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NEW METHODS OF OBTAINING BLOOD AND CEREBROSPINAL FLUID FROM MARINE TURTLES

DAVID WM. OWENS AND GEORGITA J. RUIZ

ABSTRACT: New methods are described for taking blood and cerebrospinal fluid from marine turtles. The blood is obtained via the paired dorsal cervical sinuses while the cerebrospinal fluid sampling procedure requires the insertion of a needle through the foramen magnum into the brain's 4th ventricle. Both methods have been extensively employed by the authors and have been found to cause little stress or damage to the animals.

Key words: Reptilia; Testudines; Marine turtles; Blood; Cerebrospinal fluid; Sampling

PREVIOUS hematological studies have utilized the following methods for obtaining blood from marine turtles.

1. Cardiac puncture is the most common method and involves inserting a needle through the proximal part of the hind limb (Frair, 1977*a*) or the plastral seam directly over the heart (Dozy et al., 1964; Frair, 1964; Frair, 1977*b*; Frair and Prol, 1970). This technique, however, has the disadvantage of possible contamination with pericardial fluid which, as Frair (1977*a*) has pointed out, is abundant in turtles. The approach to the heart through the hind limb requires long needles that are adapted to connect to vacuum tubes. Furthermore, this technique requires considerable experience or extensive probing in order to locate the heart. We have noted that some hatchlings die immediately after heart puncture, presumably due to damage to the sinoatrial node or other nervous structures. Also, many individuals experience hemorrhaging into the pericardial sac with subsequent clot formation.

2. Blood collection directly from the carotid artery has been referred to by Berkson (1966). Although he does not fully describe the technique, we assume it would involve a complex procedure.

3. Decapitation and post-sacrificial bleeding (Dozy et al., 1964) have also been employed. This technique necessitates killing the animal which should be avoided when endangered or threatened species are sampled. Euthanasia also eliminates the option of obtaining repeated samples from an individual. Two further problems are associated with decapitation. First, it is necessary to work quickly to prevent the blood from clotting. Second, the fluid obtained may be adulterated, as the esophagus, pericardium, lungs, spinal cavities, cranial cavities, and extravascular fluid spaces may also drain.

These considerations led us to utilize an alternative bleeding technique for marine turtles which would not harm the animal and which would be quick and easy. The dorsal cervical sinus bleeding technique was developed by Edward Scura and associates at Cayman Turtle Farm, Ltd., Grand Cayman Island, British West Indies. The technique is simple, works well under field conditions, and is easily adapted to various situations.

With regard to the cerebrospinal fluid (CSF) sampling technique, we know of no published methods for obtaining CSF from live chelonians.

PROCEDURES

In experiments conducted at Cayman Turtle Farm, Ltd., Puerto Angel, Mexico, and Colorado State University, more than 500 green turtles (*Chelonia mydas*) from 200 g

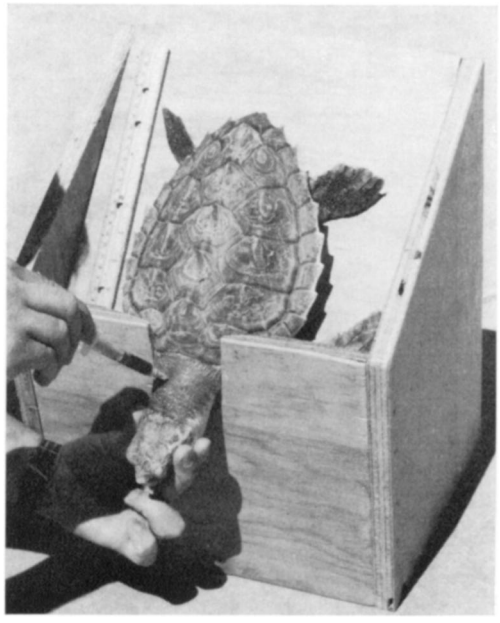

FIG. 1.—Method of taking blood from *Caretta caretta* using a restraining table.

to 250 kg were sampled via the bilaterally located cervical sinus. The same procedure was used to sample loggerheads (*Caretta caretta*) weighing 1–15 kg, Pacific ridleys (*Lepidochelys olivacea*) weighing approximately 20 kg, and hawksbills (*Eretmochelys imbricata*) weighing approximately 10 kg. William Rainey (pers. comm.) has sampled the leatherback (*Dermochelys coriacea*) using the same method.

The only essential equipment is a syringe and needle of appropriate size (i.e., ⅝ inch 25 gauge for a 200 g turtle, or 1.5 inch 21 gauge for a 200 kg turtle). An optional item which facilitates sampling is an angled table designed to restrain the turtle (Fig. 1). The table permits a single investigator to do the sampling. In addition, evacuated blood collecting tubes (Vacutainers R: Becton, Dickinson and Co.) with multiple sample needles will facilitate taking more than one sample from an individual.

Blood sampling.—The turtle is restrained in the holding table or on the ground with the sand removed from under the animal's head. The head is pulled gently forward and downward until the neck is fully outstretched. Such positioning is important as it presumably facilitates filling of the bilateral cervical sinus. The needle is inserted one cm from the dorsal-cervical midline on either side of the line's midpoint. When sampling smaller turtles, needle insertion may be as colse as 0.5 cm to the cervical midline. The angle of entry is perpendicular to the dorsal surface of the neck. It is important to insert the needle lateral to the midline to avoid the possibility of striking the vertebral column.

When the needle is in place, a small amount of suction is applied using the plunger. As the blood starts to flow, the apparatus is held still until a sufficient sample is obtained. It may be necessary to search for the sinus by adjusting the depth of the needle between 1–3 cm, while continuing gentle suction. Searching for the sinus by rotating the needle should be avoided to minimize internal damage. If the sinus is not located initially, the needle should be removed and the procedure repeated in a more lateral (occasionally slightly medial) position. If further attempts to obtain blood are unsuccessful, the opposite side of the neck should be tried.

The most common cause of failure is poor positioning of the animal. If at all possible, the turtle's head should extend below the level of the plastron. In turtles that continue to struggle, the animal's head should be released so that it can breathe in the usual head-lifted position. Following this manipulation a blood sample may be more readily obtained.

Cerebrospinal fluid sampling.—The turtle is placed in the same position as discussed for the cervical sinus blood sampling procedure. For turtles of about 1 kg a 1 inch 25 gauge needle is used, whereas a 25 gauge 2 to 3 inch needle is needed for turtles of 20–40 kg body weight. We have sampled 50 animals using this procedure. However, the technique has not been attempted on individuals larger than 50 kg.

With the turtle's head firmly restrained in the fully-extended, downward position, the needle is inserted in the neck just posterior to the skull's supraoccipital protrusion at an angle of approximately 135° to the dorsal surface of the turtle's head. The insertion point must be exactly midsagittal in order for the needle to pass directly over the atlas, through the foramen magnum, and into the 4th ventricle. If bone is struck with the needle, the syringe should be removed immediately and reinserted following a careful recheck of the entry angle. Occasionally the needle penetrates a small amount of cartilagenous-like material immediately prior to entry into the 4th ventricle.

The depth the needle is inserted depends on the size of the animal. For example, a 30 kg turtle requires penetration to 5–6 cm. Using gentle suction on the syringe the sample is rapidly withdrawn. Initially a small amount of blood may enter the syringe. This may be eliminated by replacing the syringe while maintaining the position of the inserted needle. If more than 0.25 cm^3 of blood contamination is evident, the CSF sample is rejected because the needle has not adequately penetrated the 4th ventricle. If there is a small amount of contamination, centrifugation is used to remove blood cells from the CSF. An estimate of the degree of contamination can then be made. We have successfully withdrawn a 2–3 cm^3 sample of CSF without apparent detriment to the animal. In the case of a good entry into the 4th ventricle the turtle appears to experience a spinal block type of anesthesia.

Harvesting serum.—Samples were routinely transported on ice in the field. Serum may be harvested by allowing the whole blood to clot at 21–25°C for one hour followed by centrifugation. Extensive refrigeration prior to serum harvesting often results in hemolysis. Plasma may be obtained by use of heparinized syringes. We prefer to use serum because the heparinized plasma tends to clot erratically after freezing and subsequent thawing.

Discussion

The dorsal cervical sinus blood sampling technique is a considerable improvement over other sampling procedures for marine turtles. The technique reduces stress and risk of permanent injury, requires minimal equipment, is readily learned by a novice, and produces a pure blood sample.

We had the opportunity of sampling several marine turtle species ranging from 200 g to 250 kg. In one instance, a group of 120 individuals weighing approximately 40 kg were each sampled twice with complete success (Owens et al., 1978). Similarly, in Puerto Angel, Mexico numerous individuals of two species were sampled with minimal difficulty. In a few specimens (particularly males) the sinus could not be located and sampling by this technique was not accomplished. Hatchlings of less than 100 g also proved difficult to sample. Thus in small turtles cardiac puncture is the technique of choice because of the ease in penetrating the plastron with the sampling needle.

The CSF sampling procedure was particularly successful with immature *C. mydas* at Cayman Turtle Farm. For example, in a group of 40 animals of approximately 30 kg body weight we obtained samples from all but five individuals. None of the animals on which this technique has been attempted have shown any immediate abnormalities. Groups of four animals were also observed for 24 h and 48 h after sampling with no signs of motor difficulties. A 1.6 kg loggerhead showed no ill effects and continued to grow and behave normally more than one yr after CSF sampling.

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Department of Biology, Texas A&M University, College Station, Texas 77843, USA, and Calle Colina #145, Lomas Bezares, Mexico 10, D.F., Mexico

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TEMPERATURE RELATIONSHIPS AND MOVEMENTS OF SNAKES (*ELAPHE OBSOLETA, COLUBER CONSTRICTOR*) IN A CAVE HIBERNACULUM

OWEN J. SEXTON AND SUE R. HUNT

ABSTRACT: A cave hibernaculum in western Missouri, USA, was used by *Elaphe obsoleta* and *Coluber constrictor*. Thermal characteristics of the hibernaculum were recorded over 4 hibernation seasons. Air and surface temperatures near the entry of the hibernaculum were higher than at the rear of the den during early fall and spring but the thermal gradient was reversed in winter. Locations of snakes were correlated with these thermal clines. Our data support the hypothesis that entry into and exit from dens by snakes are dependent on reversals in the thermal gradient between surface and subsurface.

Key words: Reptilia; Serpentes; Colubridae; *Coluber*; *Elaphe*; Hibernation; Missouri; Thermal gradient

HIBERNATION dens or hibernacula of snakes have long attracted field studies on species composition (e.g., Hirth and King, 1968; Parker and Brown, 1973; Woodbury et al., 1951) and seasonal movements to and from dens (e.g., Brown and Parker, 1976; Fitch, 1958; Hirth et al., 1969; Viitanen, 1967). Thermal characteristics of hibernacula relative to location and activity of hibernating snakes have received less attention. Drda (1968) recorded long-term activities of unconfined snakes in a cave, and Brown et al. (1974) reported short-term thermal observations of snakes in a subsurface soil hibernaculum. Two investigators (Aleksiuk, 1970; Viitanen, 1967), have suggested that entry into and emergence from hibernation are related to seasonal reversals in thermal gradients between deep ground and surface temperatures. The present study reports on activities of two species of colubrid snakes, the rat snake (*Elaphe obsoleta*) and the racer (*Coluber constrictor*), relative to thermal characteristics of a cave hibernaculum. We wished to test the hypothesis that annual entry into and exit from