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Alternatives to Retroorbital Blood Collection in Hispid Cotton Rats (*Sigmodon hispidus*)

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Cotton rats (*Sigmodon hispidus*) are a valuable animal model for many human viral diseases, including polio virus, measles virus, respiratory syncytial virus, and herpes simplex virus. Although cotton rats have been used in research since 1939, few publications address handling and sampling techniques for this species, and the retroorbital sinus remains the recommended blood sampling site. Here we assessed blood sampling methods that are currently used in other species and a novel subzygomatic sampling site for their use in *S. hispidus*. The subzygomatic approach accesses a venous sinus that possibly is unique to this species and that lies just below the zygomatic arch of the maxilla and deep to the masseter muscle. We report that both the novel subzygomatic approach and the sublingual vein method can be used effectively in cotton rats.

Blood sampling in rodent species is an integral procedure in the research setting, but it is often problematic due to the small body size and delicate anatomic structures in these species. In many cases, large numbers of animals must be sampled at one time, and methods need to be technically simple, yield rapid diagnostic samples of sufficient volume, and cause minimal distress to the animals. Retroorbital plexus bleeding has historically been used because of the easy access and large sample size that can be obtained; however, acute complications such as corneal damage, ocular discharge, orbital bone fractures, abnormal eye positions, and intraocular alterations may occur.[2,6,22] Chronic adverse effects of this technique include keratitis, enophthalmia, microophthalmia, abscessation, and blindness.[2,6] Due to these adverse effects, this method is considered to cause more than momentary pain or distress, and these conditions should be relieved with analgesics or anesthetics.[6] Because of this unfavorable effect, many IACUC now discourage retroorbital sampling or request scientific justification for its use.[6,15,18] Other sampling sites including the tail, lateral saphenous, submandibular or facial, and sublingual veins bleeding have been explored in laboratory rats and mice and found to be more humane than retroorbital sampling, but they have not been described in the literature for cotton rats.

Submandibular, lateral saphenous, and tail vein bleeding have become preferred methods for blood collection from mice and rats.[2,7,8,10-12,27] For submandibular bleeding, the mouse is held by gathering the skin of the neck so that the cheek area is pulled taut, then a 4.0- to 6.0-mm lancet (GoldenRod Animal Lancet, Medipoint Mineola, NY) or 23- to 25-gauge hypodermic needle is used to puncture the skin where the orbital and submandibular–facial veins meet to drain into the jugular vein. When the handler is proficient, this method is rapid, yields more than 0.3 mL of blood, and appears to cause little stress or discomfort to the mouse when released back into cage.[10] Tail vein bleeding is performed either by puncturing the distal vein and allowing drops to flow into a tube or pipette or by clipping the distal portion of the tail to induce bleeding.[2,12,15] This method is used most often in rats because of their large tail vein size, and depending on handler preferences, the tail may be warmed to increase blood flow.[21] The submandibular method is used often in mice because it has been found to cause less pain and distress and does not cause permanent damage as does tail-tip clipping.[7,8,10,12] For blood sampling from the lateral saphenous vein, the mouse or rat does not need to be anesthetized, although anesthesia often is included for restraint purposes and ease of handling. Awake animals can be restrained by hand or immobilized in a restraint tube or cone, with a hindleg exposed. The hair on the dorsolateral aspect of the hindlimb can be shaved or moistened with 70% ethanol or rubbing alcohol to visualize the vein. Either animal lancets or a hypodermic needle can be used to puncture the vein, and blood is collected as it forms drops on the skin.[11,12]

Sublingual vein bleeding is another alternative to retroorbital venipuncture that may minimize tissue damage and distress to rats and mice when performed as recently described.[1,10] In 1998, a group at Novartis (Basal, Switzerland) published a refinement of the technique which included clipping the sublingual vein with small scissors on anesthetized or unanesthetized rats.[27] The original method, when used for serial blood draws, was thought to induce damage to the vein and surrounding tissues, leading to unacceptable pain and distress in the animals. The refinement involved anesthetizing the rat with isoflurane and having one handler hold the rat by the skin of the neck and a second retracting the tongue as the vein is pierced with a 23-gauge hypodermic needle.[27] Proponents of the revised sublingual method feel that it has advantages over the submandibular method, which uses a 'blind stick' and poses possible damage to important glandular or vascular structures.[10]

The cotton rat (*Sigmodon hispidus*) has been used for many years as an animal model for human respiratory diseases caused by poliovirus, measles virus, and respiratory syncytial virus and zoonotic viral diseases such as Venezuelan equine encephalomyelitis virus, bacterial, fungal, and parasitic diseases, as well as toxicologic and oncolytic adenovirus studies of importance to human health.[5,14,16,19,25] Previous studies have shown that cotton rats are anatomically and immunologically more similar to humans than are mice in the response to many

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infectious diseases, and this increased similarity leads to more accurate efficacy and safety studies involving vaccines and therapeutics.[4] Cotton rats also are being explored as a model for genital herpes and hantavirus infections, as well as monkeypox infections and preventatives.[3,26] The development of cotton-rat–specific reagents for use in detecting and quantifying immune function and response to disease demonstrates the likelihood that this animal model will continue to be used in modern research.[3] Regardless of their usefulness in research, cotton rats have unique husbandry requirements. They have retained many of their wildtype traits and will panic with attempts to catch them and will bite when they are grasped. In addition, cotton rats have a particularly large flight zone and tend to jump vertically with attempts to hand-catch them, often leading to escape from the primary enclosure if the handler is unprepared.[24] When grasped by the tail, cotton rats spin to free themselves and the skin of their tail may slip, or in some cases, the tail may break off completely; consequently this method of capture is not recommended.[17,24] For blood collection, the retroorbital method is the only one described for use in cotton rats, and publications addressing alternative, nonterminal bleeding methods in cotton rats could not be found. One report noted that the submandibular and tail vein approaches had not been successful, despite the author having extensive experience with this species.[16]

The current study investigates various alternative sampling methods to identify one that is easy to learn, rapid, humane and yields a sufficient diagnostic sample. We hypothesized that one or more of the sampling methods investigated would enable collection of acceptable blood samples, cause minimal signs of pain or distress in the rats, and provide a viable alternative to the retroorbital method in cotton rats.

Materials and Methods

Animals. This study was undertaken at an AAALAC-accredited animal facility in the Division of Scientific Resources, Animal Resources Branch (Centers for Disease Control and Prevention, Atlanta, GA). All procedures and husbandry followed recommendations in the *Guide for the Care and Use of Laboratory Animals*, and the protocol was fully IACUC-approved.[13] Female cotton rats (*S. hispidus*; *n* = 52; age, 5 to 7 wk; weight, 60 to 70 g; Harlan Sprague–Dawley, Madison, WI) were housed in groups of 2 to 5 in static filter-top polycarbonate cages or microisolation rat boxes on a ventilated rack (Double Decker Rat Greenline IVC, Techniplast, Buguggiate, Italy) under controlled laboratory environmental conditions (64 to 79 °F [17.8 to 26.1 °C], 30% to 70% humidity, 12:12-h dark: light cycle). They were provided corncob bedding (Bed O'Cobs, The Andersons, Maumee, OH), standard rat chow (Lab Diet 5001 Rodent Diet, Brentwood, MO) and either bottled or automatic water purified through reverse osmosis and UV light ad libitum. These rats were assigned to a measles vaccine protocol that involved a pilot study for vaccine route exploration (15 rats) and a main study for postvaccine immunology analysis (37 rats), both of which required serial blood sampling for antibody levels but with a desire for a more acceptable bleeding method than retroorbital.

Experimental design. Five blood collection methods and sites currently used in rodent species, including tail vein, lateral saphenous vein, sublingual vein, submandibular, and retroorbital sinus, were compared in the initial phase of the study. A researcher experienced in bleeding another wildtype rodent species, deer mice (*Peromyscus* spp.), suggested collection from an area more rostral and proximal to the lateral canthus of the eye. This method proved successful, and the anatomic landmarks of this novel site then were established to allow reliable duplication across animals. Landmarks included the ventral aspect of the caudal rim of the zygomatic arch and the ramus of the mandible where it meets the zygomatic bone. The angle formed rostral to the intersection of these bones is the ideal puncture site and, in light of its anatomic location, this method was referred to as the subzygomatic approach and was included as a sixth sampling site for comparison in the study. For positioning, the anesthetized rat was held in the nondominant hand by gathering the dorsal skin from the base of the tail with the fourth and fifth fingers and rolling the hand forward to grasp the skin over the neck with the thumb and forefinger, thus encouraging venous congestion toward the head and pulling the skin firmly over the cheek (Figure 1). The sublingual and the novel subzygomatic techniques were used for the second sampling session and then the subzygomatic method alone was used for all subsequent blood collections because it best fulfilled the needs of the vaccine investigation. At each sampling session, the volume of blood collected and the number of attempts to collect that volume were recorded. Maximal volumes were calculated as 7.5% of total blood volume (50 to 70 mL/kg for rats) based on the animal's weight for weekly collections as recommended by the facility's IACUC and other literature.[6,15] Hypodermic needles (25 to 18 gauge) or animal lancets (5.0 mm) were used for all of the sampling procedures except retroorbital, for which a microhematocrit tube was used as described elsewhere.[2,12,22] The rats were euthanized at the end of the study, and gross necropsies were performed to examine the anatomic features of this species. Histopathologic examination was performed on a small subset to further characterize the vascular structure discovered in the pilot study and to determine any effects on tissues surrounding the puncture site after serial bleeding.

Anesthesia. The behavior of these rats is not conducive to easy handling, and anesthesia was planned for all procedures. The animals were transferred into a rodent anesthesia box (E-Z Systems, Palmer, PA) and exposed to a regulated flow of oxygen and isoflurane anesthetic. Previous publications have described special tools for catching and transferring the rats from cage to cage or an anesthesia chamber for induction.[17] This particular device was not available for this study, so the rats were transferred in their plastic or cardboard nesting tubes directly into the anesthesia box while still inside of the primary enclosure. In most instances the rats remained quiet and still for this transfer, although if they panicked and scattered they were still contained inside the primary cage and could then be encouraged into the nesting box again. We found that if slow, quiet movements were used, the rats would stay huddled within the nesting tube while it was slowly lifted and gently placed into the anesthesia box (Figure 2). The lid was shut and the rats transferred to the work surface, where the chamber was attached to the isoflurane machine. This procedure was used to minimize the stress of handling and prevent dropping or escape of the rats during transfers. Once anesthetized, they received an injectable anesthetic to facilitate the prolonged sedation periods required for the measles vaccine study. The injectable anesthetics consisted of either ketamine (75 mg/kg IM; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10 mg/kg IM; AnaSed 20 mg/mL, Shenandoah, IA) or ketamine (40 to 70 mg/kg IM) and dexmedetomidine (0.136 to 0.235 mg/kg; Dexdomitor, Pfizer, New York, NY) intramuscularly. The different mixtures were used to determine which would be most efficacious for this species and the vaccine study's needs (data not shown).

Figure 1. An anesthetized cotton rat held in the correct position for bleeding, with cheek fur shaved. The yellow line approximates the zygomatic bone, and the green line approximates the top edge of the mandibular ramus. The angle made by the intersection of these landmarks just below the caudal aspect of the eye outlines the area of the puncture site.

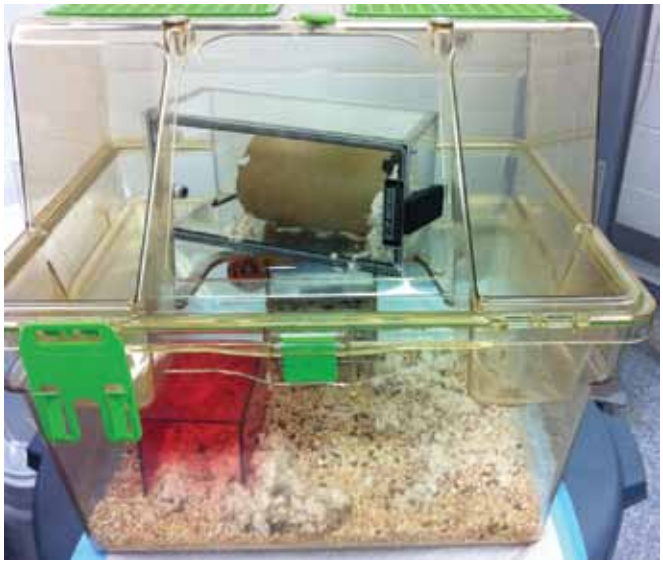

Figure 2. Cotton rats transferred into the anesthesia chamber in their nesting structure while inside the primary enclosure, to minimize stress and possibility of escape.

Results

Blood collection. Four of the 5 blood collection techniques that were compared in the initial phase, including the submandibular, lateral saphenous, tail vein and retroorbital methods, were determined to be unsuitable in cotton rats. Blood could not be obtained after multiple attempts in the region described for submandibular bleeding in *Mus* and *Rattus* species, leading us to conclude that the vascular anatomy in cotton rats was not comparable to that in the other species. The lateral saphenous vein method was attempted in 3 rats by using 25-gauge hypodermic needles and rat-sized animal lancets, but this method was ruled out due to the very small size of the vein and the inability to induce any blood flow from the puncture sites. The tail vein method was unsuccessful due to the dark tail pigmentation, which limited the ability to visualize the vein, and concerns about the previously documented potential for tail slip.[16,17] Retroorbital bleeding led to concerns that the already prominent eye globe in this species would easily proptose with scruffing of the neck, and additional pressure at the medial or lateral canthus of the eye from the microhematocrit tube would exacerbate this potential. Three attempts were made at inserting the microhematocrit tube into the medial conjunctiva, but the method subsequently was abandoned because the globe tended to evert out of the socket before sufficient pressure could be applied to induce blood flow. In consideration of the historically acknowledged pain and distress factors for this technique, further attempts were not made by using this route.

The sublingual approach was similar to what has been described in *Rattus* rats, although the vein was not as prominent. When the vein was punctured, blood collected in the cheek space of the oral cavity instead of flowing out of the mouth as described in *Rattus* rats. In addition, the tongue of the cotton rat was short, based more caudally in the oral cavity as compared with that in other species, and frequently slipped out of the holder's fingers, all of which required repositioning and allowed clotting to occur before the full sample could be collected. In addition, this approach required 2 handlers: one to hold the rat and stretch the tongue up and to the side and the other to puncture the vein. This approach yielded only a small sample with the 25- or 23-gauge needles, unlike what previously has been described in mice and rats. However, use of a 22-gauge needle did increase the sample size to a useable amount, and this method was the most successful technique during the initial comparison (Figure 3). Hemostasis occurred after light pressure at the base of the tongue with a gauze sponge for 10 to 15 s.

The novel subzygomatic method discovered after the first session, was the most viable method investigated, and fulfilled the blood collection requirements sought. The landmarks were easily palpated; when the cheek was shaved, the anatomy could be visualized and the vein seen as a slight outward bulge (Figure 4). The appropriate positioning encouraged venous congestion toward the head and allowed increased blood flow. Vascular puncture was made in a firm but controlled manner to a depth of 2 to 4 mm with the rat on its side on the work surface. As soon as blood started to flow, the rat was lifted and turned so the punctured cheek faced the work surface, to catch the drops with the microhematocrit tube (Figure 5). The technique required practice with animal lancets and needles of different sizes before the correct site could be accessed in only 1 or 2 attempts and yielded sufficient blood flow. Initial puncture attempts with a lancet yielded only very small drops of blood, possibly because the flat puncture edge did not create a sufficiently large track through the masseter muscle. A 20-gauge, 3/4-in. needle allowed for blood to flow easily after initial puncture, but additional attempts were needed because blood often stopped flowing or clotted before the required volume had been collected. An 18-gauge hypodermic needle was ideal for allowing large blood droplets to flow quickly while minimizing early clotting. After sample collection, the rat was placed on a table, pressure on the neck skin was released, and the puncture site gently pressed with a gauze pad to encourage hemostasis. This technique easily produced volumes in the 0.3- to 0.5-mL range. The subzygomatic method ultimately was used as the sole sampling method for the majority of the study, and proficiency continued to improve (Figure 6).

Gross necropsy. The skin covering the cheek was removed carefully. Hemorrhage was either absent or present only in small amounts on the surface of the masseter muscle where the needle had been inserted. The masseter muscle was dissected free by releasing its attachments to the underside of the zygomatic arch and the underlying tissues and maxillary bone. When the muscle was removed, a large venous sinus lay deep to it, at the

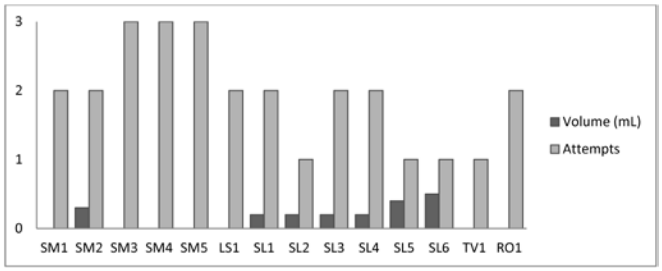

Figure 3. Number of collection attempts and the volume (mL) of blood collected for the 5 methods tested during the first session (the subzygomatic site had not yet been discovered). Note that attempts at the lateral saphenous (LS), tail vein (TV), and retroorbital (RO) sites did not yield any measureable amounts of blood. The submandibular (SM) method yielded only 1 or 2 drops from 1 of 5 attempts. The sublingual (SL) method was the only technique that ultimately yielded measureable amounts of blood during this sampling session.

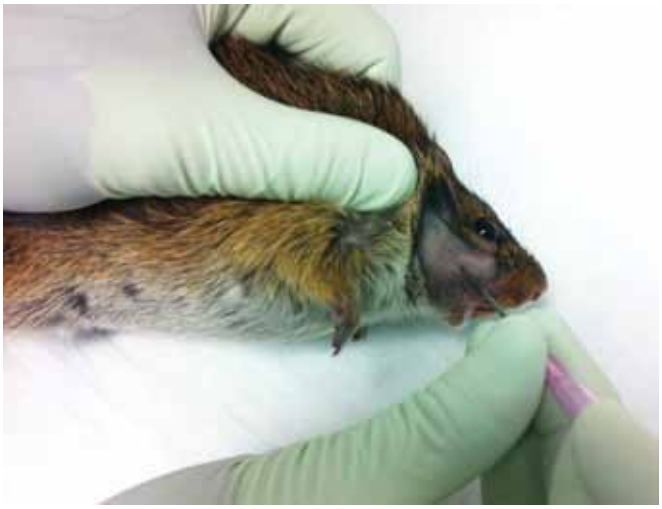

Figure 4. The 18-gauge needle indicates the correct location (just rostral to the junction of the zygomatic bone and ramus of the mandible) for subzygomatic sampling in anesthetized rats.

Figure 5. Elevating a cotton rat for the collection of blood flowing from the subzygomatic site into a microhematocrit tube.

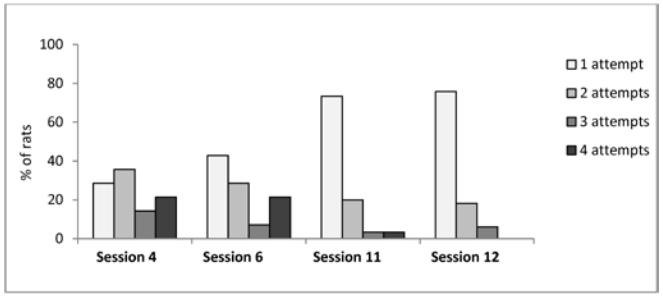

Figure 6. Number of attempts needed to collect the required sample by using the novel subzygomatic method from all animals during multiple sessions over time. Initially, the desired amount of blood was collected in 2 or fewer attempts (considered to cause only momentary pain or distress in an awake animal [USDA pain category C]) in only 64% of the rats; by the final session, the desired amount of blood was collected in 2 or fewer attempts from 94% of rats, and no rat required 4 attempts. Every sampling session is not shown due to incomplete data collection or participation/training of different sampling personnel at those times.)

intersection of the caudoventral aspect of the zygomatic bone and caudal ramus of the mandible. This structure appeared to be closely connected to the retroorbital sinus, because pressure applied externally to the globe pushed blood into and distended the vascular structure (Figure 7).

Histopathology. All rats underwent gross dissection, and 12 were selected randomly for additional tissue sectioning to examine the subzygomatic area. The histopathologic characteristics of the lesions present at the of site blood collection were highly variable and dependent on several factors, including the time interval between blood collection and necropsy, level of a given cross-section, and presence or absence of introduced hair shafts. The following lesions were limited primarily to the subcutaneous tissue of the face, masseter muscle, and associated fascia. The lesions that occurred immediately after blood drawing were characterized by tracts of hemorrhage with focal skeletal myocyte necrosis. In rats with longer evaluation periods, the histopathology ranged from fine, linear tracts with minimal fibrosis (Figure 8), followed by prominent foci of granulation tissue (Figure 9) in various stages of organization, to well-organized fibrosis. Prominent regeneration of myocytes (Figure 9) was present in the skeletal muscle of some rats. When present, inflammation was mixed, with a predominance of mononuclear cells (macrophages, lymphocytes, and rarely plasma cells) with small numbers of neutrophils. In one case, a hair shaft had been introduced into the muscle, eliciting a focal foreign body reaction (Figure 10). One rat had an organizing thrombus in the sinus, and 5 animals showed evidence of mild inflammation in glands near the sinus, but neither could be directly connected to the puncture lesions. The cross-sections examined failed to confirm continuation of the subzygomatic sinus into the retroorbital sinus, so further exploration is warranted.

Discussion

Phlebotomy techniques for many of the small mammals used in research today continue to improve, and investigators and veterinarians should use the most humane and least distressing method available that are consistent with research aims. Based on the current exploration of refinements on blood collection in hispid cotton rats, both the sublingual and the newly described subzygomatic techniques appear to be viable alternatives to retroorbital collection.

The sublingual vein technique has been explored in other rodent species and found to be rapid and humane with minimal adverse effects noted.[10,27] This site was more technically difficult in *S. hispidus* than other species due to anatomic differences in the placement and structure of the tongue and oral cavity. However, we were able to obtain blood from this site, which is preferable to the retroorbital plexus from an animal welfare standpoint. Although the sublingual site has been used in other

Figure 7. Gross dissection of the cotton rat cheek with the masseter muscle removed, showing the large, blood-filled vascular structure (circled).

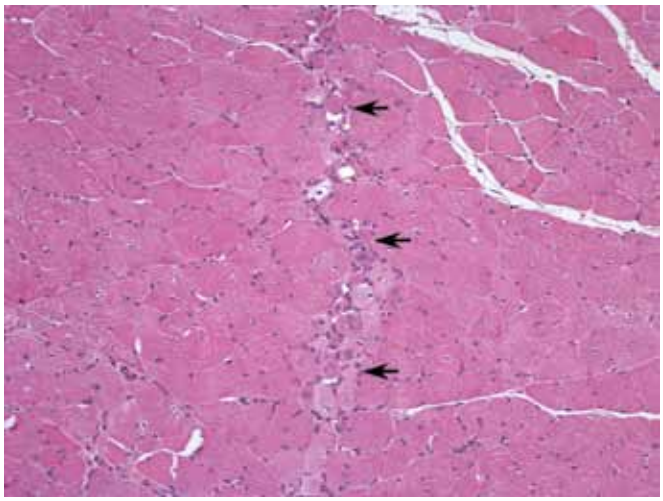

Figure 8. A section of masseter muscle showing a healing linear tract with minimal fibrosis as evidenced by the lighter staining myocytes with only a few surrounding fibroblasts (arrows). Hematoxylin and eosin stain; original magnification, 100×.

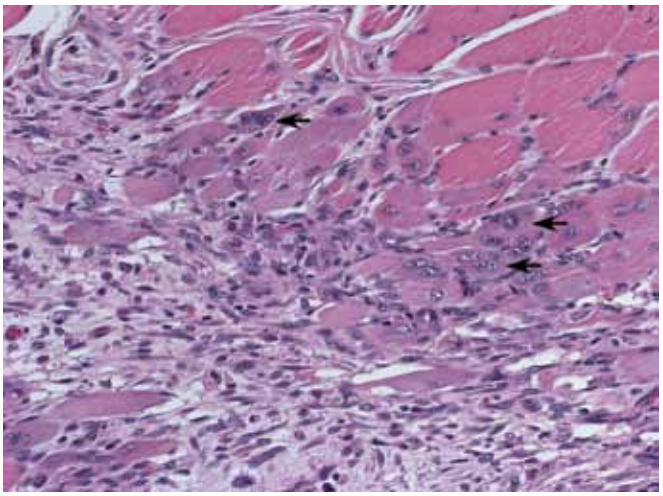

Figure 9. A section of masseter muscle with granulation tissue characterized by abundant fibroblasts and minimal neovascularization. Some skeletal myocytes have a pale basophilic cytoplasm with multiple nuclei, consistent with myocyte regeneration (arrows). Hematoxylin and eosin stain; original magnification, 200×.

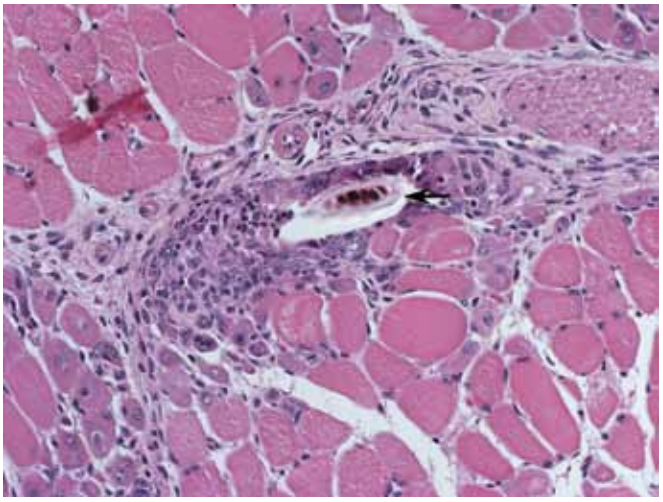

Figure 10. A section of masseter muscle with an embedded hair shaft (arrow) surrounded by macrophages and neutrophils. Hematoxylin and eosin stain; original magnification, 200×.

species without sedation, its use in cotton rats is not recommended without anesthesia or sedation due to their tendency to struggle and bite when held. Although the sublingual method requires 2 handlers, it allows for sufficient sample collection, and further investigation into the utility of the technique should be explored.

The novel subzygomatic method described here provides access to a lateral facial venous sinus that is not present in *Rattus* or *Mus* species. This method was a humane and efficient way to collect blood in cotton rats and has the added advantage of being easily carried out by a single veterinarian, technician, or researcher. We preferred the subzygomatic sinus over the sublingual vein because blood was easy to collect as it flowed from the lateral cheek area, the cheek is more accessible than the base of the tongue, and hemostasis was rapid after only light pressure on the cheek with gauze. Use of the described holding method was important, because the blood flowed more readily when the rat was fully supported than when it was only held by the skin over the neck, with the caudal body unsupported. Handlers must be careful not to pull the neck skin too tight, because doing so can cause ocular proptosis, and not to grasp the tail, because it is fragile and may break or slough. All rats recovered with no apparent signs of pain or irritation at the sampling site, and all body weights increased throughout the study (data not shown). An important point to remember is the allowable amount of blood that can be collected in rodents. The subzygomatic approach allows blood to flow rapidly once the site is punctured, and investigators must pay close attention to the amount they have collected, especially if they are already planning on acquiring close to the maximal limit. We typically found that 1 or 2 additional drops flowed from the puncture site during the time that elapsed between setting down the collection tube, releasing the full-body hold on the rat, and applying pressure to the cheek for hemostasis. This loss did not appear to have any adverse effects on the rats but may account for an extra 25 to 50 µL of blood in addition to what was collected. This effect was lessened with the use of needles of a smaller gauge, but that modification usually disproportionately increased the difficulty in collecting the necessary amount of blood. We speculate that a large-gauge needle is needed for adequate blood flow because the vascular structure lies beneath muscle that will immediately reseal after puncture if the needle track is too small.

Throughout this study, rats showed normal self-grooming, digging for and eating seed mix treats, nest rebuilding, and gnawing on provided wood blocks after anesthetic recovery, suggesting that they experienced little, if any, pain or distress. Some rats performed a yawning or gagging action by opening the mouth widely and having abdominal contractions as they were recovering from anesthesia. We first thought this behavior to be a sign of jaw or facial pain, but it also occurred during anesthesia recovery in animals that were not bled. This behavior may be related to possible nausea due to the chemical anesthetics used. Some rats vocalized during the procedure even though they appeared to be sedated appropriately, as indicated by lack of toe withdrawal and blink reflexes. Vocalization occurred not only while puncturing the cheek but also while handling the animal for noninvasive procedures such as cheek shaving or administering the vaccines, so it could not be attributed solely to pain associated with venipuncture. These vocalizations may be a paradoxical response to the dissociative sedatives used, but further investigation is warranted. The submandibular method in mice is considered to be only momentarily painful and does not require anesthesia or analgesia, although some handlers do immobilize animals to decrease defensive movement and improve the quality of the blood samples.[7,10] Because sedatives or anesthetics usually are used to handle cotton rats, momentary pain would not prompt additional control measures. No other abnormalities related to the bleeding method, such as abscesses, jaw dysfunction, ocular damage, and oral injury, were noted throughout the study.

Histopathologic examination was performed to further evaluate this method of blood collection. Many publications have documented the adverse clinical and behavioral outcomes and the microscopic damage that occurs when the retroorbital plexus is accessed.[2,6,12,15,20,22,23] The pathology observed at the sample sites in the current study were similar to those described when the submandibular technique is performed in mice and included mild acute hemorrhage, edema, and inflammation.[10] The more chronic effects noted were mild also, and tissues often showed evidence of regeneration and repair. We suspect that the described subzygomatic sinus is a ventral out-pocketing of the retroorbital sinus. This structure may be unique to cotton rats, given that it has not been described in laboratory rat and mice anatomic references.[9] However, the connection between the 2 structures could not be proven in the small number of rats we examined.

Blood collection from the lateral saphenous vein was unsuccessful but warrants further exploration in light of its increasing use in other rodent species. Our failure to obtain blood from this site in the anesthetized rats in the current study may reflect their young age and accordingly small size of the peripheral vessels at the time of collection attempts. In addition, isoflurane anesthesia and subsequent chemical sedation may have caused decreases in blood pressure that affected the flow of blood to this peripheral vein. Due to the superficial location of this sinus below the skin and away from the head of the animal, restraint without sedation perhaps could be used in cotton rats, and further investigation into collecting from this site is recommended in more mature, unsedated cotton rats.

The subzygomatic approach to the novel vascular sinus discovered in this study proved to be a rapid, simple and apparently humane new method to consider when collecting blood in *S. hispidus* rats. Further studies to explore the full anatomic boundaries of this sinus are warranted because it does not appear to have been described previously. Additional physiologic, behavioral, hematologic, and histopathologic studies also are recommended to analyze the long-term tissue effects and clinical outcomes of sampling the sinus. In particular, evaluating the effects of sampling this site multiple times in a single day and without anesthesia, as might be needed for toxicologic or pharmacokinetic studies, would be beneficial. In addition, it seems prudent to study the other methods outlined here in more detail, by having studies designed solely for this purpose that allow true comparisons across the techniques and in different sexes and ages of cotton rats; such evaluations could not be done here due to the requirements of the vaccine study on which the rats were enrolled. Regardless, the current study demonstrates that the sublingual vein and novel subzygomatic approaches are viable blood sampling techniques in cotton rats, are marked refinements as compared with the retroorbital plexus method, and should be strongly considered for blood collection in this species.

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