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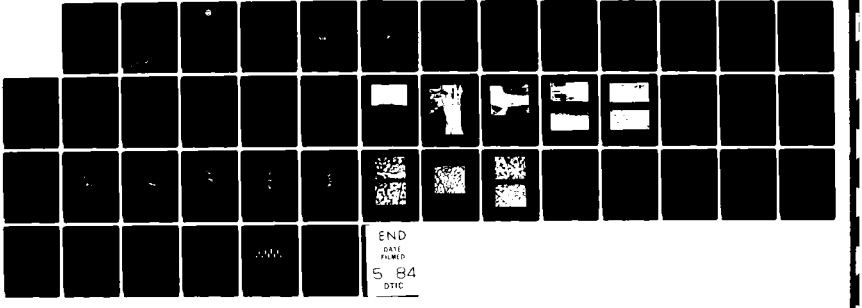

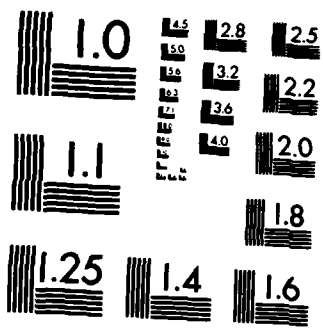

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BIOCHEMICAL AND ANATOMICAL CHARACTERISTICS OF DOLPHIN MUSCLES

H. W. Goforth, Jr.

January 1984
FY83 Final Report

Prepared for
Office of Naval Research
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An examination of the anatomical and biochemical systems involved with swimming in the bottlenose dolphin. This study produced quantitative descriptions of muscle fiber architecture, fiber type composition, and biochemical properties of tendons associated with dolphin locomotion. These data add a new dimension to the current understanding of the axial musculature of dolphins and further define its function in locomotion.

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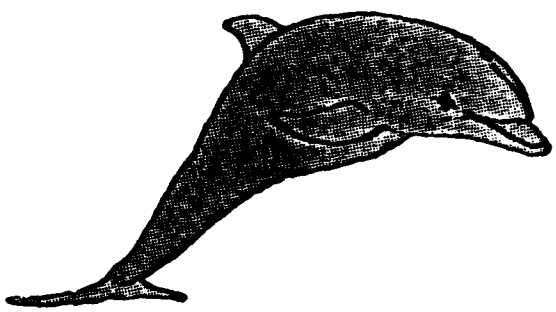
by
Harold W. Goforth, Jr.

January 1984



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APPENDIX A: Muscle Fiber Lengths and Tendon Arrangements in Dorsal and Ventral Muscles of the Dolphin *(Tursiops truncatus)* . . . A-1

APPENDIX B: Fiber Type and Fiber Size Distributions in the Axial Musculature of the Dolphin *(Tursiops truncatus)* . . . B-1

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INTRODUCTION AND BACKGROUND

Dolphins and other cetaceans are capable of swimming at high speed for extended periods of time and produce exceptionally high forces during brief periods (1-3 second bursts). This performance capacity may be a result of (a) efficient biomechanical systems for producing thrust; (b) efficient bioenergetic/metabolic systems; (c) hydrodynamic adaptations that alter resistance to flow; or more likely, (d) contributions from each (Hertel 1969). Reports of dolphins swimming at speeds greater than predicted led to the concept known as "Gray's Paradox" (Gray 1936). Gray calculated that for dolphins to swim at their reported speeds they must develop exceptionally high muscle power and/or an equivalent reduction in drag that would result in a 40% increase in their power-to-body-weight ratio. This report stimulated a number of studies designed to explain this "paradox." Several Navy-funded studies were conducted with dolphins that were trained to swim at maximum speed under controlled, free-swimming conditions (Lang and Pryor 1966; Lang and Norris 1966). These studies concluded that dolphins develop muscular power of 5-6 horsepower to swim at the observed speeds. This was not considered exceptional, being roughly equivalent to that produced by top athletes. The lack of unusual results from these studies and the need for improved telemetry technology required to conduct definitive experiments, caused U.S. research in this field of hydrodynamics to dwindle. Russian research however, continued (see Haun 1982, for review) and concluded that dolphins do possess biological adaptations which create a hydrodynamic advantage (i.e., over identically shaped rigid models). Since U.S. research in this field has lagged several years behind that in the U.S.S.R., it is currently impossible to fully evaluate the validity of the reported Russian findings.

This study was designed to expand our understanding of the power output potential of these animals by examining the anatomical and biochemical characteristics of the musculotendonous systems involved with swimming. Strickler (1980) summarized the needs in this area in his paper on the axial musculature of the La Plata River dolphin:

> "In the past, morphological data have been fitted to various hydrodynamic theories of cetacean locomotion, rather than the theories fitted to the morphology. Any sound theory of locomotion must be consistent with the morphological properties of the system; to understand the action of the muscles, one must first know their anatomy."

This study was designed to determine the muscle fiber architecture (angle of attachment, length, diameter and cross-sectional area), fiber type composition (percentage fast- and slow-twitch), and the biochemical properties of tendons. The goal was to add a new dimension to the current understanding of the anatomy of the dolphin's axial musculature and further define its function in locomotion. The findings reported in this document represent an expanded summary of the work done under a contract to Dr. V. R. Edgerton of the Kinesiology Department/Brain Research Institute at UCLA. Appendices A, B, and C contain material covering the contract work for FY83 as presented by the UCLA researchers at the 1983 Annual Meeting of the American Physiology Society. The figures from these presentations are referred to in the text of this summary and are located in their respective appendix (i.e., A, B, or C).

The ultimate objective of this research is to provide the quantitative data needed to calculate the energy consumed (i.e., oxygen uptake and substrates used) and forces produced at the tendonous attachments (spine and tail fluke) during swimming. In order to design and conduct a dynamic (free swimming) test of the hydrodynamic efficiency of a dolphin, as discussed at the "Dolphin Hydrodynamics Workshop" (Haun 1983), a knowledge of the cardio-respiratory, energy production, and biomechanical systems during exercise is required. These data will then be used to quantitatively evaluate and resolve the questions regarding the dolphin's hydrodynamic efficiency.

MATERIALS AND METHODS

MUSCLE ANALYSES

A frozen specimen of a bottlenose dolphin, *Tursiops truncatus*, (17 years old, female, 180 kg body weight) was thawed and fixed in 10% formalin. Serial cross-sections of the axial muscles were taken from the left side (Appendix B, figure B1) and tissue samples were taken from specific regions. These were then histochemically stained for myosin ATPase. The fiber area and fiber type were then determined using an automated image processing system (Blanco et al. 1982). Individual muscles were identified based on the descriptions reported by Strickler (1980). The muscles on the right side of the specimen were treated with a buffer followed by acid digestion, to facilitate dissection of single muscle fibers (Sacks and Roy 1982). Single fibers were then isolated for architectural analyses (fiber length, angle of attachment and arrangement of tendonous attachments) as described previously (Spector et al. 1980, Bodine et al. 1982, and Powell et al. 1982). Data were analyzed following the guidelines published by Gans and Bock (1965) in their article, "The Functional Significance of Muscle Architecture: A Theoretical Review."

TENDON ANALYSES

Frozen tendon samples were taken from the tail region of the same specimen used for the muscle characterization studies. Analyses were performed on these samples for DNA, hexuronate, noncollagenous protein, glycosaminoglycans and hydroxyproline. Fresh samples from the achilles and patellar tendons of rats also were analyzed for comparative purposes. All tendon samples were maintained in a frozen state, lyophilized and treated with papain digestion before performing the biochemical analyses (Lowry et al. 1951, Bonting and Jones 1957, Bitter and Muir 1962, and Woessner 1961).

RESULTS

Results obtained from the dead tissues of a 180 kg *Tursiops truncatus* specimen have been submitted in a preliminary report to ONR. This report repeats, confirms, and extends those findings to include results from fresh frozen biopsied tissue. The inability to obtain additional

dead specimens for analysis unfortunately limited the conclusions of this study. Marine Mammal Act regulations and Federal Court rulings (National Marine Fisheries Service vs Tuna industry) created unanticipated limitations in acquiring dead specimens. Fortunately, we were able to obtain biopsy material to augment the frozen muscle tissue. The biopsied muscle tissue was obtained using the same procedures employed with human athletes (Costill et al. 1971, Edgerton et al. 1975, Edwards et al. 1980, and Evans et al. 1982). The results are in agreement quantitatively with the fiber type data obtained from the single dead specimen. Additional dead animal tissue samples will be necessary to determine the validity of using dead animal tissues for future studies.

MUSCLE FIBER LENGTH AND ANGLE OF ATTACHMENT

The fiber lengths of the muscles in the dorsal and ventral compartments are shown in Appendix A, figure A1. The fiber lengths of the dorsal muscles ranged from 160-226 mm (avg = 190 mm). Fiber lengths of the ventral muscles had a larger range (37-185 mm), but were shorter overall (avg = 90 mm) than dorsal muscles. In addition the ventral fibers appeared to vary considerably in length in different regions of the same muscle compartment. In both the dorsal and ventral muscles the fibers towards the caudal end were longer than those in the cranial end.

The angle of attachment (pinnation) with respect to the tendon was approximately 15 degrees for most muscles. The dorsal fibers were attached to long thin tendons in a "net-like" arrangement which caudally converged with larger tendons near the tail fluke (Appendix A, figures A2 to A8). The ventral muscles appeared to have some fibers arranged in series (Roy et al. 1983). This issue requires further examination because this information has a significant bearing on the eventual force-velocity predictions.

FIBER TYPE AND SIZE DISTRIBUTION IN AXIAL MUSCULATURE OF DEAD FROZEN MUSCLE TISSUE

The fiber type composition and fiber area (square micrometers) in the individual muscles identified from six of the twenty-four serial cross-sections taken from the dolphin's left side are shown in Appendix B, figures B2a to B2f. Generally, both the dorsal and ventral muscles were composed of a 50:50% population of fast- and slow-twitch fibers (FT and ST). One dorsal muscle (*extensor caudae medialis*) had an average of 70% ST fibers. Some dorsal and ventral muscles located at the caudal end of the body (near the tail fluke) were composed of up to 70% FT fibers (Bello et al. 1983).

The cross-sectional area (CSA) of the ventral muscles was 65% greater than that of the dorsal muscles (1750 vs 1072 μm^2). The FT fibers were 30% and 40% larger in diameter than the ST fibers in the ventral and dorsal muscles, respectively (Appendix B, figures B3a to B3d). The diameters of both fiber types were less than those observed in the cat and other typical terrestial mammals (Burke and Edgerton 1975).

QUANTITATIVE HISTOCHEMISTRY OF FRESH FROZEN MUSCLE BIOPSIES

Three anatomical sites (two dorsal and one ventral) were selected for replicate muscle biopsies (approximately 75 mg each). A formal request was submitted to and approved by the NOSC Animal Care and Utilization Committee. The biopsies were performed by NOSC marine mammal veterinarians. Tissue was obtained from the dorsal and ventral musculature of a 16-year-old female *Tursiops truncatus* that weighed 173 kg. Quantitative histochemical analyses confirmed the fiber type composition previously determined from the single dead specimen that was used for all other analyses in this study (Bello et al. 1983). The collection of these biopsies also provided an opportunity to (a) evaluate the accuracy of the anatomical map developed from the dead specimen; (b) perfect techniques for obtaining replicate biopsies; and (c) evaluate the dolphin's behavioral responses to this procedure. All three of these criteria are important factors in the next phase of this study.

Results indicated that the map was quite accurate and could be scaled to apply to dolphins of different sizes. The fiber type compositions of the biopsied muscles approximated those previously determined from dead muscle tissue. Analysis of the succinate dehydrogenase activity (SDH), a marker enzyme for oxidative capacity (Appendix B, figure B4), revealed that the ST fibers in both the dorsal and ventral muscles were significantly more oxidative than the FT fibers. The SDH activities of the FT fibers did not overlap those of the ST fibers, suggesting that dolphins have few, if any, fast-twitch oxidative glycolytic fibers (FOG fibers). Interestingly, it is this population of fiber types that becomes more prominent after endurance training.

TENDON ANALYSES

The matrix components (collagen and proteoglycans), cellularity (DNA), and noncollagenous protein of dolphin tendons involved with locomotion (achilles and patellar) in terrestrial animals (Appendix C, figures C1 to C6). The dolphin tail tendon was found to be higher in collagen and proteoglycan content, slightly higher in cellularity, and equivalent in noncollagenous protein. Detailed analysis of the proteoglycans revealed that dolphin tendon has a more equal amount of galactosamine (Gal) and glucosamine (Glu) with a ratio of 0.96, compared to 2.8 for rat tendon. The high DNA value suggests that dolphin tendons have a greater capacity for cell turn-over and represent a more dynamic metabolic tissue (i.e., greater rates of synthesis and degradation) than tendons of terrestrial animals. Figure C6 presents a three-axis summary graph comparing the biochemical properties of dolphin and rat tendons. These findings have been previously reported at the American Physiological Society Annual Meeting (Russel et al. 1983).

CONCLUSIONS

MUSCLE FIBER TYPE, SIZE AND ANGLE OF ATTACHMENT

The 50:50% (FT:ST) composition of dolphin swimming musculature indicates a capacity for (a) brief, rapid, forceful contractions, and (b) continuous, slower, less powerful fatigue-resistant contractions. This composition has been suggested to be the best for competitive swimmers (Saltin 1973) and essentially is similar to the 43:57% (FT:ST) reported in an epaxial muscle of a dead Pacific white-sided dolphin (Ponganis and Pierce 1978). The rationale to support this suggestion is that intensive endurance training can improve the oxidative capacity and endurance of the FT fibers resulting in powerful fatigue-resistant fibers. The two specimens used for this study had both been in captivity for a number of years and their physical activity had been limited to slow swimming or occasional short rapid bursts. Neither of these dolphins had been engaged in what could be classified as endurance training immediately prior to tissue sampling. This is further suggested by the lack of overlap in the oxidative enzyme capacity of FT and ST fibers (figure B4). The presence of smaller sized fibers may be an adaptation to decrease the diffusion distance between the capillaries and the center of the fiber. This arrangement would enhance oxygen transfer and aerobic capacity. The occurrence of smaller-diameter fibers may also represent a state of detraining that may be reversed by vigorous physical conditioning. The angle of pinnation (15 degrees) is similar to that reported for most terrestrial mammals (10-20 degrees) and would not significantly affect muscular efficiency.

The differences in fiber length between the dorsal and ventral musculature definitely contribute to a difference in functional capacity. The longer dorsal fibers are designed to produce greater displacement and velocity of movements in that direction. The shorter ventral fibers are designed to produce greater forces at the expense of reduced velocity of muscle shortening. These findings have strong implications regarding the relative importance of the upward and downward strokes of the tail and fluke during swimming (Hertel 1969, Smith et al. 1976, and Strickler 1980). This architectural characteristic supports suggestions that the downward stroke is the primary power stroke. This is in agreement with findings reported by Russian researchers derived from kinematic studies conducted on free-swimming dolphins (Pershin and Tomlin 1974).

The larger-diameter fibers of the ventral muscles indicate a greater force-producing potential than the dorsal muscles. The greater percentage of FT fibers in both the dorsal and ventral muscles near the tail region provides a capability to produce rapid, high forces in a body region requiring contractions of this type during rapid locomotion. The muscle fiber sizes, lengths, and fast-twitch composition appear to represent a coordinated adaptation to allow the ventral muscles to generate a large downward force. However, the eventual objective is to be able to estimate absolute forces and velocities and then match this informationn with measured forces and velocities during swimming. To do this, detailed information on each muscle contributing to forward propulsion must be known and this information must be based on samples taken from healthy animals.

QUANTITATIVE HISTOCHEMISTRY OF FRESH MUSCLE TISSUE

The successful and uncomplicated acquisition of biopsy material was encouraging and suggests that its inclusion in proposed future work will parallel that of human studies. The absence of highly oxidative fast-twitch fibers was somewhat unanticipated since dolphins have been found to possess high levels of fat in their blood (Patton 1975). This generally is associated with the facilitation of a fat-oriented (oxidative) metabolism in muscles (Newsholme 1977). Replication of this finding from tissues of both the live and dead specimens suggests that either dolphins possess only fast fatigable fibers (used only for sprints) or that both animals studied were significantly deconditioned (Holloszy and Booth 1976, and Burke and Edgerton 1975). A sample from a regularly exercised or a newly captured dolphin, which has the biochemical adaptations associated with endurance training, should provide the information to resolve this question. Plans are being made to obtain such a sample.

TENDON ANALYSES

The biochemistry of the dolphin tendon suggests that this tissue is well adapted to withstand large forces and significant deformation (stretch). The combination of capacities for strength and elasticity, coupled with high cellularity, produces a tendon that is adapted for the types of stresses associated with dynamic muscular activity. The relatively high elastic properties of dolphin tendons have significant implications regarding energy efficiencies (Morgan et al. 1978, Alexander et al. 1980, and Ker 1981).

FUTURE STUDIES

To evaluate the hydrodynamic efficiency of a free-swimming dolphin, it will be necessary to determine the energy expended by the animal to swim between two fixed points. A knowledge of the cardio-respiratory and metabolic responses to exercise can be used to indirectly determine oxygen uptake (energy consumption) of the free-swimming dolphin. Direct continuous measurement of oxygen uptake of free-swimming dolphins presents numerous technical problems. These problems may be reduced if cardio-respiratory responses to various workloads can be correlated with oxygen uptake by dolphins in a more controlled setting (Fedak et al. 1981). The direct measurement of respiratory gases of a dolphin exercising against a calibrated workload will provide the conditions needed to correlate cardio-respiratory and metabolic parameters with oxygen uptake and relative workload. This is the same technique used by human physiologists and cardiologists to evaluate the oxygen uptake of athletes and patients when direct determination is impractical (Astrand and Rodahl 1976).

Another future need is for energy substrates and metabolic characteristics of dolphin muscles to be determined under conditions of dynamic exercise. The relative importance of the energy pathways and substrates used by dolphins requires a knowledge of the levels of critical metabolites in living tissue during exercise at selected work intensities. To date, studies of dolphin muscle metabolism have relied upon dead frozen tissue or opportunistic "fresh" tissue obtained from recently stranded animals (Storey and Hochachka 1974, Ponganis

and Pierce 1978, and Castellini et al. 1981). To date there have been no attempts to conduct biopsies on dolphins in conjunction with an exercise stress test (as is frequently done with humans). The combination of factors required to conduct such a study is available to few researchers. Fortunately, the Biological Sciences Division (Code 514) of NOSC has the appropriate facilities: i.e., holding facilities, dolphins, training expertise, marine mammal veterinarians, exercise physiologists, biochemists, and bioengineering support. Additionally, NOSC, through its joint research projects with UCLA and UCI, has access to researchers who are recognized experts in the fields of neuromuscular physiology, connective tissue, and muscle biochemistry. A detailed proposal outlining the goals, approach, milestones, and costs for planned FY84 research has been submitted to the Office of Naval Research (Codes 422CB and 441).

BIBLIOGRAPHY

Alexander, R. McN., A. S. Jayes, and R. F. Ker. Estimates of energy cost for quadrupedal running gaits. *J. Zool.* (London) 190: 155-192, 1980.

Astrand, P. O. and K. Rodahl. *Textbook of Work Physiology*. New York: McGraw-Hill, 1976.

Bello, M. A., R. R. Roy, I. Oxman, T. Martin, and V. R. Edgerton. Fiber type and fiber size distributions in the axial musculature of the dolphin *(Tursiops truncatus). Physiologist* 26: A-26, 1983.

Bitter, T. and H. M. Muir. A modified uronic acid carbazole reaction. *Analyt. Biochem.* 4: 330, 1962.

Blanco, C. E., V. R. Edgerton, and K. Cashteman. Apparent regional differences in the oxidative capacity of single muscle fibers in the cat hindlimb. *Amer. Physiol. Society* Abstract, 1982.

Bodine, S. C., R. R. Roy, D. A. Meadows, R. F. Zernike, R. D. Sacks, M. Fournier, and V. R. Edgerton. Architectural, histochemical and contractile characteristics of a unique biarticular muscle: The cat semiteninosus. *J. Neurophysiol.* 48: 192-201, 1982

Bonting, S. L. and M. Jones. Determination of microgram quantities of deoxyribonucleic acid and protein in tissues grown in vitro. *Arch. Biochem. and Biophys.* 66: 340, 1957.

Burke, R. E. and V. R. Edgerton. Motor unit properties and selective involvement in movement. *In: Exercise and Sport Science Reviews.* New York: Academic Press, Vol. 3: 31-70, 1975.

Castellini, M. A., G. N. Somero, G. L. Kooyman. Glycolytic enzyme activities in tissues of marine and terrestrial mammals. *Physiol. Zool.* 54(2): 242-252, 1981.

Costill, D. L., K. Sparks, R. Gregor, and C. Turner. Muscle glycogen utilization during exhaustive running. *J. Appl. Physiol.* 31(3): 353-356, 1971.

Edgerton, V. R., B. Essen, B. Saltin, and D. R. Simpson. Glycogen depletion in specific types of human skeletal muscle fibers in intermittent and continuous exercise. pp. 402-415 *In:* H. Howald and J. R. Poortmans eds. *Metabolic Adaptations to Prolonged Physical Exercise.* Birkhauser Verlag Basel, Lausanne, 1975.

Edwards, R., A. Young, and M. Wiles. Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. *New Eng. J. of Med.* 302(5): 261-271, 1980.

Evans, W. J., S. D. Phinney, and V. R. Young. Suction applied to a muscle biopsy maximizes sample size. *Med. Sci. Sports Exercise.* 14(1): 101-102, 1982.

Fedak, M. A., L. Rome, and H. J. Seeherman. One-step N^2-dilution technique for calibrating open-circuit VO_2 measuring systems. *Amer. Physiol. Soc.* 51(3): 772-776, 1981.

Gans, C. and W. J. Bock. The functional significance of muscle architecture: A theoretical analysis. *Ergeb. Anat. Entwicklungsgesch.* 38: 115-142, 1965.

Gray, J. Studies in animal locomotion. VI. The propulsive powers of the dolphin. *J. Exp. Biol.* 13: 192-199, 1936.

Haun, J. E. Dolphin hydrodynamics: A review of the literature. Naval Ocean Systems Center, San Diego, CA, TN 1183, Sept 1982.

Haun, J. E., Editor. Proceedings of Dolphin Hydrodynamics Workshop, Naval Ocean Systems Center, TD 569, Apr 1983.

Hertel, H. Hydrodynamics of swimming and wave-riding dolphins. pp. 31-63 *In* H. T. Andersen ed. *The Biology of Marine Mammals.* Academic Press, New York, 1969.

Holloszy, J. O. and F. W. Booth. Biochemical adaptations to endurance exercise in muscle. *Ann. Rev. of Physiol.* 38: 273-291, 1976.

Ker, R. F. Dynamic tensile properties of the plantaris tendon of sheep *(Ovis aries) J. Exp. Biol.* 93: 283-302, 1981.

Lang, G. T. and K. S. Norris. Swimming speed of a Pacific bottlenose porpoise *(Stenella attenuata). Science* 151: 531-533, 1966.

Lang, G. T. and K. Pryor. Hydrodynamic performance of porpoises *(Stenella attenuata). Science* 152(3721): 531-533, 1966.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265, 1962.

Morgan, D. L., U. Proske, and D. Warren. Measurements of muscle stiffness and the mechanism of elastic storage of energy in hopping kangaroos. *J. Physiol.* 282: 253-261, 1978.

Newsholme, E. A. The regulation of intracellular and extracellular fuel supply during sustained exercise. *Annals N. Y. Acad. Sci.* 301: 81-91, 1977.

Patton, G. S. Metabolic studies on marine mammals: Characterization of alpha- and beta-cell function. PhD dissertation, Univ. of S. Cal., 1975.

Pershin, S. V. and A. G. Tomlin. Biochemical fundamentals for controllable hydroelastic effect in fins, the wing supporting surfaces of cetaceans (dolphins and whales). *Funktsional' naya morphologiya mlekopitayushchikh* 54: 266-305, 1974.

Ponganis, P. J. and R. W. Pierce. Muscle metabolic profiles and fiber type composition in some marine mammals. *Comp. Biochem. Physiol.* 59B: 99-102, 1978.

Powell, P. L., R. R. Roy, P. Kanin, M. Bello, and V. R. Edgerton. Predictability of skeletal muscle tension output from architectural determinations. *Physiologist* 26: 259, 1982.

Roy, R. R., M. A. Bello, I. Oxman, and V. R. Edgerton. Muscle fiber lengths and tendon arrangements in dorsal and ventral muscles of the dolphin *(Tursiops truncatus). Physiologist* 26: A-26, 1983.

Russell, M., A. C. Vailas, V. R. Edgerton, C. Sasson, A. Nekooi, and J. Durivage. Dolphin tendon matrix components. *Physiologist* 26: A-26, 1983.

Sacks, R. D. and R. R. Roy. Architecture of the hind limb muscles of cats: functional significance. *J. Morphol.* 173: 185-195, 1982.

Saltin, B. Metabolic fundamentals in exercise. *Med. Sci. in Sports* 5(3): 137-146, 1973.

Smith, G. J. D., K. W. Browne, and D. E. Gaskin, Functional myology of the harbor porpoise, *Phocoena phocoena* (L.). *Can. J. Zool.* 54(5): 716-729, 1976.

Spector, S. A., P. F. Gardiner, R. F. Zernike, R. R. Roy, and V. R. Edgerton. Muscle architecture and the force velocity characteristics of the cat soleus and medial gastrocnemius: Implications for motor control. *J. Neurophysiol.* 44: 951-960, 1980.

Strickler, T. L. The axial musculature of *Pontoporia blainvilli* with comments on the organization of this system and its effect on fluke-stroke dynamics in the cetacea. *Am. J. Anat.* 157: 49-59, 1980.

Woessner, J. F. The determination of hydroxyproline in tissues and protein samples containing small proportions of this amino acid. *Arch. Biochem. Biophys.* 93: 440-447, 1961.

APPENDIX A

MUSCLE FIBER LENGTHS AND TENDON ARRANGEMENTS IN DORSAL AND VENTRAL MUSCLES OF THE DOLPHIN *(Tursiops truncatus)*

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OVERVIEW

The architecture of the dolphin axial musculature may be an important factor in this mammal's ability to swim. To examine this question, the architecture of dorsal and ventral axial muscles was studied. A frozen specimen (~17 years old and 180 kg body weight) was thawed and fixed in 10% formalin. The muscles were removed and digested in acid (Sacks and Roy, *J. Morphol.* 173: 185, 1982). Fiber lengths in the dorsal muscles ranged from 160-226 mm ($\bar{X}$ = 190 mm). Fiber lengths in the ventral muscles had a larger range (37-185 mm), were shorter ($\bar{X}$ = 90 mm), and appeared to vary considerably in different regions of the same compartment. The fibers toward the caudal end were longer than those in the more cranial end in both the ventral and dorsal muscles. Angle of pinnation with respect to the tendon was ~15° in most muscles. The dorsal fibers were attached to thin, long tendons in a "net-like" arrangement which eventually converged with the larger tendons near the fluke. In the ventral muscles, some fibers appeared to be arranged in series. These differences may have functional implications with respect to proposed differences in the upward and downward strokes during swimming.

METHODS

A frozen specimen (~17 years old and 180 kg body weight) was thawed and fixed in 10% formalin. Muscles were then carefully disected, removed and digested in acid (Sacks and Roy, *J. Morphol.* 173: 185, 1982). Single muscle fibers were isolated and fiber and sarcomere lengths were measured.

RESULTS

Results are shown in figures A1 through A8.

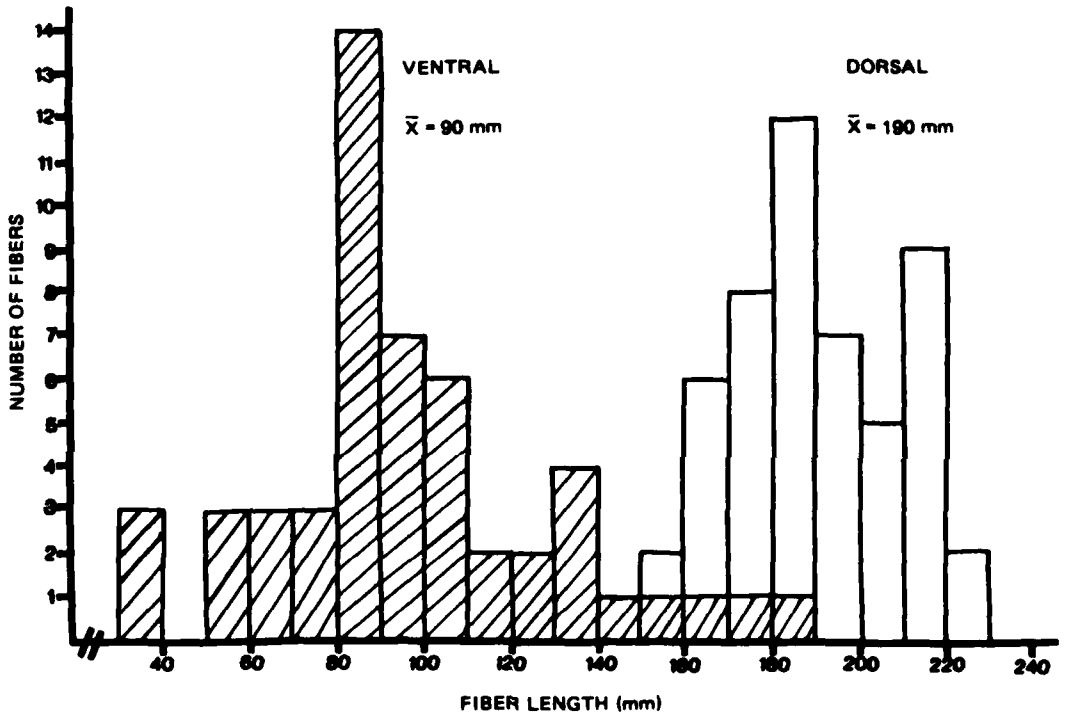

Figure A1. Muscle fiber lengths recorded in muscles taken from both the dorsal and ventral compartments.

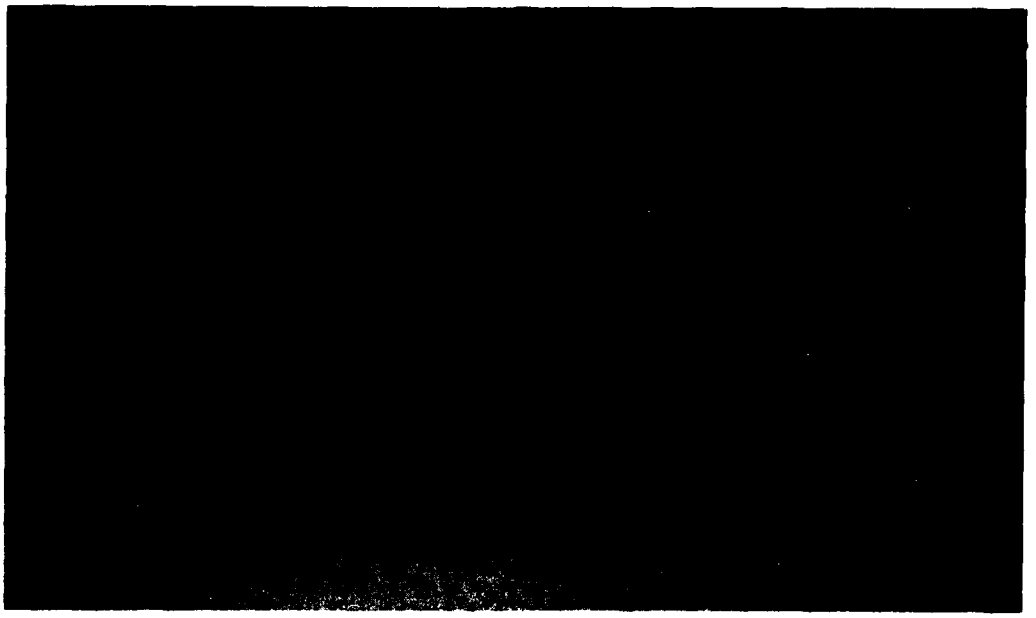

Figure A2. Muscle fiber attachment to a sub-branch of a tendon.

Note: Several bundles of muscles converge on a common tendon sub-branch (also see figure A7). Many sub-branches converge in the caudal direction with large tendons becoming the most prominent at the caudal-most point. Smaller fiber bundles were teased to obtain the fiber lengths shown in figure A1.

Figure A3 The typical tendon-muscle arrangement present throughout the axial musculature

Note Fibers are present in multiple layers, each in a feather-like arrangement. In most muscles, the angle of pinnation of the muscle fiber with respect to the tendon was 15 degrees

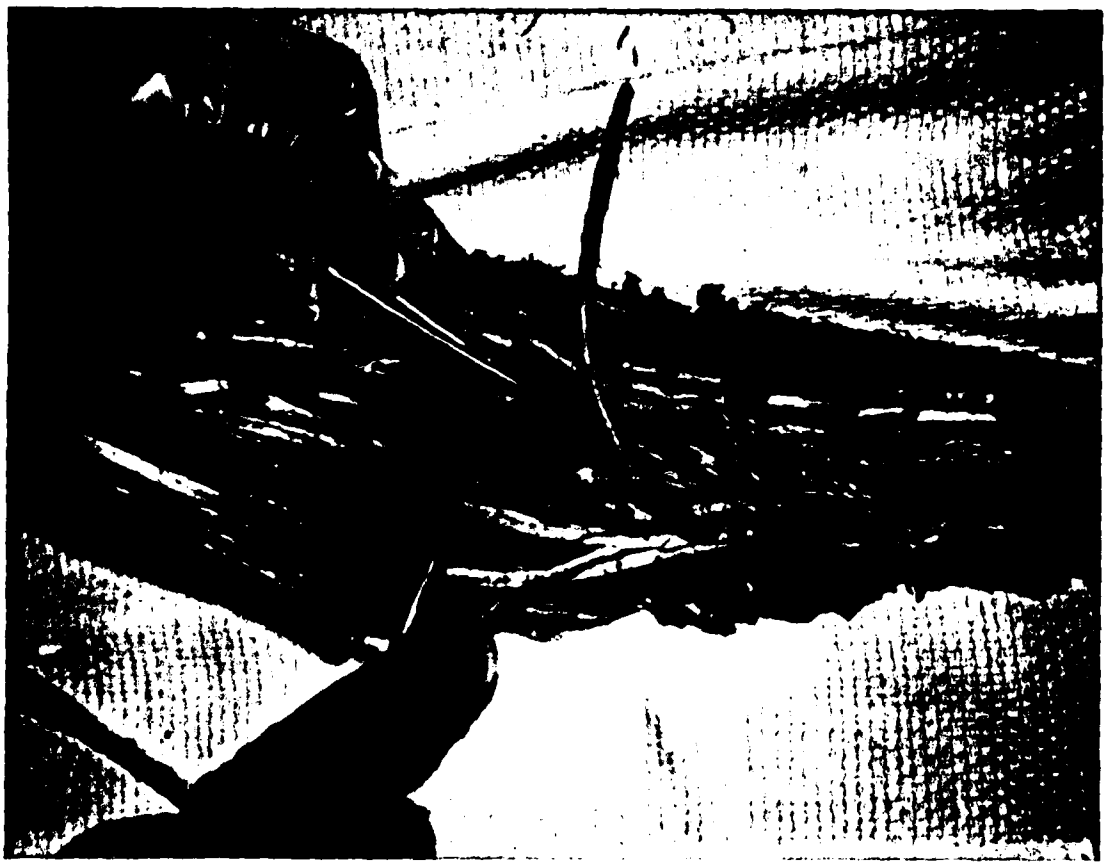

Figure A4. Shorter muscle fiber lengths of *about* 35–40 *mm* (exposed between the forceps above) were observed within the ventral musculature.

Note: Thick connective tissue ensheaths the tendons.

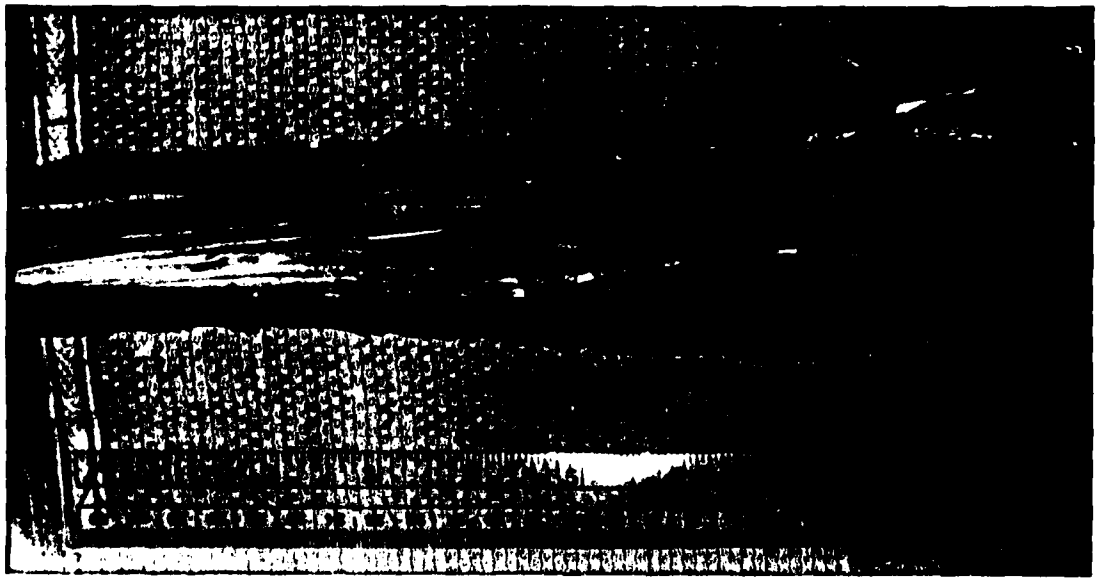

Figure A5. Gradual convergence of tendons to form thick tendon bundles caudally.

Note: Each of these tendinous bundles can be teased into fine individual tendons and traced to its muscle fiber attachment.

Figure A6. The lattice arrangement of connective tissue in which the fibers are intermeshed.

Note: The unique tendinous convergence begins as fine individual tendons branched throughout the muscle with each small branch being a site of muscle fiber attachment.

Figure A7. The intricate interwoven pattern of tendons from muscles of different compartments.

Note: In some instances, as shown above, the tendinous arrangement suggests an "in series" fiber arrangement.

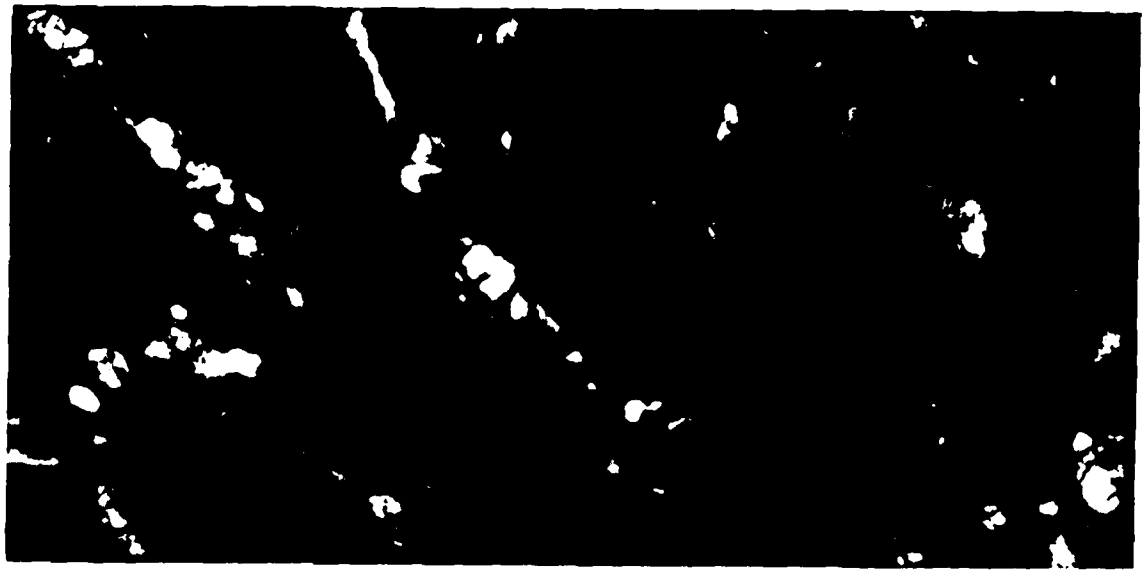

Figure A8. The tendon arrangement in muscles from the dorsal compartment of the axial musculature.

Note: The tendinous arrangements are illustrated with a trichrome stain. Many tendon branches of various sizes are found throughout the muscle. These tendons are often aligned providing distinct separations of the muscles.

CONCLUSIONS

1. Fiber lengths in the dorsal muscles were longer ($\bar{X}$ = 190 mm) than the fiber lengths in the ventral muscles ($\bar{X}$ = 90 mm).

2. The ventral compartment had a larger range of muscle fiber lengths.

3. Each muscle consists of multiple layers of fibers in a feather-like arrangement.

4. Each tendon consists of multiple levels of sub-branches with each sub-branch forming a site of attachment for muscle fibers along its length in a pinnate fashion.

5. The differences in length of the muscle fibers in the dorsal and ventral musculature imply a difference in the muscle's ability to produce displacement and velocity versus force with the shorter fibers implying a bias toward force. These differences may have functional implications with respect to proposed differences in the upward and downward strokes during swimming.

APPENDIX B

FIBER TYPE AND FIBER SIZE DISTRIBUTIONS IN THE AXIAL MUSCULATURE OF THE DOLPHIN *(Tursiops truncatus)*

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OVERVIEW

To begin to understand the bioenergetic efficiency of the swimming dolphin, muscle fiber size and type of the axial musculature were investigated. All tissue samples were taken from a single specimen (~17 years old and 180 kg body weight). "Fast" (F) and "slow" (S) fiber types were identified from frozen sections stained for myosin ATPase. Fiber area and type were determined using an automated image processing system. Generally, both the dorsal and ventral muscles consisted of 50% S and 50% F fibers. One dorsal muscle (*extensor caudae medialis)* (Strickler, *Am. J. Anat.* 157:49, 1980) had one region that consisted of about 70% S. The caudal end of the dorsal and ventral muscles had about 70% F fibers. Mean cross-sectional area (CSA) of the fibers in the ventral muscles was ~65% greater than in the dorsal muscles (1750 vs 1072 μm^2). The F fibers were 40% (2200 vs 1317 μm^2) and 30% (1213 vs 879 μm^2) larger than the S fibers in the ventral and dorsal muscles, respectively. These fiber sizes are smaller than for most terrestrial mammalian muscles. The observation that the ventral muscles had larger and shorter fibers (Roy et al., *Physiologist,* 1983) suggests that they are specifically designed for force production. In contrast, the dorsal muscles are designed to optimize velocity and displacement.

METHODS

Tissue samples were taken from a single specimen (~17 years old and 180 kg body weight). "Fast" (F) and "slow" (S) fiber types were identified from frozen samples, taken from specified cross-sections of the animal, and stained for myosin ATPase. The fiber area and type were determined using an automated image processing system.

RESULTS

Figures B2a through B2f show the results of the fiber analyses of the muscle cross-sections shown in figure B1. These analyses were performed on the Automated Image Processing System (AIPS). Each sample was taken from the indicated site within each cross-section and stained for myosin ATPase, using alkaline preincubation, (pH 8.8) as shown in figure B3a. The fiber area of individual muscles was determined by outlining each fiber on the AIPS computer. Individual muscles were identified based on previous studies by Strickler (1980).

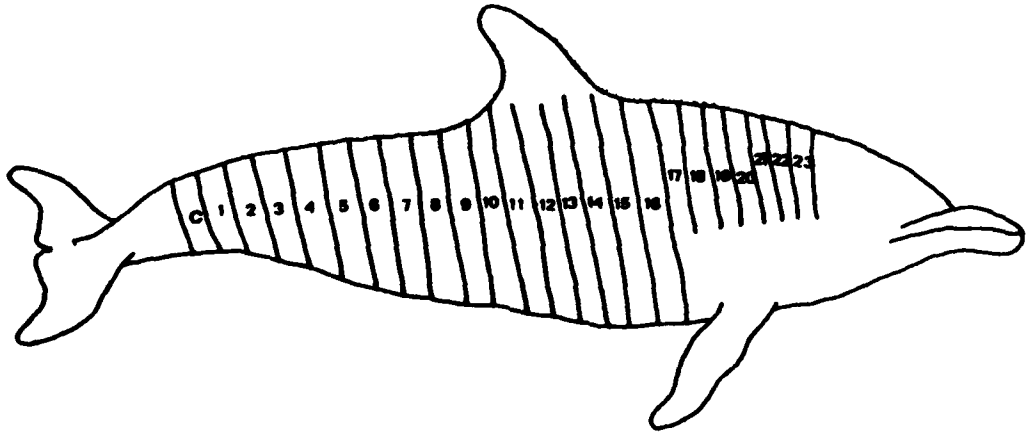

Figure B1. Sites where the twenty-four cross-sections of muscle were taken to determine the fiber type composition and cross-sectional area of the axial muscles.

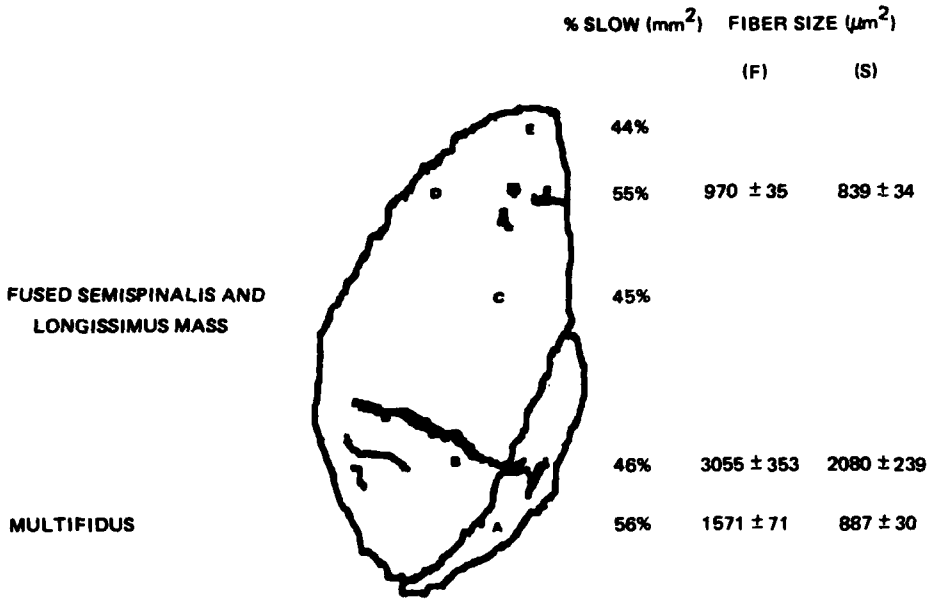

Figure B2a. Fiber type composition and area of muscles in cross-section number 23.

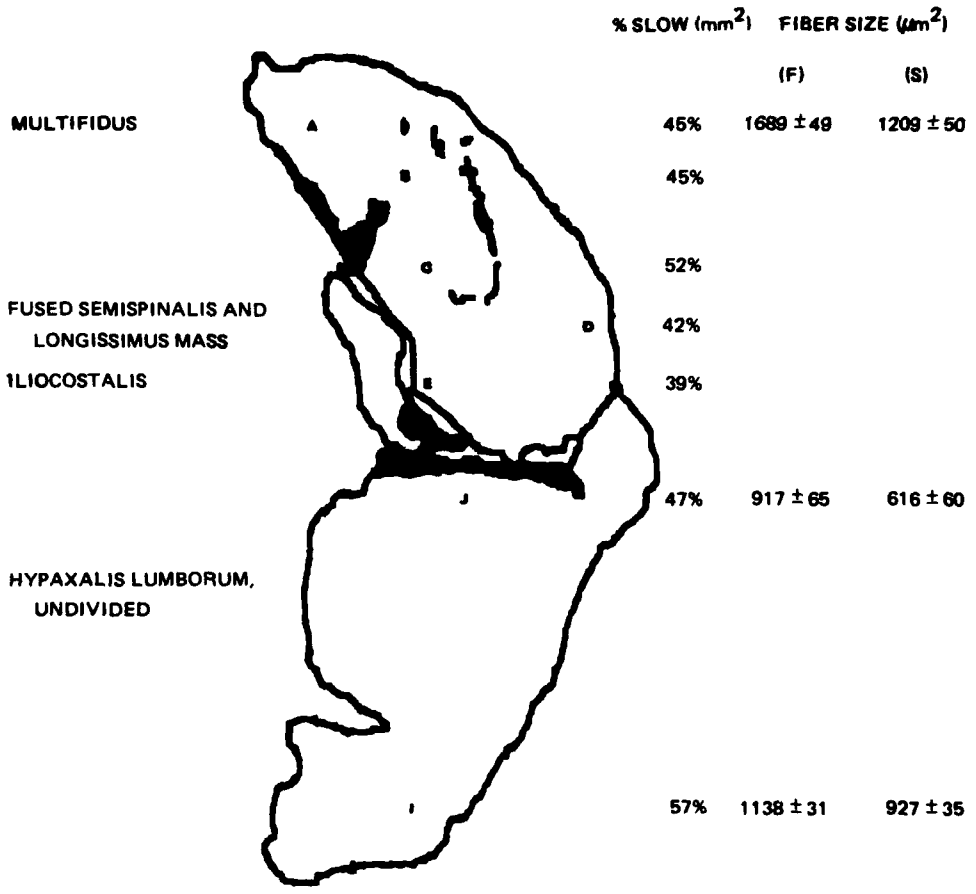

Figure B2b. Fiber type composition and area of muscles in cross-section number 13.

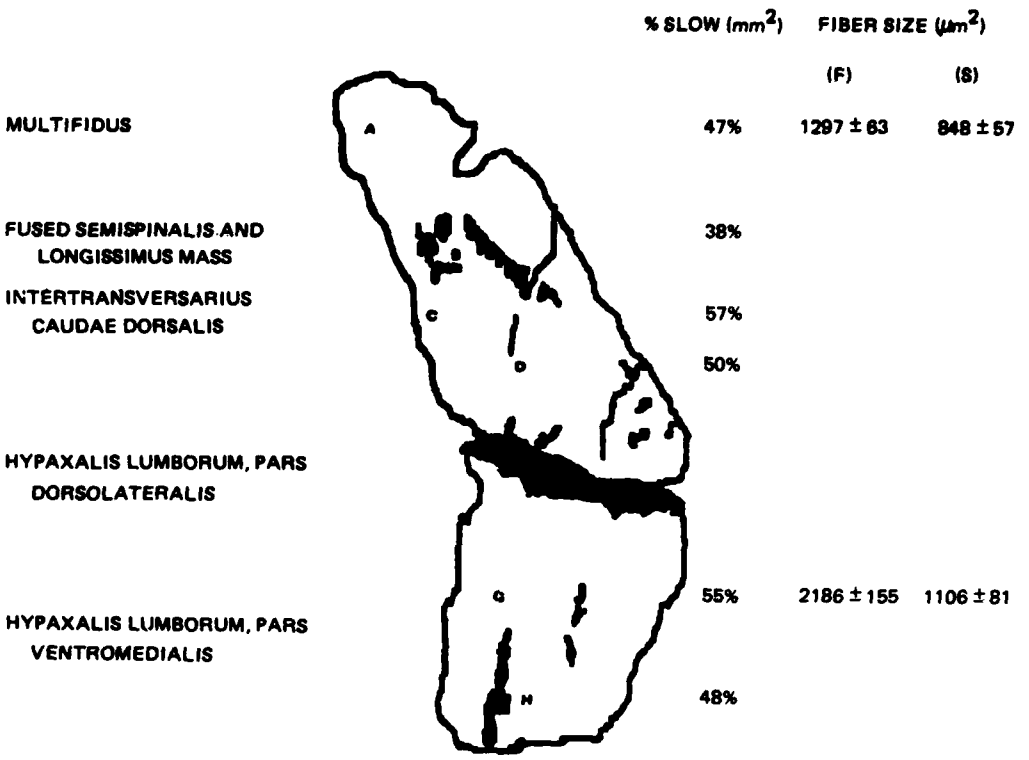

Figure B2c. Fiber type composition and area of muscles in cross-section number 8.

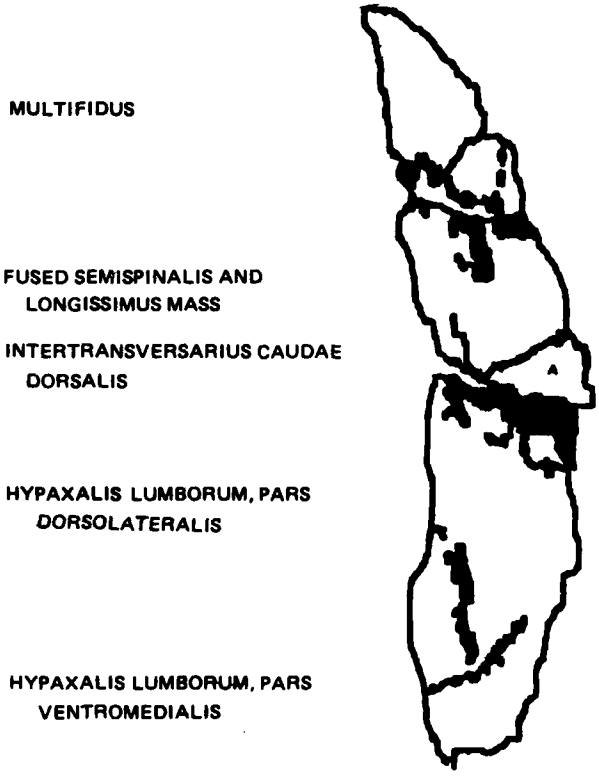

Figure B2d. Fiber type composition and area of muscles in cross-section number 5.

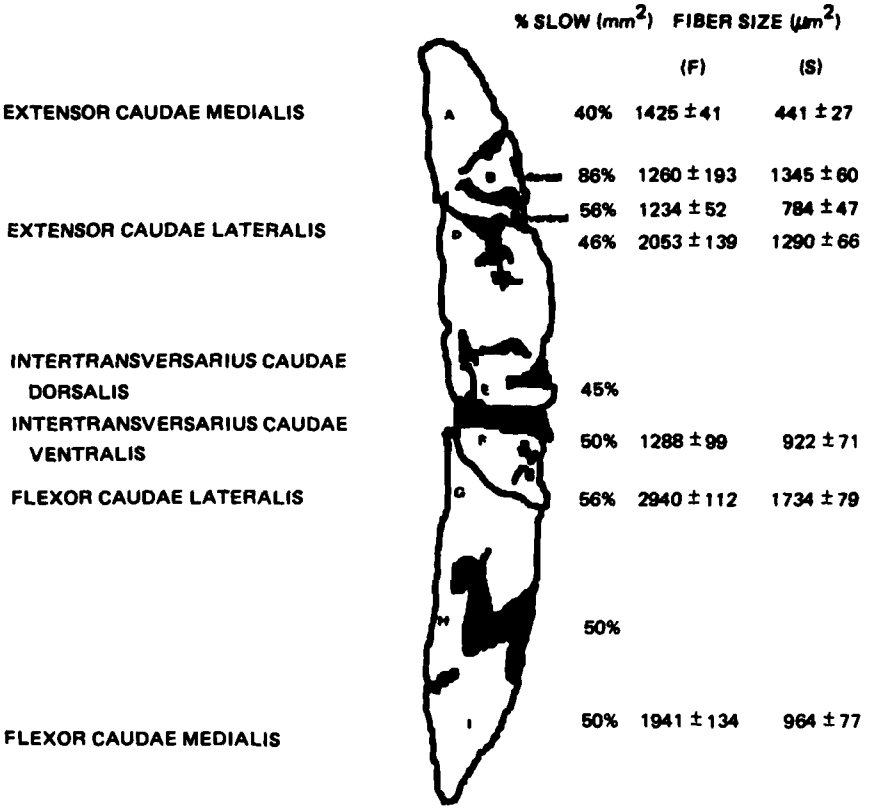

Figure B2e. Fiber type composition and area of muscles in cross-section number 2.

	% SLOW	FIBER SIZE (μm^2) (F)	FIBER SIZE (μm^2) (S)
EXTENSOR CAUDAE MEDIALIS	25%	977 ± 40	283 ± 26
EXTENSOR CAUDAE LATERALIS	31%		
INTERTRANSVERSARIUS CAUDAE DORSALIS	36%	2490 ± 89	1196 ± 59
INTERTRANSVERSARIUS CAUDAE VENTRALIS	38%	1547 ± 70	673 ± 33
	47%		
FLEXOR CAUDAE LATERALIS			
FLEXOR CAUDAE MEDIALIS	50%	1822 ± 73	940 ± 39

Figure B2f. Fiber type composition and area of muscles in cross-section number C.

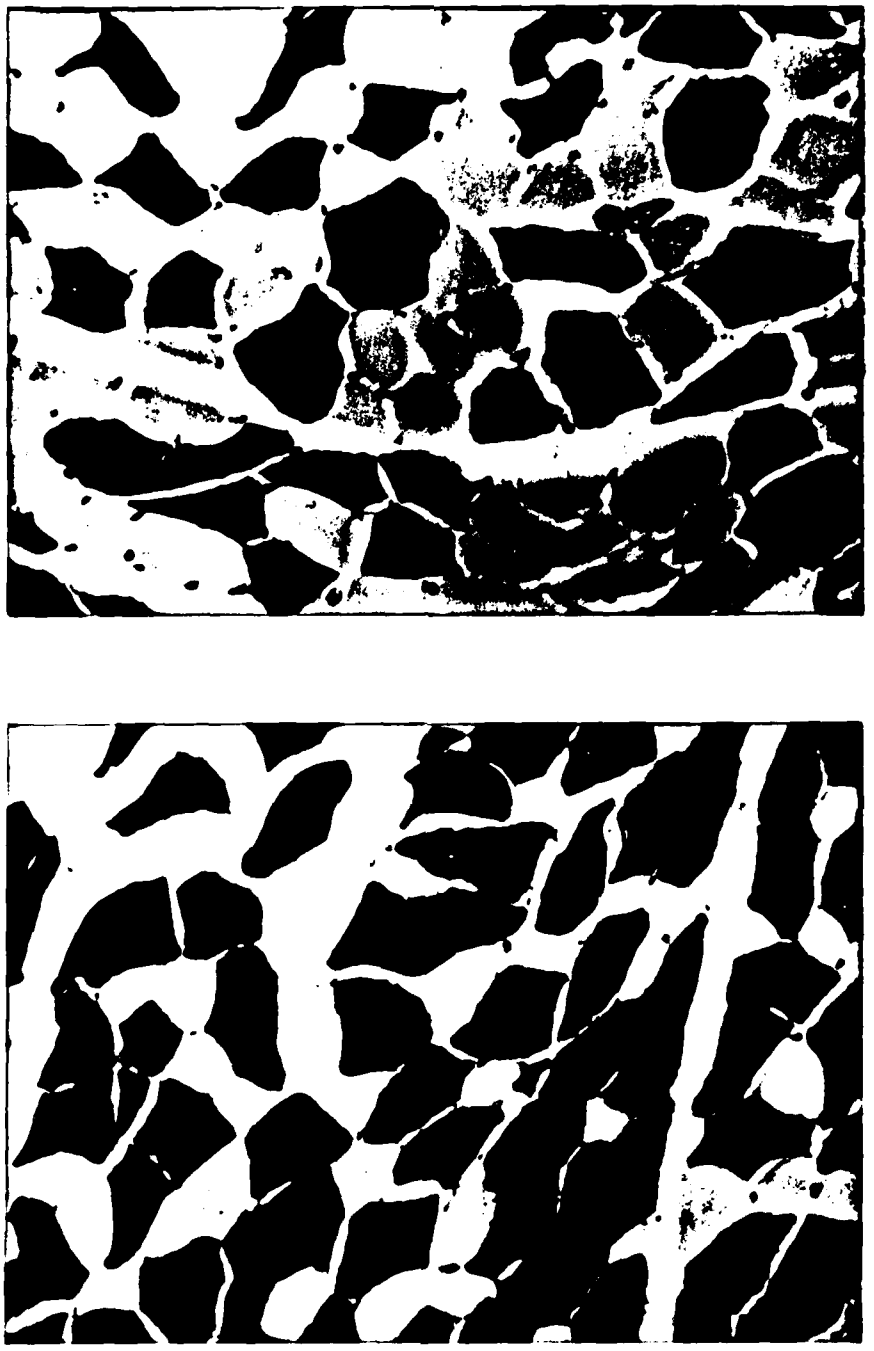

Figure B3a. Muscle fiber type of the cross-section of muscles from the caudal end of the dorsal compartment (designated A).

Note: These photographs illustrate the muscle fiber type by histochemically staining for myosin ATPase. The fast (F) fibers stained dark and the slow (S) fibers stained light.

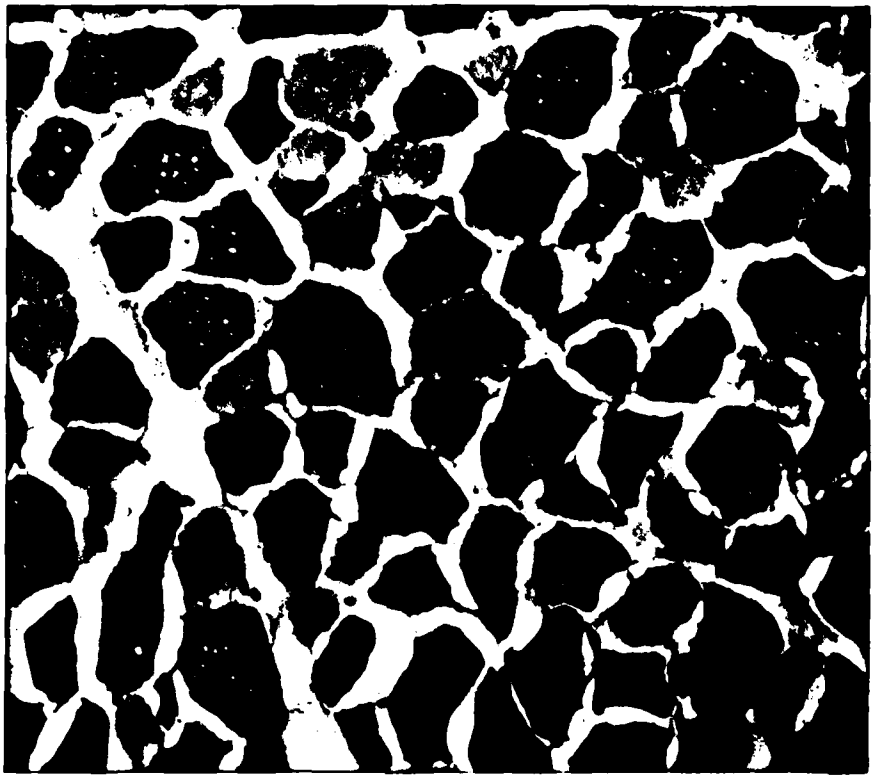

Figure B3b. Muscle fiber type of the cross-section of muscles from the rostral end of the dorsal compartment (designated 13B).

Figure B3c. Muscle fiber type of the cross-section of muscles from the caudal end of the ventral compartment (designated F).

Figure B3d. Muscle fiber type composition of the cross-section of muscles from the rostral end of the ventral compartment (designated 131).

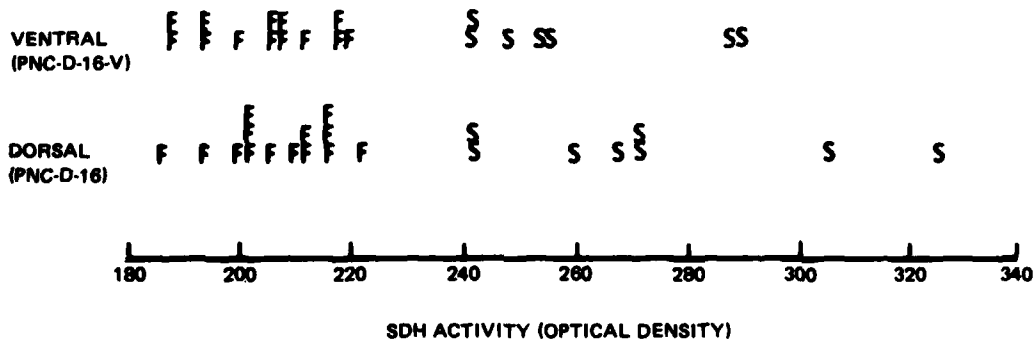

Figure B4. Results of quantitative histochemical analyses of succinate dehydrogenase activity (SDH) in fresh frozen muscle biopsies taken from the dorsal and ventral compartments of a bottlenose dolphin (*Tursiops truncatus*).

CONCLUSIONS

1. Most dolphin muscles consist of approximately 50% "fast" and 50% "slow" fibers as identified histochemically by myofibrillar ATPase.

2. The ventral muscle fibers were significantly larger than the dorsal fibers.

3. The "fast" fibers were larger than the "slow" fibers.

4. Considering the muscle size along with the muscle architecture, the dorsal muscles seem to be slightly better suited for velocity while the ventral muscles are slightly better suited for force.

APPENDIX C

DOLPHIN TENDON MATRIX COMPONENTS

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OVERVIEW

Very little is known about the important tendonous structures that are involved in the transmittance of muscular forces to the fluke of a dolphin. The purpose of this preliminary study is to characterize the matrix components in tendons of the dolphin tail region and make comparisons to similar components in tendons from lower extremities of terrestrial mammals. The matrix components collagen, proteoglycans, noncollagenous protein, and cellularity were evaluated from papain digests of tendons obtained from the tail region of the dolphin and compared to achilles and patellar tendons of rats. In contrast to rat tendon, dolphin tail tendon was higher in collagen and proteoglycan content, slightly higher in cellularity, and equivalent in noncollagenous protein. Analysis of proteoglycan composition in rat tendon revealed a substantially greater amount of galactosamine than glucosamine, with a GAL:GLU ratio of 2.8. For dolphin tendon, however, the GAL:GLU ratio of 0.96 indicated nearly equal amounts of the two hexosamines. These data suggest a biochemical difference in dolphin and rat tendon, which may be of relevance to the unique myofascial design of the dolphin.

METHODS

Dolphin tissue was taken from the tail tendon region of one frozen specimen. Assays were performed using 17 such samples for the DNA, hexuronate, and noncollagenous protein measurements, 10 samples for the glycosaminoglycans, and 6 samples for the hydroxyproline. Rat tissue was taken from the achilles and patellar tendons (2 each per rat) of freshly sacrificed animals. Assays were performed on 17 such samples for the DNA, hexuronate, and noncollagenous protein measurements, 10 samples for the glycosaminoglycans, and 9 samples for the hydroxyproline. All tissues were frozen, lyophillized, treated with papain digest, and subjected to the follow assays:

- DNA: S. L. Bonting and M. Jones. *Arch. of Biochem. & Biophys.* 66:340, 1957.
- Noncollagenous Protein: O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. *J. of Biol. Chem.* 193:265, 1951.
- Hexuronate: T. Bitter and H. M. Muir. *Analyt. Biochem.* 4:330, 1962.
- Hydroxyproline: J. F. Woessner. *Arch. of Biochem. & Biophys.* 93:440, 1961.

RESULTS

Results of the analyses are presented in table C1 and figures C1 through C6. Table C1 presents a key to the abbreviations used on the figures.

Table C1. Legend for the graphs in figures C1 through C6.

Legend	
RAT AT	= Rat achilles tendon
RAT PT	= Patellar tendon
DOLPHIN	= Dolphin tail tendon
UGMG	= Micrograms per milligram dry weight
DATA BARS	= Standard deviation
GAL:GLU	= Ratio of galactosamine to glucosamine content

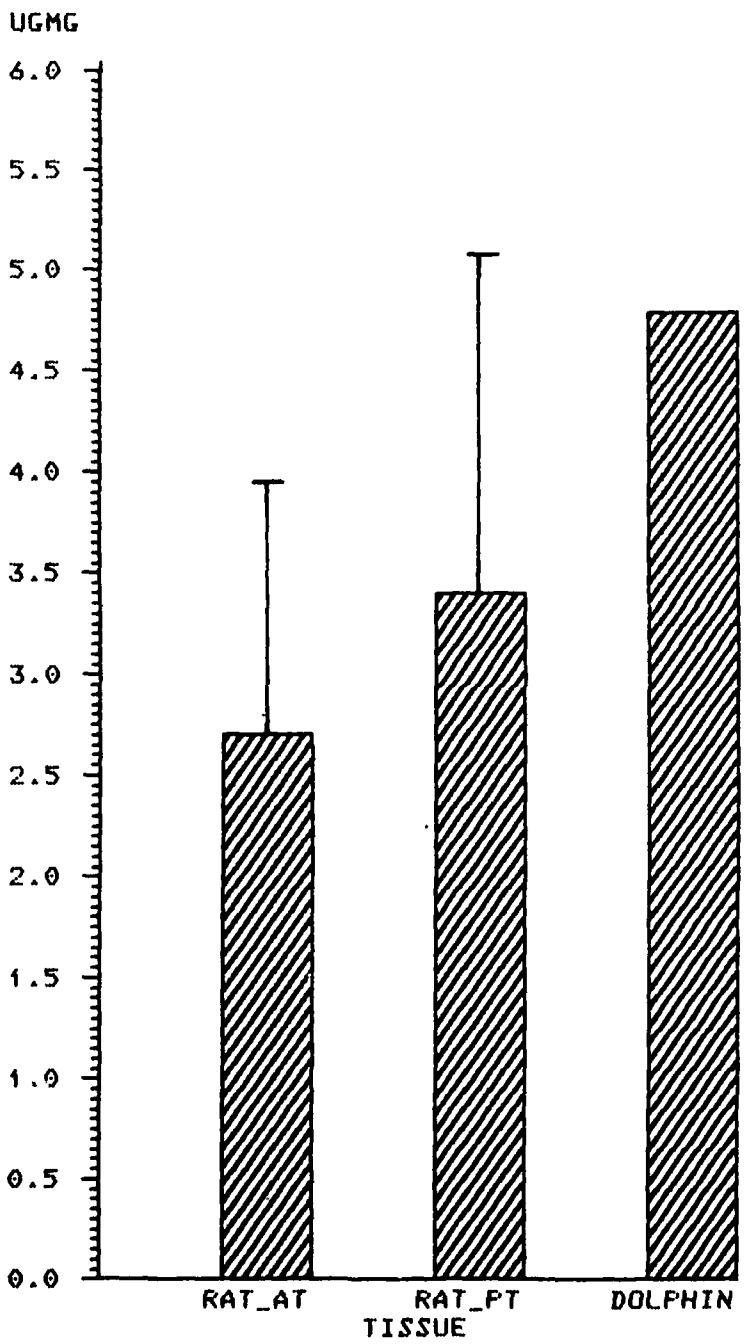

Figure C1. DNA content of dolphin and rat tendons.

HEXURONATE

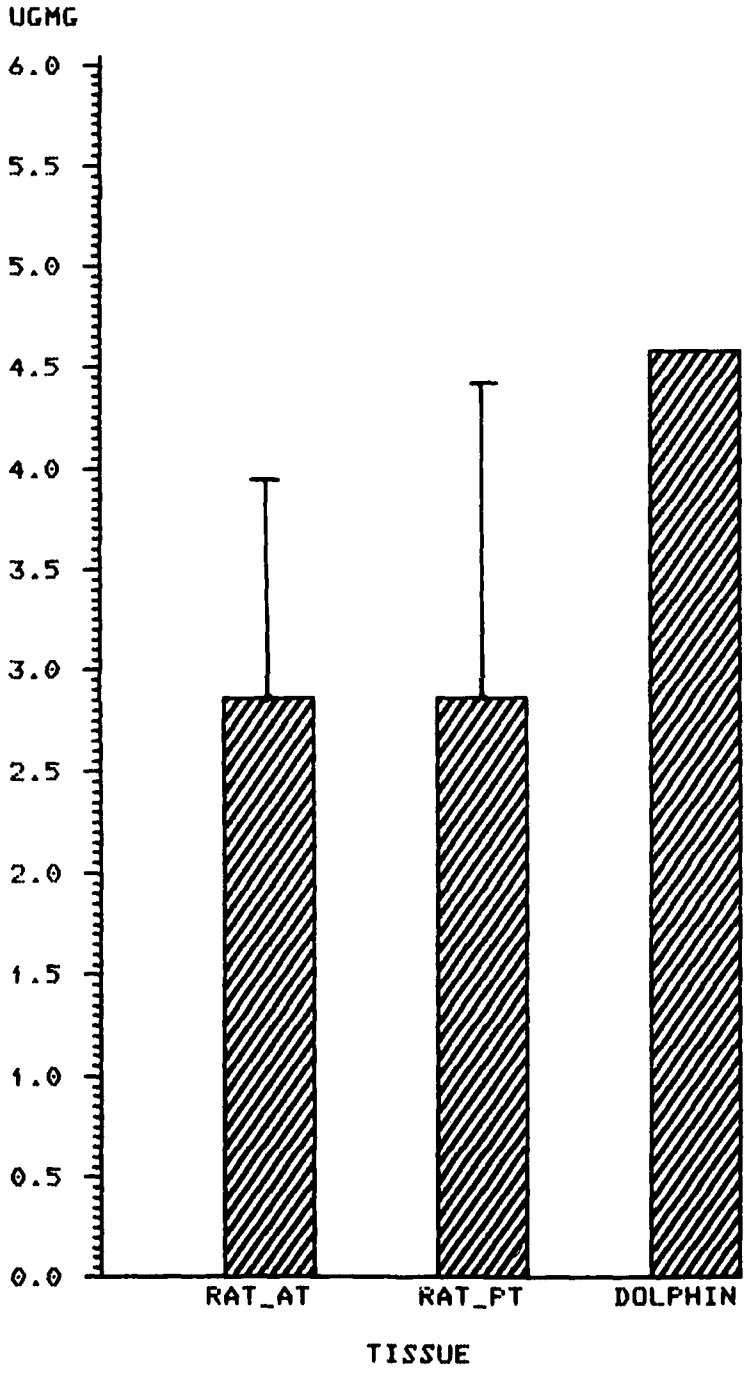

Figure C2. Hexuronate content of dolphin and rat tendons.

HYDROXYPROLINE

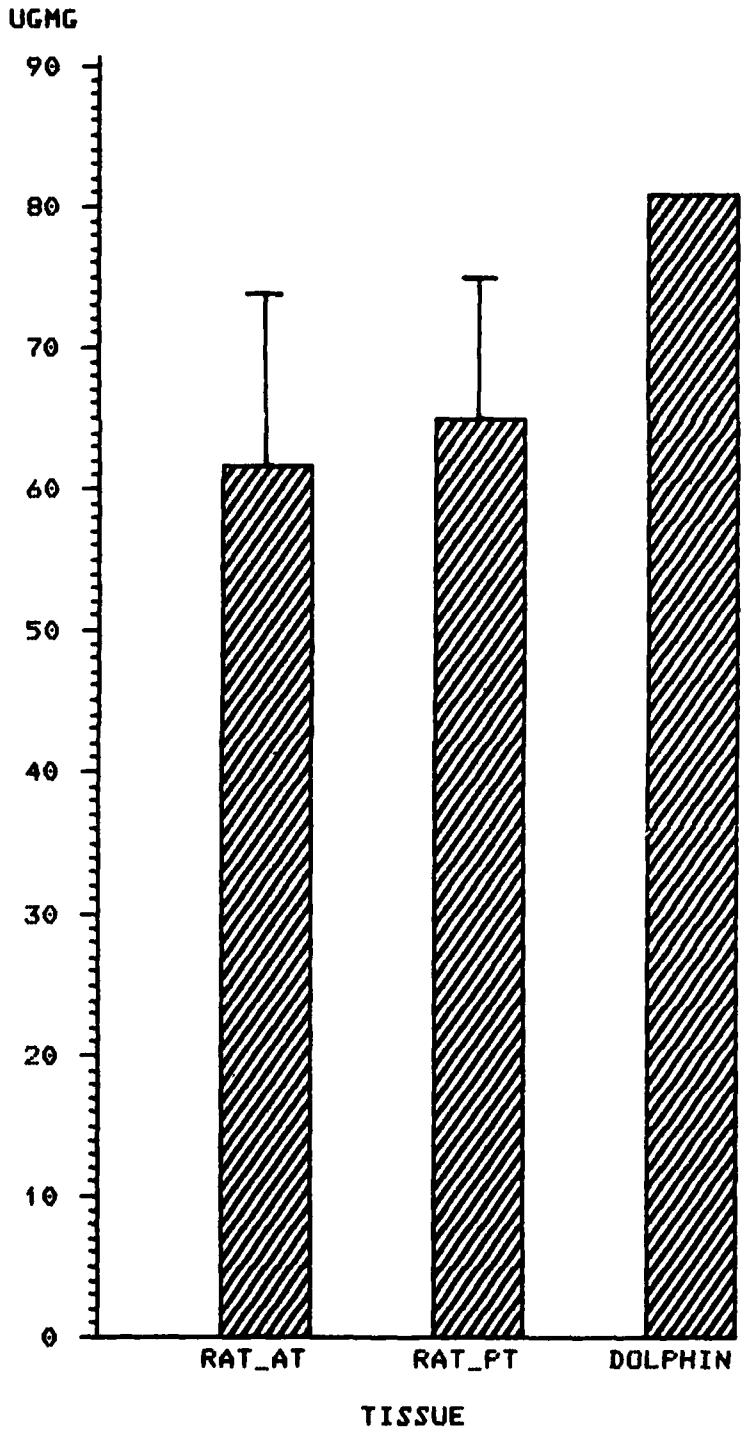

Figure C3. Hydroxyproline content of dolphin and rat tendons.

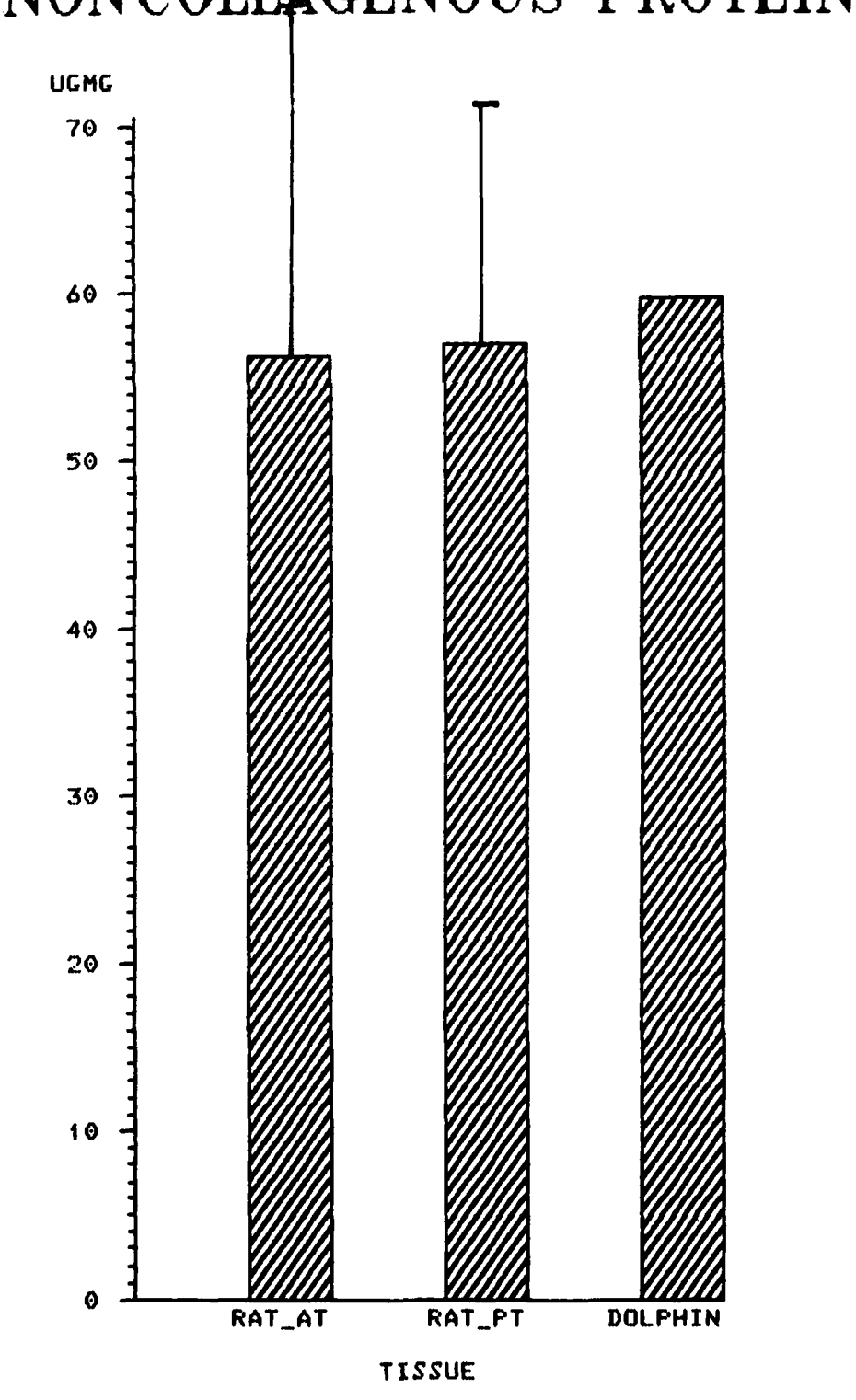

Figure C4. Noncollagenous protein content of dolphin and rat tendons.

GLYCOSAMINOGLYCANS

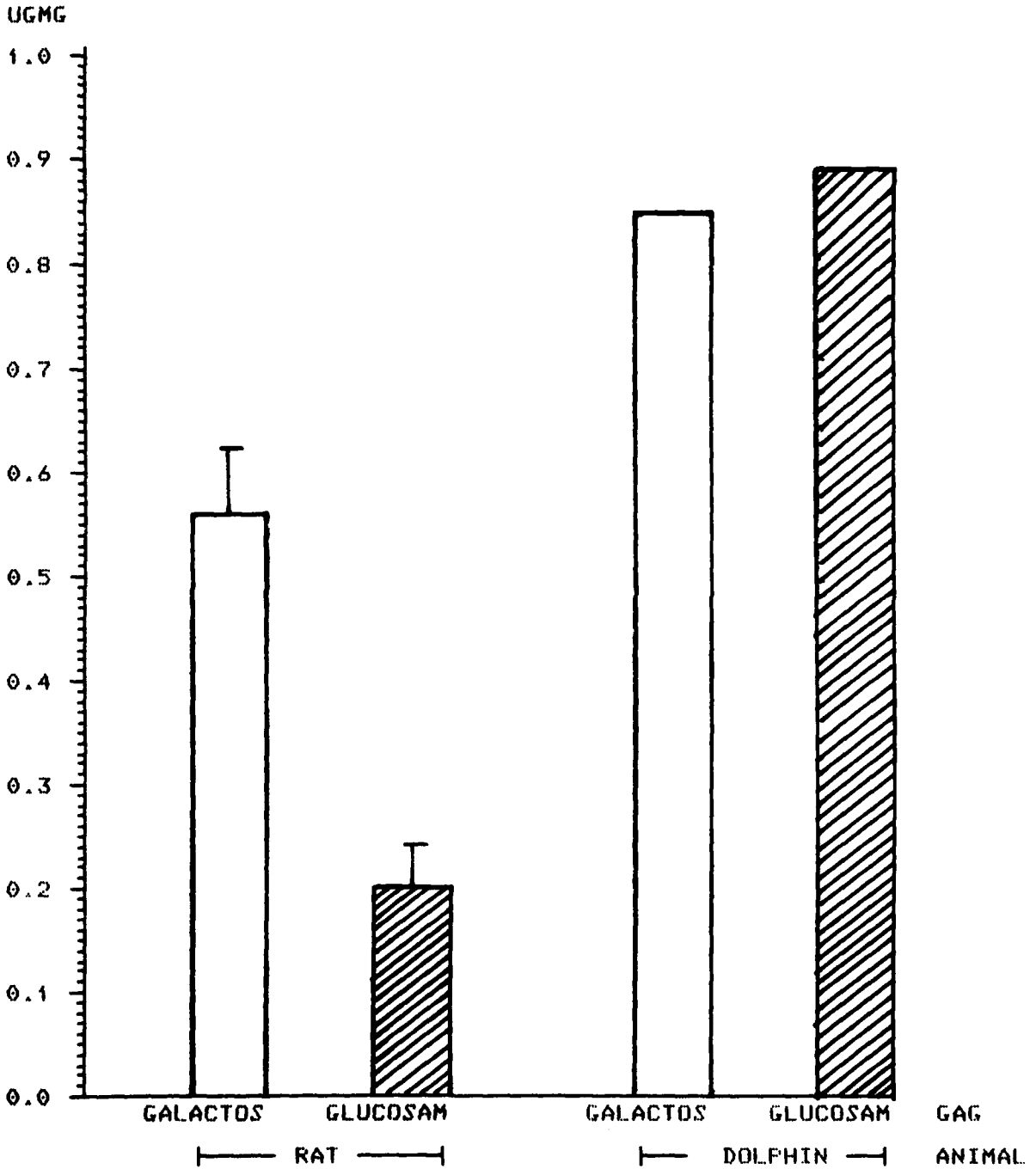

Figure C5. Glycosaminoglycans (galactosamine and glucosamine) content of dolphin and rat tendons.

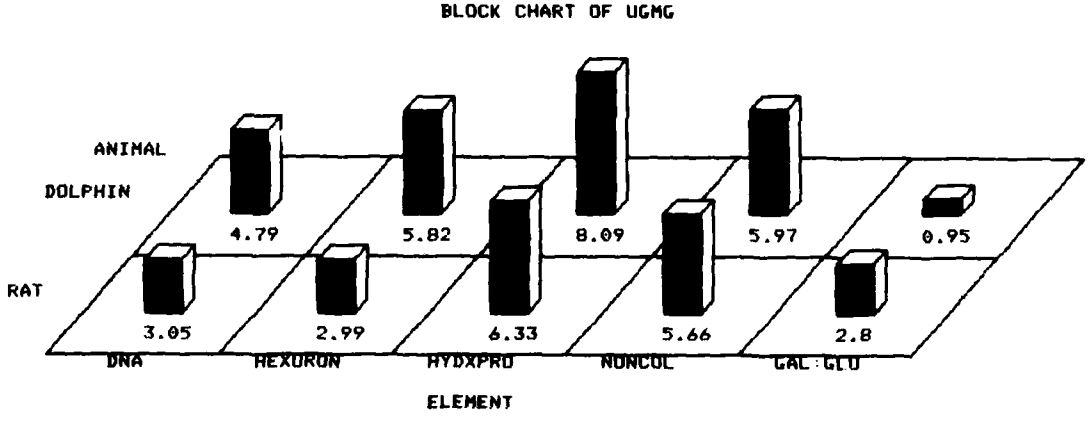

Figure C6. Summary chart of the matrix components of dolphin and rat tendons.
Note: Values for hydroxyproline are reduced by a factor of 10.

CONCLUSIONS

Connective tissues contain each of the essential components measured in this study: cells — as indicated by DNA content; collagen — as indicated by hydroxyproline content; noncollagenous protein — including elastin, fibronectin, and cellular proteins; and proteoglycans — complex macromolecules containing a core protein to which are bound glycosaminoglycan (GAG) chains. The GAG chain is a polymer of repeating disaccharides, each disaccharide containing a hexosamine and either a carboxylated hexose (a hexuronate) or a sulfated hexose. The predominant hexosamines are glucosamine and galactosamine. Collagen is a material of high tensile strength and low extensibility, particularly in comparison with elastin. Elastin is found in elastic tissues subject to consistent repetitive distension such as the aorta and the *ligamentum nuchae.*

Tendon is composed largely of collagen fibers arranged in parallel to transmit tension developed by muscle action. The greater amount of collagen (hydroxyproline) and the equivalence of noncollagenous protein (e.g., elastin) suggest that dolphin tendon has a relatively greater need for strength rather than stretch in its dynamic function.

Proteoglycans, with their high charge density and very large capacity to hold water, function in part to provide resiliency in conditions of compressive loading, such as in articular cartilage and in the aorta. Proteoglycans perform many other functions as well, some which are known and others yet to be clarified. Their greater presence in dolphin relative to rat tendon and the equivalent ratio of galactosamine to glucosamine may indicate a need to absorb compressive forces such as could occur in the rapid and powerful undulations of the dolphin tail. Although more study is needed for clarification, the biochemical differences between dolphin and rat tendon indicated by this study bring up interesting questions concerning the dynamic role of tendon in movement.