained an average of 8.1% slow-twitch fibers. The fast-twitch fibers could be used to protract the wing into gliding position and then the isometric contraction of the slow fibers could be used to maintain wing position.

Body Support

The pectoralis is ideally located to support the body between the wings during gliding. Electromyographic studies of the herring gull (Goldspink et al. '78), American kestrel (Meyers '93) and budgies (*Melopsittacus undulatus*; Tobalske and Dial '94) found this muscle to be active while the birds were gliding. I examined several areas of the pectoralis and only found slow-twitch fibers in the area analogous to the deep layer found in other gliding species. The total number of fibers in this region was very small and it is unlikely that these fibers alone could function to support the body during gliding flight. Since the pectoralis in birds is very large, it is difficult to sample the entire muscle. Additional slow fibers may have been missed during sampling. Different areas of the pectoralis may activate at different times allowing fatigued areas to recover, thus maintaining contraction for long periods of time. The fast-twitch fibers in this muscle are highly oxidative and this may allow for these fibers to function for a longer time without fatigue (see Kaplan and Goslow '89).

Studies conducted on herring gulls have produced conflicting results with regard to slow-twitch fibers in the pectoralis. Talesara and Goldspink ('78) found the pectoralis to contain approximately 6% slow-twitch fibers. They sampled one gull and did not specify the area where the fibers were found. Rosser and George ('86b) examined three different areas of the pectoralis in two herring gulls. Samples were taken from the most superficial fibers on the proximal one-half of the

muscle, deepest fibers of the proximal one-half of the muscle (deep to the previous sample) and deepest fibers of the distal one-third of the muscle. They found these areas to be uniformly fast with no slow fibers. Similar results were described by Caldow and Furness ('93), who also sampled three regions of the pectoralis, but only in one herring gull. Samples were taken near the cranial edge of the muscle. One sample was superficial and the other two were from successively deeper regions. They did not find any slow fibers in the areas sampled. It has been suggested that slow-fibers in the pectoralis may be disadvantageous for flapping flight (Talesara and Goldspink '78).

The divided pectoralis is a specialization found in some gliding and soaring species. The pectoralis in these species is divided into two parts: superficial and deep. It has been suggested that the deep layer should be comprised of slow tonic fibers to aid in soaring (Pennycuick '82). Studies of the deep layer in the turkey vulture (Rosser and George '86a) and the white pelican (Rosser et al. '94) found the deep layer to be comprised of entirely slow fibers while the superficial layer was completely made up of fast-twitch fibers. The deep layer has also been described in albatrosses and petrels (*Diomedea* sp., *Macronectes* sp., *Procellaria aequinoctialis*, *Pachyptila desolata*; Pennycuick '82), anhingas (*A. anhinga*; Owre '67), cranes and storks (*Grus americana*; Fisher and Goodman '55; *Leptoptilos crumeniferus*; Vanden Berge '70), frigatebirds and boobies (*Fregata magnificens*, *Sula nebouxii*; Kuroda '61), Aegypiine vultures (Pennycuick '82), some gulls (Laridae; Hudson et al. '69). The deep layer is important for gliding because it allows the bird to use the superficial pectoralis layer for flapping flight and then switch over to the deep layer to support the body during gliding. Previous work on

pelicans (Rosser et al. '94) and vultures (Rosser and George '86a) are the only studies that histochemically examine the deep layer in gliding species. To date, there have been no electromyographic studies performed on species with the divided pectoralis.

The majority of birds with the deep layer are larger birds like the albatross, pelican and vulture. *Larus californicus* is a smaller bird and this may account for the absence of the deep pectoralis layer. A larger bird might require the pectoralis to support more weight and this may explain the presence of a deep layer in these species. To explore this further, I examined the pectoralis morphology of a larger gull, the great black-backed gull (*L. marinus*), which is in the same weight class as the turkey vulture and frigatebirds (Dunning '93). This gull weighs an average of 1829.0 g, which is three times the weight of *L. californicus*. The great black-backed gull has a similar pectoralis morphology to *L. californicus* and also lacks the deep pectoralis layer. This poses the question of how much gliding is required to need a specialization such as a divided pectoralis and slow fiber populations. If the bird spends less time gliding it may be able to maintain body support by utilizing highly oxidative fast-twitch fibers in the pectoralis and other gliding muscles involved in wing position.

Fast Fibers and Isometric Contraction

The lack of slow fibers in muscles that are important in gliding posture suggests that there is another mechanism used by *L. californicus* to maintain wing position while gliding. I found two different types of

fast fibers in the muscles sampled. One fiber type stained moderately for NADH activity while the other stained intensely. Bird muscle is highly oxidative and this may account for the lack of slow fibers found. The reason that oxidative fast fibers may be able to perform this function is due to the rate at which energy is replenished during contraction. Awan and Goldspink ('71) found that the "fast" biceps muscle of the Syrian hamster could replenish its energy supply during contraction faster than the "slow" soleus muscle. If fast fibers are used to maintain gliding position, then due to their high oxidative capacity they could hold the desired contraction for a substantial amount of time. Awan and Goldspink ('71) also found that most of the energy used during isometric contraction was used during the development of tension. Far less energy was needed for the maintenance of isometric contraction. This means that a muscle with a small number of slow fibers may be able to maintain the desired wing position. The CBC contained only 6% slow-twitch fibers which may be enough to maintain wing protraction. If the fast fibers were used to move the wing into desired position, slow fibers could then take over and maintain contraction.

Conclusions

The muscles sampled in this study were expected to contain substantial amounts of slow fibers to reduce energetic costs associated with gliding. The lack of slow fibers indicates that another mechanism is involved in maintaining wing position. Physiological studies of these

muscles are needed to determine if fast fibers are being used to maintain wing position. Additional studies are also needed to determine if small slow-fiber populations can maintain isometric contraction when gliding.

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Figure Legends

Figure 1: Serial sections of ATPase histochemistry of *Larus californicus.* a) acid preincubation of pH 4.2. b) alkaline preincubation of pH 10.4. c) Nicotinamide Adenine Dinucleotide Diaphorase Activity. 1 = slow -twitch fiber; 2 = fast-twitch fiber. Scale = 100 μm

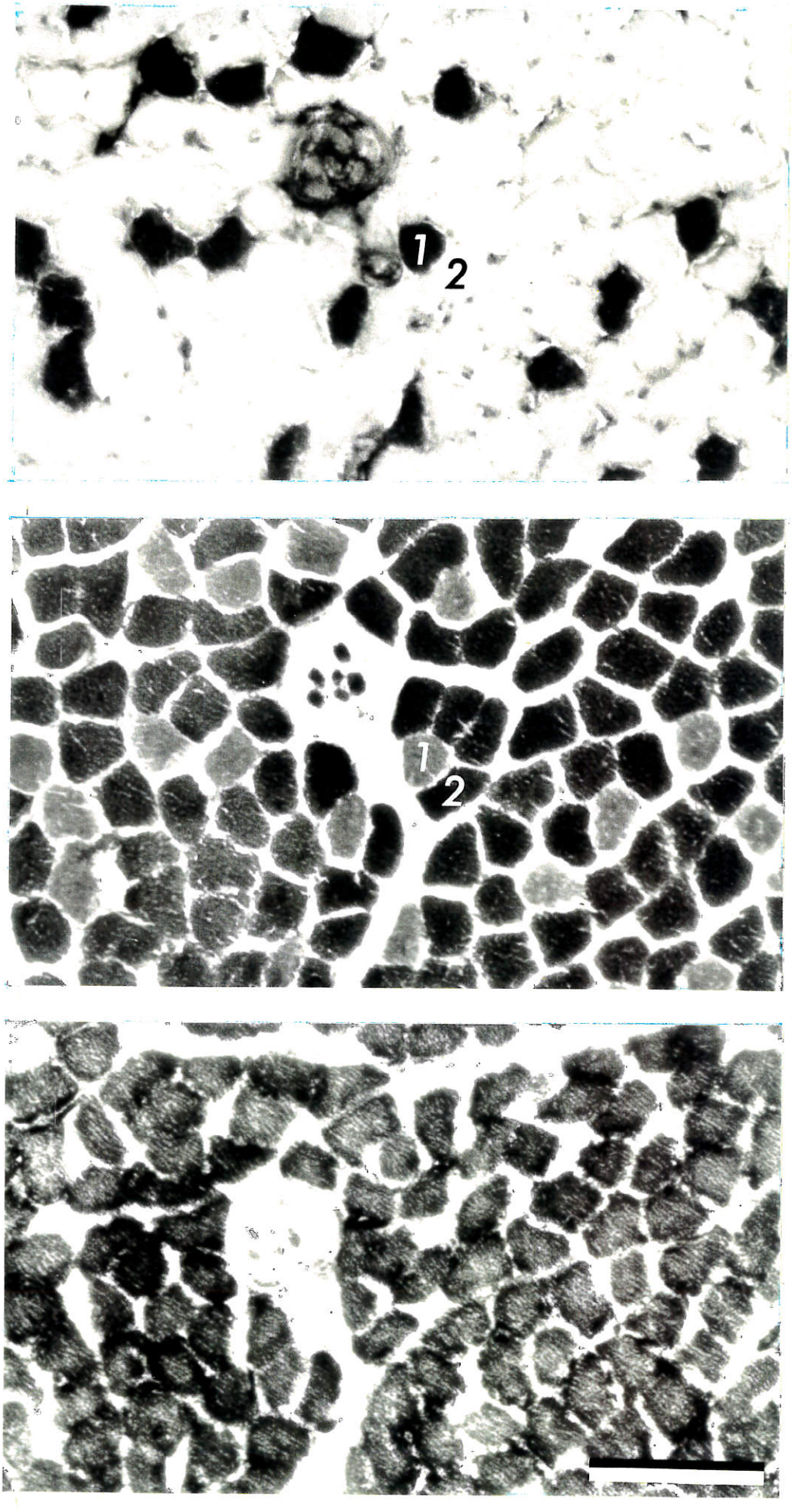
Figure 2: Whole muscle cross-section of M. coracobrachialis cranialis at pH 4.2 preincubation ATPase histochemistry showing the distribution of fast and slow-twitch fibers. Cranial is to the left, dorsal is to the top. Scale = 1 mm

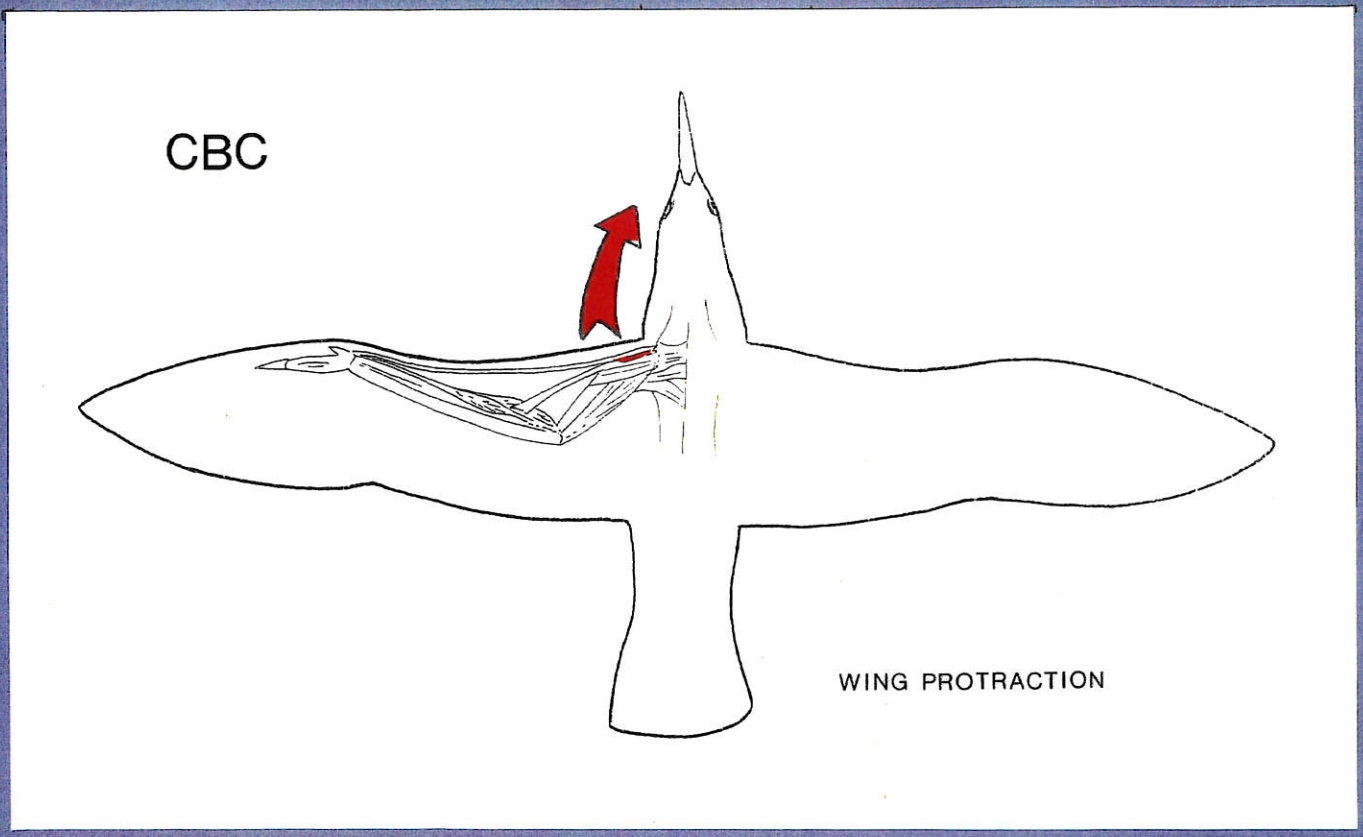
Figure 3: Whole muscle cross-section of M. extensor metacarpi radialis ventralis and dorsalis at pH 4.2 preincubation ATPase histochemistry showing distribution of fast and slow-twitch fibers. For dorsalis, cranial is to the right and dorsal is to the top. For ventralis, cranial is to the right and dorsal is to the bottom. Scale = 1 mm

Figure 4: Cross-section of M. pectoralis sample taken from the area analogous to the deep layer found in other gliding species at pH 4.2 preincubation ATPase histochemistry. Slow fibers are located within the brackets. Cranial is to the left, dorsal is to the top. Scale = 1 mm

Figure 5: Whole muscle cross-section of pH 4.2 preincubation ATPase histochemistry showing distribution of fast and slow-twitch fibers. a) M. triceps humeralis. Lateral is to the right, dorsal is to the top. b) M. triceps scapularis. Cranial is to the right, dorsal is to the top. Scale = 1 mm

Fig. 1

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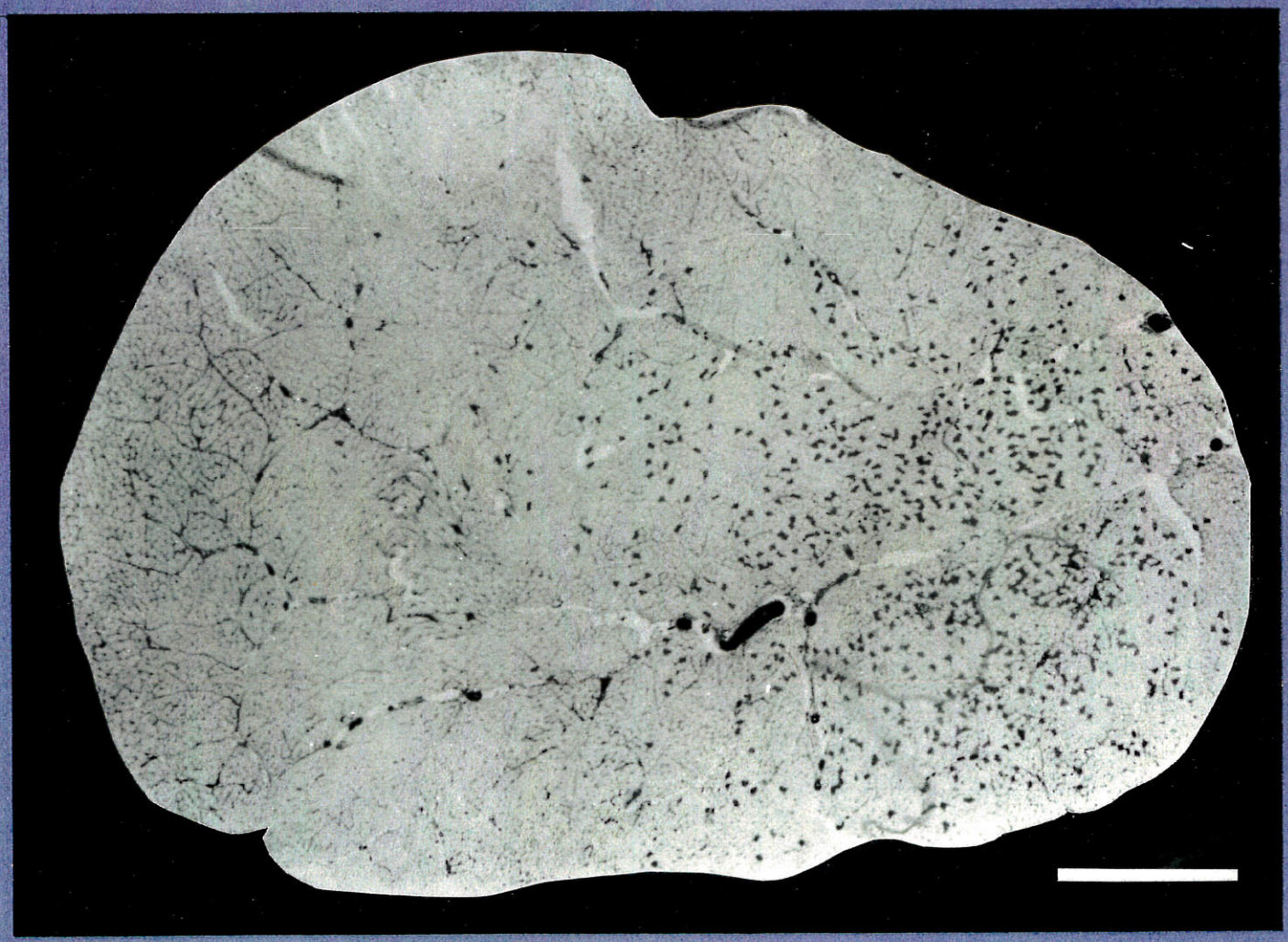

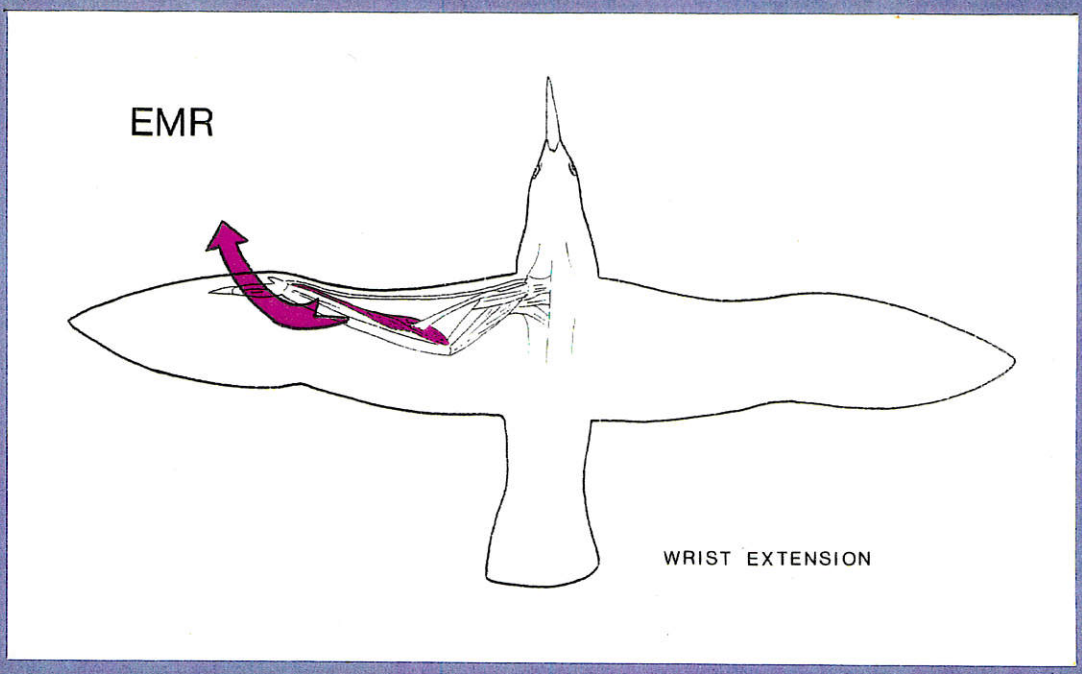
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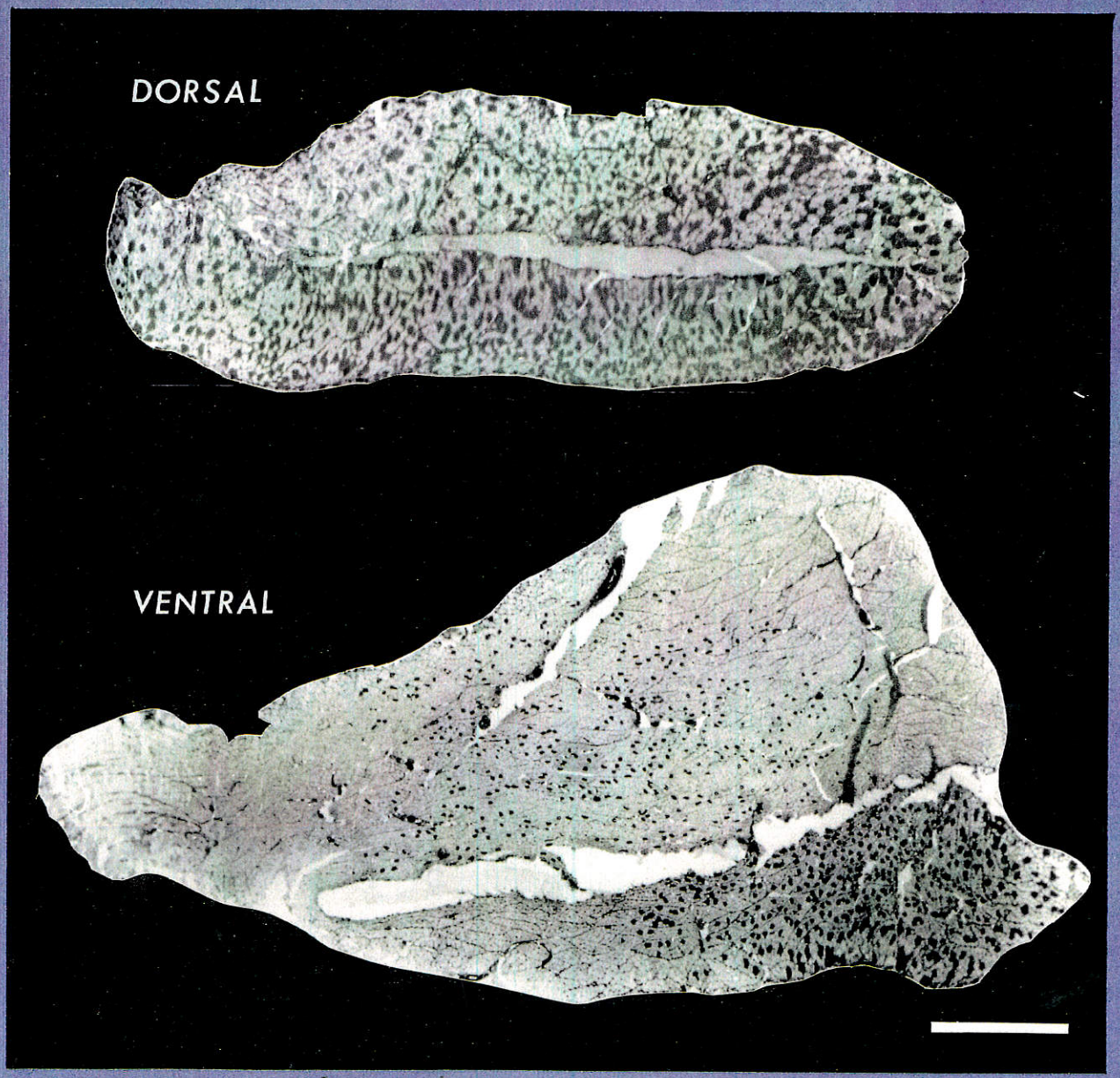
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VENTRAL

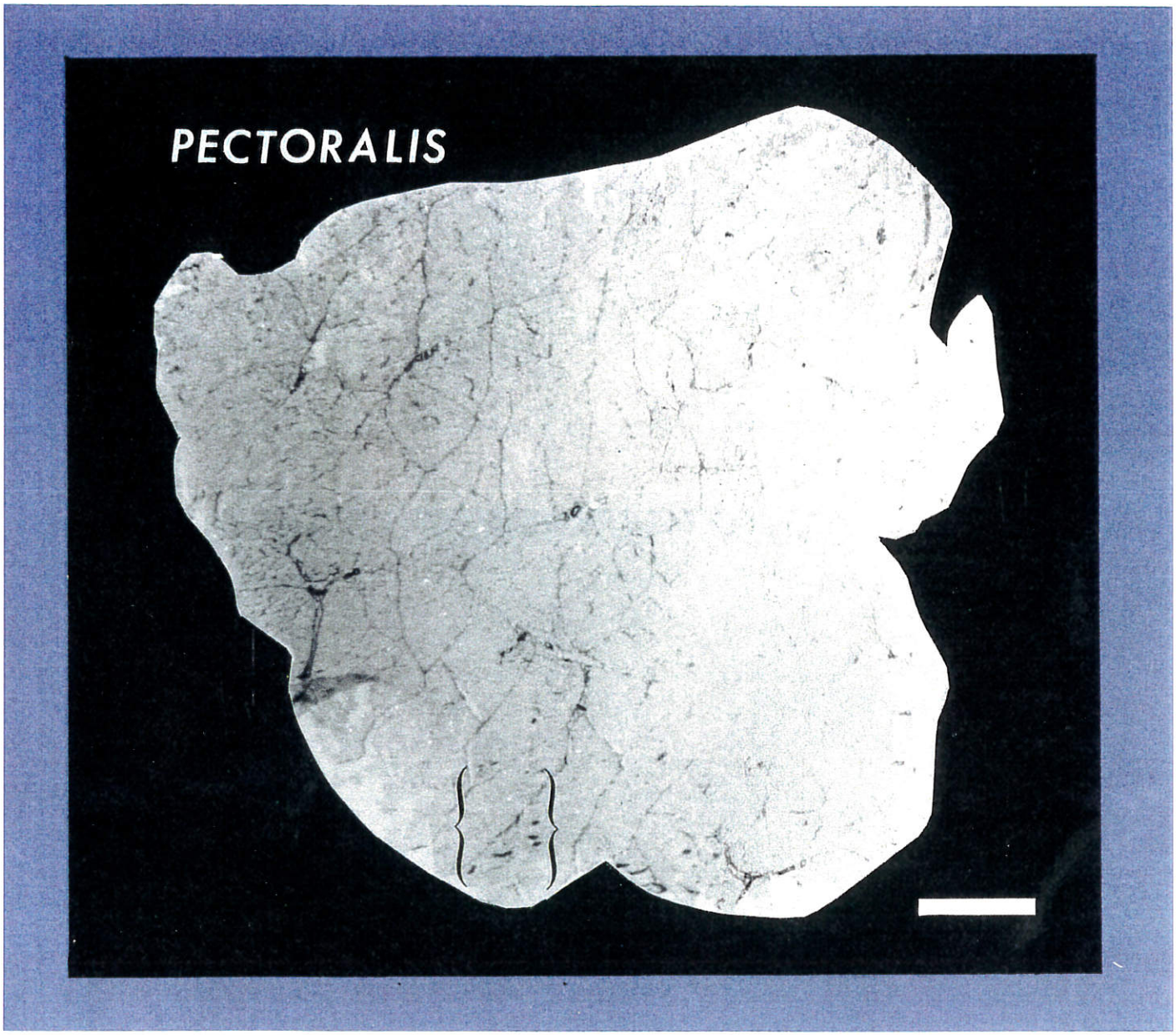
PECTORALIS

Fig. 5

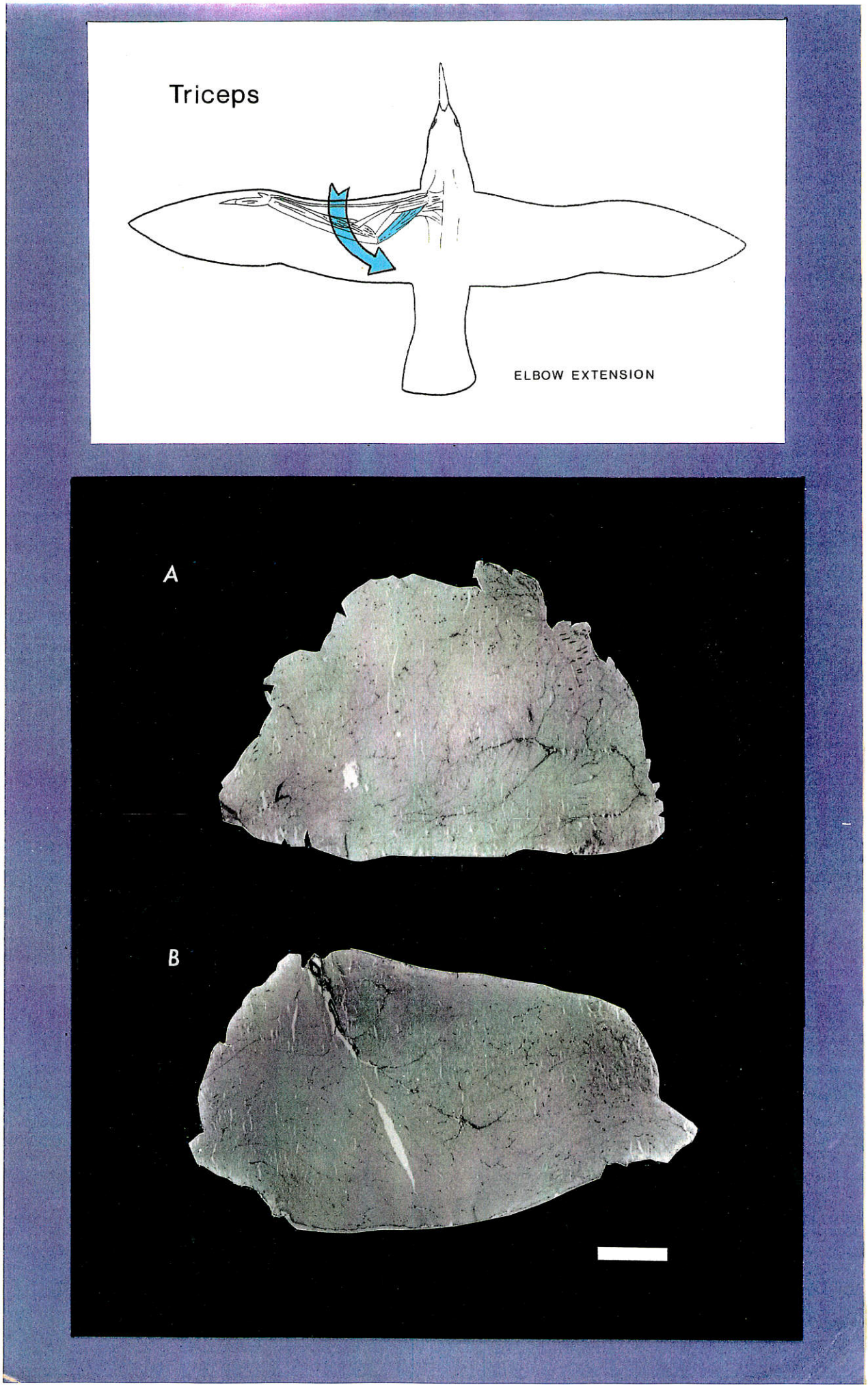

Table 1: Total fiber numbers, Relative fiber percentages, and Standard error of the mean for coracobrachialis cranialis, extensor metacarpi radialis and ventralis in *Larus californicus*

Muscle	Gull	# of Fibers		% of Slow Fibers	Mean % of Slow Fibers ± SE
		Slow	Fast		
Coracobrachialis cranialis	A	1059	11,030	8.7	
	B	660	13,680	4.6	8.1 ± 1.85
	C	1163	9484	10.9	
Extensor metacarpi radialis dorsalis	A	929	2595	26.4	
	B	1433	3180	24.8	28.6 ± 3.0
	C	933	1761	34.6	
Extensor metacarpi radialis ventralis	A	668	10,346	6.1	
	B	800	15,088	5.0	5.8 ± 0.38
	C	852	12,916	6.2	