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A Tethered-Restraint System for Blood Collection from Ferrets

Robert K Jackson, Victor A Kieffer, Jerome J Sauber and Gregory L King

The laboratory ferret, *Mustela putorius furo*, recently has come into prominence as a laboratory animal for use in biomedical research. Our laboratory has adopted the use of this species because the ferret's emetic response to radiation occurs at a lower dose and has a more rapid onset than that of dogs (1, 2, 3). One approach for determining the physiological basis of this response is to measure serum levels of various circulating substances before and after irradiation. However, blood collection from the ferret can be difficult because the lack of easily accessible veins and seasonal accumulation of subcutaneous body fat.

Previously described methods for blood collection in this species (toenail clip, retro-orbital or cardiac puncture, caudal tail or jugular venipuncture and bleeding from the ventral tail artery) all require chemical or physical restraint (4). These restraint methods are not well suited for studies in which an active response is to be observed during blood withdrawal or drug administration. Sedation inhibits radiation-induced emesis in other species (1, 2) and sling-restraint interferes with radiation-induced emesis in the ferret (5). Therefore, we developed a means by which ferrets could be restrained which would have minimal effect on the emetic response.

This report describes a method of tethered-restraint for the ferret in which an in-dwelling venous jugular catheter is implanted for withdrawing blood samples. No interference with the animal's normal activities occurs during the sampling procedure. Each animal is conditioned to the tethered-restraint prior to surgical placement of the catheter. The technique provides a minimally stressful method of restraint. A similar tethering system has been used successfully on several other animal species, such as non-human primates (6) and rats (7).

Thirty-three commercially obtained adult male, fitch (sable), castrated and descented ferrets (1 to 1.5 kg), were used[1]. All animals were housed in modified stainless-steel rabbit or cat cages and fed dried cat or ferret diet *ad libitum*. The animal quarters were maintained at

From the Department of Veterinary Science (Jackson, Sauber) and Physiology (Kieffer, King), Armed Forces Radiobiology Research Institue, Bethesda, MD 20814.

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the following temperature, relative humidity and photocycle respectively: 15–21°C, 45–55% and 12 hours light/12 hours dark.

Each animal was caged individually and fitted with a modified guinea pig harness and tether produced by Alice King Chatham Medical Arts (8)[2]. The harness fit around the animal's upper torso and was attached to a stainless-steel flexible-spring tether through which the catheter was threaded. The distal end of the tether was connected to a miniature fluid swivel which was attached to the catheter and was clamped to the cage top. The only rotation occurred between the swivel shaft and the housing of the swivel. This arrangement prevented twisting or kinking of the catheter. The system restricted movement somewhat, but did not interfere with normal activity once the animal became accustomed to tethered-restraint (Figure 1).

The catheter material was flexible tubing, type MRE-040, 0.040" (.88 mm) o.d. × .025" (.55 mm) i.d., approximately 90 cm in length[3]. One end was beveled for insertion into the jugular vein while the end to be exteriorized was sealed with a stainless-steel 23 ga. pin. A collar of a 5–10 mm piece of slightly larger diameter polyethylene tubing (1.36 mm o.d. × 0.99 mm i.d.) was glued around the catheter 10 cm from the beveled end with a silicone sealant and allowed to dry overnight. Catheters were gas sterilized.

Conditioning to tethered-restraint began after the ferret's arrival and acclimation to its new environment. Ferrets were first fitted to the harness alone, followed by the addition of the tether. Each animal was fitted with a small or medium size jacket designed for guinea pigs. The larger size of the ferret necessitated the enlargement of the leg openings by cutting four small slits radially around the inner edges. This modification helped prevent irritation caused by normal motion of the legs. To fit the jacket, the ferrets were held by the skin of the neck while the forepaws were fed through the leg openings. The jacket was then pulled snugly to the back where a 4 × 7 cm piece of Velcro® held the ends together. The jacket, composed of lightweight nylon, was laced by a string to clasps on each side.

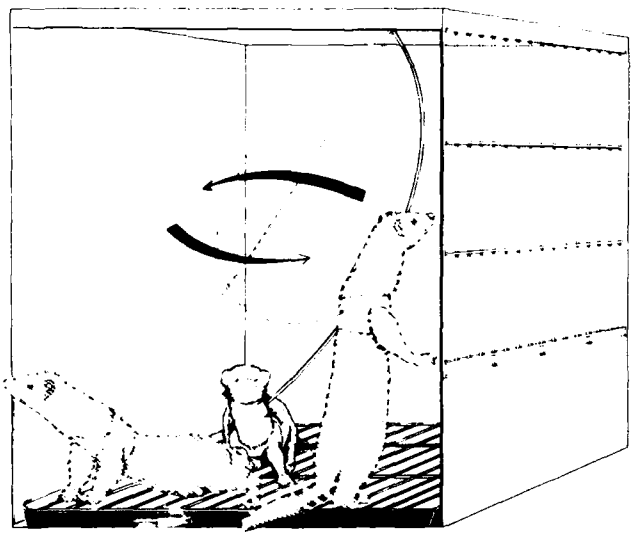

Figure 1 The length of the tether is sufficient to allow the ferret access to all areas of the cage. The system restricts movement somewhat, but does not interfere with normal activity once the ferret has become accustomed to tethered-restraint.

Conditioning was for short periods of time initially and progressed until the animal tolerated continuous tethered-restraint. Full adaptation was defined by a normal pattern of activity which included grooming, eating, sleeping and inquisitive behavior. During the initial fitting and conditioning each animal was observed for the first hour. For conditioning periods longer than 1 hour, ferrets were monitored intermittently. At the first signs of distress the animal was removed from the restraint apparatus. Those animals that did not adapt (e.g., tolerance time to restraint lessened rather than lengthened) were removed from the study. Animals did not undergo catheterization until accustomed to at least 24 hour restraint. All pre-operative conditioning procedures required individual housing of the ferrets.

On first fitting the nylon jackets to the ferrets, there was some initial discomfort as evidenced by rolling over and scratching at the jacket. This initial response subsided within a few minutes. Of the 33 animals in the study, only one animal was withdrawn because it would not tolerate the jacket.

During the next stage of conditioning, animals were tether-restrained for 4 days with periods of restraint progressing from 1 to 8 hours. Those animals that adapted rapidly were tethered for 5 to 6 hours on day two. By days three to five, each animal was tether-retrained for at least one 24 hour period. Tethered-restraint did not interfere with normal eating, sleeping or exploratory behavior, thus meeting the criteria for full adaptations.

Twelve to sixteen hours prior to surgery each animal was assessed for the status of its general health and food was withheld. Each animal was preanesthetized with an IM injection of ketamine hydrochloride (30 mg/kg) and acetylpromazine maleate (25 mg/kg). This placed the overall injected dose of both preanesthetic compounds within the middle of the recommended dosage ranges (i.e., 20–35 mg/kg ketamine and 0.2–0.35 mg/kg acetylpromzine) for the ferret (4).

Early in the study, induction and maintenance of a surgical plane of anesthesia was accomplished by masking the animal and gassing with 0.75 – 1.25% halothane in 0.8 L/min of N_2O and 0.4 L/min of 0_2. Respirations and mask placement/retention over the snout were monitored visually throughout the procedure. In more recent procedures, ferrets were intubated using a 3.0 I.D.x4.3 O.D. endotracheal tube and maintained on 1.0 – 1.5% isoflurane in 0.5 L/min of 0_2 using a Bickford PC-2, pediatric, non-rebreathing system[4].

The use of gas anesthesia helped to maintain a safe plane of surgical anesthesia and to ensure rapid postoperative recovery. Altering the anesthetic technique to the use of isoflurane, intubation, and a pediatric, non-

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rebreathing system enhanced the ability to control the plane of anesthesia and further shortened the recovery period. N_2O has been used for jugular catheterization in the ferret (9) and no complications were noted with it in our study. Nevertheless, its use was discontinued due to the risk of expansion of any possible air embolism secondary to the jugular cutdown procedures.

One death occurred due to a presumed hypersensitivity reaction to the pre-anesthetic medications. This particular 1.1 kg ferret received the calculated pre-anesthetic dose of ketamine and acetylpromazine, was returned to the holding cage in the surgical preparation area and expired prior to retrieval for surgical preparation. Attempts to resuscitate the animal were unsuccessful.

The surgical approach to the jugular vein in the ferret did not offer significantly from the approach in the dog and cat, and various techniques have been described previously in the ferret (10, 11). The saline-filled catheter was inserted through the jugular incision and advanced until the collar passed just beyond the incision site. Cannula patency was tested by drawing blood into the catheter and then flushing with approximately 1 ml of heparinized saline (10 mg/1000 ml). Once the catheter was determined to be patent, it was sealed with a sterile stainless-steel pin. The catheter was secured in place by tying two silk ligatures on either side of the collar. The distal end of the catheter exited through a dorsal midline incision between the scapulae. In our experience, subcuticular sutures are better tolerated than skin sutures in the ferret, and minimize the chance for catheter complications secondary to handling the animal to remove non-absorbable skin sutures. After closing both incisions, the exposed end of the catheter was threaded through the sterile flexible tether. The harness was fitted and fastened, and the catheter uncapped and attached to the sterile, sealed swivel.

Post surgically, each animal was ventilated briefly with 100% O_2, and then allowed to breathe room air before being returned to its cage. The swivel was attached to the overhead clamp and the tether's length (53 cm) allowed the ferret access to the entire cage interior. Animals were monitored constantly until sufficiently recovered to maintain sternal recumbancy. All animals recovered from the effects of the anesthesia within an hour and were eating and active by the end of the day. They were observed frequently over the next 24 hours to ensure complete recovery from anesthesia and to monitor for excessive bleeding. Each animal was also checked for possible seroma or hematoma formation in dependent areas and for proper jacket fit.

The catheter was checked daily to ascertain its patency by aspirating until blood was visualized and then flushing with approximately 1.0 ml of sterile heparinized saline solution. Sterile gloves were worn and aseptic techniques used for this procedure (e.g., sterile syringes and needles).

Within 24 to 48 hours after recovery from surgery, blood was drawn for control samples. One to two days after control samples were drawn, the animals were irradiated and additional blood samples were taken. Aseptic technique was used to withdraw all samples. Sampling was done from outside the cage by manipulating the distal end of the catheter where the tether attached to the swivle assembly, thus avoiding the need to handle the ferret. During sampling, no more than 4 ml of blood was drawn at any one time. An equal volume of normal saline was infused afterward to maintain the intravascular blood volume.

Of the 32 animals adapted to tethered-restraint and implanted with catheters, blood samples were drawn successfully from 30. Of these, both control (pre-irradiation) and experimental (post-irradiation) samples were drawn from 25, while control samples only were collected from five. With one exception, control blood samples were taken at 1 to 2 days postoperatively, and experimental samples were drawn 2 to 9 days postoperatively. In the one case, the control sample was taken at day 18 postoperatively and the experimental sample drawn at day 21. The volume of blood taken during a single withdrawal ranged from 2 to 4 ml.

We were unable to obtain any blood from two ferrets. In these two cases, the problem was attributed to clotting at the end of the catheter, and was confirmed at necropsy. The clotting presumably resulted from occlusion of the jugular and subsequent loss of blood flow around the catheter tip. This problem was corrected by lengthening the intravascular portion of the catheter to 10 cm, so that its tip would rest where the left and right jugular veins join to form the precava. With the remaining five animals, complications arose while drawing the experimental sample. In one of these animals, the catheter had become crimped in the subcutaneous tunnel. In another, the catheter was not blocked at necropsy although it was impossible to flush or withdraw *in situ*. The probable cause was thought to be a fibrin tag acting as a one way valve. No necropsy was performed on the other three animals.

At the conclusion of the experiment, each animal was euthanatized with an IV injection of T-61® (0.3 ml/kg)[5].

The principal conclusion to be drawn from these studies is that the ferret is amenable to tethered-restraint. This is evidenced by the 97% success rate (n = 32/33) for adaptation after conditioning. Restriction of movement induced by the tether is minimal. All animals exhibited normal behavior patterns of sleeping, eating, grooming and exploration. Alternative restraint systems, such as a lucite tube (12) or a sling (5) prevent such activity.

A major advantage of tethered-restraint is the apparent lack of stress that has been observed as compared with other means of physical restraint. Tether-restrained ferrets exhibited no observable difference in activity levels or behavioral traits from non-restrained animals. In addition, the emetic responses of the tethered ferrets did not differ in latency from those of non-restrained ferrets (3). From these observations, it appears that the stress of tethered-restraint in the ferret is no greater that of single housing them in standard laboratory animal caging.

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Given the small diameter of ferret veins and the difficulty with locating them (10), intermittent venipuncture of these vessels (12) requires periods of restraint that add to the stress of venipuncture and may alter physiologic parameters under study. Even the jugular catheterization technique described by Greener and Gillies (11) requires a degree of physical restraint during sampling. The conditioning of ferrets to tethered-restraint should minimize stress-induced changes in levels of numerous circulating hormones, vasoactive compounds and hemotological components (13, 14). Additionally, it allows the investigator to administer compounds and withdraw samples unassisted.

The major complications arising from this study have been associated with catheter length. In two early cases, the catheter blocked with clotted blood. This problem was corrected by lengthening the indwelling portion of the catheter so that its tip would extend into the precava, thus maintaining blood flow away from the end. Once this problem was corrected, the overall success rate for blood withdrawal from the remaining ferrets was 83% (n = 25/30). The one instance in which blood was drawn 18 and 21 days postoperatively suggests this method may be amenable for chronic studies and warrants additional investigation.

Other complications resulted from the lack of precise postoperative fitting of the jacket. If the jacket fit too snugly, postoperative edema occurred and skin irritation around the neck and axillary regions resulted. If the jacket fit too loosely, the animals could remove the jacket and possibly dislodge the catheter. Careful monitoring and adjustment of the jackets for the first 24 hours prevented these problems.

In conclusion, we find that ferrets easily adapt to the tethered-restraint system, and that the behavior of tethered ferrets is not discernibly different from non-tethered ferrets. Additionally, tethered-restraint allows blood samples to be drawn by one person, reduces the stress normally associated with other means of physical restraint, and avoids the need for tranquilization or sedation during sampling.

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Correspondence concerning this manuscript should be sent to G.L. King, Ph.D., Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

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Footnotes

[1]Marshall Research Animals, Inc., North Rose, NY
[2]Alice King Chatham Medical Arts, Los Angeles, CA
[3]Microrenathane®; Braintree Scientific, Inc., Braintree, MA
[4]A.M. Bickford, INC., Wales Center NY
[5]T-61®; American Hoechst Division, Somerville, NJ