Differences in hematocrit of blood samples obtained from two venipuncture sites in sharks

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Objective—To evaluate differences in Hct between 2 venipuncture sites in captive and free-ranging sharks.

Animals—32 healthy adult captive sharks (*Carcharhinus melanopterus*, *Carcharhinus plumbeus*, *Stegastoma fasciatum*, *Orectolobus japonicus*, and *Triaenodon obesus*) and 15 captured free-ranging adult sharks (*Carcharhinus limbatus* and *Carcharhinus acronotus*).

Procedures—Blood samples were collected from the caudal tail artery followed by collection from the sinus located immediately caudal to the cranial dorsal fin. The Hct was determined for each sample and results were compared. Additionally, results for sharks that were highly active and used aerobic metabolism were compared with results for sharks that were less active and tolerant of anaerobic conditions.

Results—Mean Hct for all sharks was significantly less (8% less) in blood samples obtained from the cranial dorsal fin sinus, compared with the Hct for samples obtained from the caudal tail artery. When compared on the basis of metabolic class, sharks that were more tolerant of anaerobic conditions had lower Hct values and smaller differences between the 2 venipuncture sites.

Conclusions and Clinical Relevance—Hct values were significantly lower in blood samples collected from the cranial dorsal fin sinus compared with values for samples collected from the caudal tail artery. It is important to recognize this difference when evaluating hematologic variables in sharks and when establishing reference ranges for Hcts for shark populations. Sharks that were more active and relied on aerobic metabolism had higher Hct values than did anaerobic-tolerant sharks, and the difference in Hct values between venipuncture sites was more pronounced. (*Am J Vet Res* 2006;67:1861–1864)

The field of elasmobranch medicine is in its infancy, and there is a paucity of information on hematologic values[1-4] despite the growing need for veterinary care of cap-

tive sharks. Sites for collection of blood samples in sharks are typically chosen in accordance with the method of animal handling. When a shark is large or difficult to manually restrain, it is most convenient to obtain a blood sample from the dorsal sinus, an area located at the caudal aspect at the base of the dorsal fin. This method allows handlers to maintain the shark in ventral recumbency. When a shark is easily restrained or anesthetized, blood collection from the caudal tail vein or artery is usually chosen because the shark can be placed in dorsal recumbency, which facilitates access to the vessels. In the authors' clinical experience, Hcts differ in samples collected from these 2 sites for use in routine health screening of captive shark populations. To assess the importance of this observation, a prospective study was initiated that used both captive habituated sharks and captured free-ranging (wild) sharks.

Sharks can be classified into metabolic categories on the basis of their method of respiration. Pelagic sharks (ie, open-water sharks) rely on ram ventilation. These sharks ensure ventilation by forcing water over their gills and, hence, are obligated to swim constantly. Conversely, nonpelagic sharks (ie, reef-oriented species of sharks) are capable of pumping water over their gills and can rest on the ocean bottom for extended periods. The nonpelagic species have a higher tolerance for anaerobic situations than do the pelagic sharks.[5,6] The Hct in tuna, a high-energy, ram-ventilating teleost, is considerably higher than the Hct in most other teleosts because tuna have a high requirement for oxygen.[7] Therefore, we elected to assess whether similar differences existed in pelagic and nonpelagic sharks.

Materials and Methods

Animals—Blood samples were collected from 47 adult sharks. The sharks comprised 2 groups (32 captive habituated sharks and 15 captured free-ranging wild sharks). Sharks were considered adults on the basis of secondary sex characteristics; standard and total length measurements; and, when possible, body weight.

Collection of blood samples—Captive habituated adult sharks were acclimated to captive conditions for at least 1 year, and all were handled or anesthetized during that time period. Samples were obtained twice from some sharks. Animals included 4 brown sharks (*Carcharhinus plumbeus*), 13 blacktip reef sharks (*Carcharhinus melanopterus*), 3 wobegong sharks (*Orectolobus japonicus*), 4 zebra sharks (*Stegastoma fasciatum*), and 8 whitetip reef sharks (*Triaenodon obesus*). All sharks were considered healthy on the basis of results of physical examination, routine hematologic tests, and serum biochemical analysis, as determined by use of in-house reference ranges.

Blood samples were collected from captive habituated sharks during routine biannual examinations. Sharks were anesthetized in water that contained tricaine methane sulfonate[a] (125 mg/L) buffered with an equal amount of sodium bicarbonate. Sharks were then moved into water that contained 100 mg of tricaine methane sulfonate/L for maintenance of anesthesia.

Two blood samples were obtained from each shark. An arterial blood sample was obtained from the caudal tail artery. This artery was easy to access because of its anatomic location on the ventral midline and immediately adjacent to the vertebral column (**Figure 1**). Arterial samples were verified by use of blood gas analysis. Arterial samples were collected by use of various sizes of needles and syringes appropriate for each shark (typically a 3-mL syringe with a 20-gauge, 1.5-inch needle). A blood sample was also obtained from the sinus located immediately caudal to the cranial dorsal fin at the point where the fin met the dorsum. For these samples, a 1-mL syringe with a 26-gauge, 0.75-inch needle was typically used.

Free-ranging wild sharks were captured by use of a hook and line during a typical collecting trip in the coastal waters of southern Florida. All free-ranging sharks were captured within 35 km of Marathon, Fla. Animals included 7 *Carcharhinus acronotus* and 8 *Carcharhinus limbatus*. After capture, sharks were immediately brought alongside the boat, netted, lifted onto the boat deck, and manually restrained. Blood samples were opportunistically collected during removal of the hook and line; samples were collected in the same manner and location as described for the captive habituated sharks. Time from initial capture until venipuncture was approximately 1 to 2 minutes.

For both groups of sharks, all blood samples were placed in sodium heparin.[b] Samples were analyzed within 2 hours after collection.

Analysis of blood samples—The Hct was analyzed in heparinized blood samples. For each shark, the blood sample was divided and placed into 2 microhematocrit tubes. Tubes were centrifuged at $11,000 \times g$ for 5 minutes,[1] and Hct values were determined manually. The mean value for both tubes was calculated and used for statistical analysis. Because of other research needs, only the Hct was evaluated, although full hematologic and biochemical analyses for samples obtained from each site would have been ideal.

Statistical analysis—Data were analyzed by use of Student paired 2-tailed *t* tests[c] and an ANOVA[d] to compare Hct values between sites in captive and wild populations. Data were additionally analyzed on the basis of metabolic category of shark by use of the Wilcoxon rank sum test.[e] Sharks considered aerobic respirators were *C plumbeus*, *C acronotus*, *C melanopterus*, and *C limbatus*. Sharks considered anaerobic respirators were *S fasciatum*, *O japonicus*, and *T obesus*. For all tests, comparisons were considered significant at values of $P < 0.05$.

Results

The Hct values differed significantly between collection sites (**Figure 2**). Mean ± SD Hct for all species for the caudal tail artery was 25.56 ± 5.68% (range, 13% to 35%), whereas mean Hct of the cranial dorsal fin sinus was 16.46 ± 6.35% (range, 8% to 32%). Analysis revealed that these values differed significantly (paired Student *t* test). For all species, except *S fasciatum*, the Hct differed significantly (ANOVA) between the 2 sites.
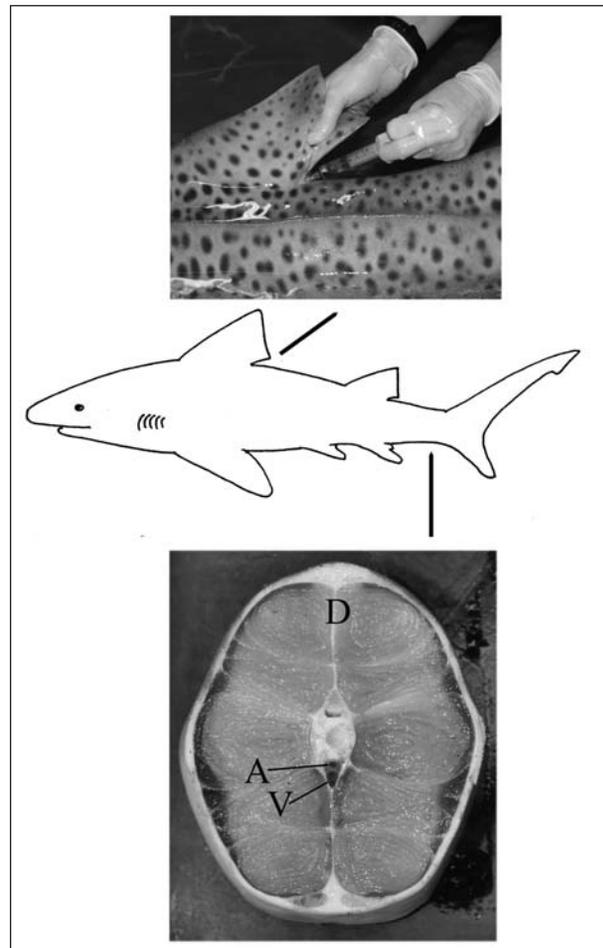

Figure 1—Illustrations depicting sites for collection of blood samples in a shark (*Stegastoma fasciatum*). Samples were obtained from the sinus located immediately caudal to the cranial dorsal fin (middle and top) and the ventral tail artery (middle and bottom). In the cross-sectional view, notice that the ventral tail artery (A) is located between the vertebral column and ventral tail vein (V). D = Dorsal.
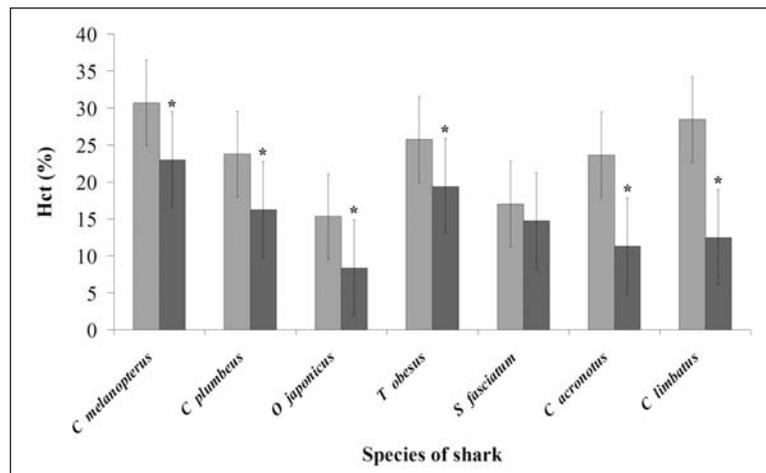

Figure 2—Mean ± SD Hct for blood samples collected from the ventral tail artery (light gray bars) and the sinus located caudal to the cranial dorsal fin (dark gray bars) for various captive habituated and free-ranging wild sharks. Species and number of sharks represented include *Carcharhinus melanopterus* (n = 15), *Carcharhinus plumbeus* (4), *Orectolobus japonicus* (3), *Triaenodon obesus* (8), *S fasciatum* (4), *Carcharhinus acronotus* (7), and *Carcharhinus limbatus* (8). *Within a species of shark, value differs significantly ($P < 0.05$) from the value for the ventral tail artery.

When sharks were grouped on the basis of metabolic category (eg, aerobic vs anaerobic), there was a significant ($P < 0.001$) difference between Hct values for the cranial dorsal fin sinus and caudal tail artery. The Hct for the caudal tail artery differed significantly ($P < 0.001$) between metabolic groups, but the Hct for the cranial dorsal fin sinus did not differ significantly ($P = 0.837$) between metabolic groups.

Discussion

Similar to diagnostic testing in other animals, the Hct is a rapid, simple diagnostic test that can be performed in sharks and is an important component of a patient's minimum database. During routine collection of blood samples for biannual examinations of the sharks reported here, we noticed that there were differences in quality of samples obtained from 2 common blood collection sites (the caudal tail artery and the cranial dorsal fin sinus). Blood obtained from the dorsal sinus was a lighter red color and less viscous than blood obtained from the artery. Examination of the Hct of these samples revealed that blood from the dorsal sinus had a lower Hct than that of blood from the tail artery. Assuming that only the value for the blood sample from the dorsal sinus was used to evaluate a shark, a spurious anemic condition would have been suspected. This concern prompted us to prospectively evaluate the Hct during the next scheduled physical examinations.

Differences in hematologic values exist between venipuncture sites in other species, including rats, tortoises, and dogs.[8-10] However, it is most typical that there are no significant differences in values for blood samples collected from various venipuncture sites, as indicated in studies[11-13] in rhinoceri, cats, and chameleons. In rats and dogs, the differences were attributed to local tissue damage from samples collected from peripheral vascular sites. Differences in Hct between venipuncture sites in turtles and other reptiles have been attributed to lymph contamination as a result of the close proximity of lymphatics to blood vasculature.[12,14]

In sharks, several sites have been used for antemortem blood collection, including the caudal tail vessels, heart, and cranial dorsal fin sinus. Cardiac collection is not recommended because of the anatomic barriers surrounding the heart and potential morbidity that may result when cardiac tissue is damaged. Samples obtained from the caudal tail artery were used for the baseline Hct in the study reported here because this vessel is an important component of the central vascular system and is the site most commonly used for blood collection in elasmobranchs. The Hct value for *C limbatus* was 27.5% in another study,[15] which is similar to our mean value of 28.44%. In *C plumbeus*, our mean value was 23.63%, which is also similar to a value of 22.8% (PCV of 23.8% with a 1% buffy coat) reported elsewhere.[1] In both of those studies,[1,15] comparisons were made on samples collected from the caudal tail artery.

We are not aware of any reports of hematologic values for the other shark species included in our study. When Hct values for samples obtained from the caudal tail artery were compared with Hct values for samples obtained from the cranial dorsal fin sinus, there were significant differences in all species, except one (*S fasciatum*). The uniformity of Hct values in *S fasciatum* may be attributable to species variation or the small number of sharks from which samples were collected. Additional research is warranted to expand the number of species and the number of sharks for each species.

Fish, including sharks, lack a lymphatic system analogous to that of the terrestrial vertebrates. In teleosts, there is an SVS[16-19] that accounts for shifts in bodily fluids in lieu of a true lymphatic system. The SVS is a parallel circulatory system that connects to the primary circulatory system via microcapillaries. It prohibits most RBCs but allows plasma and even favors entry of WBCs, which leads to a low Hct in the SVS fluids (sometimes as low as 1%).[19,20] Physiology of the SVS is being investigated, and it has been proposed that the purpose of this system in fish is nutritive (supplying the skin, gills, and gastrointestinal epithelium),[16,17] immunosupportive (providing WBCs to the skin, gills, and gastrointestinal epithelium),[17,19] or for homeostasis of tissue fluids.[17] Carp can have dramatic shifts in fluid volume during stress, with a sudden efflux into the SVS resulting in a higher Hct.[17] Other fish also can have an increase in Hct values during stress, with evidence that there are fluid shifts through the SVS.[17]

It would be convenient to attribute the differences in Hct reported here to an SVS in sharks. However, elasmobranchs (sharks, rays, and skates) do not possess the same type of anatomic SVS.[17,19] Regardless, they do have a complex subcutaneous vascular plexus that leads many researchers to believe it is possible for sharks to have an SVS. Although advanced diagnostic testing has ruled out the possibility that the same SVS anatomic similarities exist between elasmobranchs and teleosts, subcutaneous vessels in sharks do contain variable numbers of RBCs.[17] In 1 study,[f] investigators characterized the dorsal fin sinus and its association with the venous system. They found that blood flows from the sinus and subcutaneous vessels into the caudal cardinal vein; then into the postcardinal venous sinus; and, finally, into the atrium of the heart. It is also posited that the large vascular sinuses of elasmobranchs are typically filled with blood that has a low Hct.[19] Despite the lack of firm evidence for an SVS in sharks, there is sufficient evidence that passage of RBCs is impaired in some portions of the venous system in sharks. It is possible that a microanatomic or microfunctional explanation exists for the lower Hct in blood obtained from the dorsal fin vasculature. More research on the circulatory system of sharks is needed.

Pelagic (ram ventilator) sharks rely on aerobic metabolism for daily activities. They use anaerobic metabolism only during bursts of activity, such as avoiding predators or capturing prey. Conversely, nonpelagic or more sedentary sharks have a high tolerance for anaerobic metabolism and thus have a lower demand for oxygen. In the study reported here, we categorized species on the basis of their metabolic characteristics and evaluated the differences in Hct. We found that aerobic sharks were more likely to have a higher

Hct and a greater difference in Hct values between venipuncture sites. Pelagic fish, including elasmobranchs, have a higher Hct[21] because of an increased demand for oxygen-carrying capacity in high-energy fish.

Hematocrit values in fish are influenced by exhaustive exercise, capture, and stress.[21-25] Conversely, most elasmobranchs do not have the same dramatic and immediate response[15,25,26] unless they are subjected to substantial exhaustive exercise or substress.[4,15,22] In the study reported here, captive habituated and free-ranging wild sharks were rapidly restrained and samples collected immediately; therefore, Hct values likely reflected each shark's typical physiologic state. Although anesthesia has been correlated with increases in Hct in teleosts,[15,27,28] we are not aware of any published comparative studies that report this finding in sharks.

Large amounts of information still need to be gathered regarding health of elasmobranchs. The study reported here revealed that there is a significant difference in Hct values between blood collection sites that can affect the list of differential diagnoses and treatment plans for an abnormal shark. We conclude that the most reliable source for accurate measurement of the Hct in many shark species is collection of blood samples from the caudal tail artery.

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a. Tricaine methane sulfonate (MS-222), Argent Laboratories, Redmond, Wash.
b. Sodium heparin (1,000 USP U/mL), Baxter Healthcare Corp, Deerfield, Ill.
c. Microsoft Excel, Microsoft Corp, Redmond, Wash.
d. ANOVA software. Available at: www.physics.csbsju.edu/stats/anova.html. Accessed Oct 18, 2005.
e. Hammer Ø, Harper DAT, Ryan PD. PAST software: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 2001;4. Available at: palaeo-electronica.org/2001_1/past/issue1_01.htm). Accessed Jan 30, 2006.
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