Continuous Bladder Infusion Methods For Studying Voiding Function
In The Ambulatory Mouse

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Abstract

Objective
Development of a method for chronic cannulation of the mouse bladder that would enable repeated intravesical drug delivery and measurement of voiding patterns in unrestrained mice under controlled infusion conditions.

Methods
Fifteen female mice were anesthetized with halothane and implanted with 3Fr polyurethane bladder catheters. Catheters exited from the back through mesh and a polysulfone button into a spring coil that protected the catheter and tethered the mouse. A counter-balanced swivel and infusion pump permitted unencumbered mobility during continuous intravesical perfusion.

Results
Patent catheterization was consistently achieved for at least 5 weeks. Voiding patterns produced with an infusion pump were stable not only within a study session but also over the course of several weeks. Catheters remained patent but eventually withdrew from the bladder in 9 mice, at which point the mice were killed. The mesh eventually emerged from the skin in 4 animals without evidence of infection and was associated with catheter leakage at the level of mesh exposure. The subcutaneous placement of the mesh and tether assembly adequately transferred torque to the swivel without catheter obstruction. One mouse died unexpectedly during anesthesia; another was killed one week after catheter implantation because of an intraperitoneal leak. No bladder stones were identified. Urine cultures were inconclusive.
Conclusion

Continuous, patent catheterization of the murine bladder can be achieved consistently for periods of 5 weeks. When used in combination with counter-balanced swivel assemblies and electronic balance technology, these methods permit prolonged evaluation of micturition patterns in the awake, ambulatory mouse.
Introduction

In the age of molecular medicine and studies of bladder biology, the mouse provides an attractive research model. Improvements in methods for studying murine voiding behavior will likely play a pivotal role in understanding the molecular determinants of bladder function. Murine voiding patterns have been evaluated by estimating the size of urine spots on paper beneath a cage or by cystometry in anesthetized mice. While the former is labor-intensive and may be prone to human estimation error, it allows freedom of movement and has been used to generate an interesting and informative literature. The latter allows precise measurement; however, voiding patterns under anesthesia may differ from those of the awake animal.

We have developed a continuous bladder infusion system allowing control over both the type of solution infused and the infusion rate in awake, minimally restrained mice. A catheter is implanted into the bladder, exits from the animal's back and is connected to an infusion pump. Combining catheterization with our previously described automated method for measuring micturition frequency, volume, and urine production provides a sensitive and comprehensive research tool for the study of murine voiding behavior. This paper describes new methods for establishing continuous access to the bladder in awake, ambulatory mice that may be used to study the physiology of micturition under controlled infusion as well as intravesically-administered agents.
Methods

General Housing  Female CRL CD-1 (Charles River, Boston) mice weighing 30 to 40 g were housed and studied in a standard vivarium room (~23 °C) with a 12:12hr light:dark cycle. Mice were studied during the dark cycle, when most drinking, voiding, and other behavior occurs in these nocturnal animals. They were adapted to the reverse light cycle in diuresis cages (Model 650-0322; NalgeNunc, Rochester, NY) for one week before inclusion in experiments. The mice had food and tap water ad libitum in polycarbonate home cages when not actively under study. Surgical procedures and study protocols were approved by the institutional animal care and use committee.

Methods for indwelling bladder catheter placement. Mice were anesthetized with halothane via nose cone in a 27°C room. The lower abdomen and infrascapular regions were shaved before prepping with povidone iodine solution and performing the surgery as described and illustrated in Figure 1. For voiding studies, the external catheter was elongated by placing a 23 Ga connector at the end and adding a 23cm length of 3 Fr polyurethane tubing. This elongated catheter was protected by connecting to the original coil an additional 17 cm length of similar coil. This tether was secured in the swivel's clamp, and the catheter was placed over the 23 Ga stub exiting the swivel's chamber (Fig. 2A.) Three Fr polyurethane tubing from an infusion pump coursed along the counter-balanced arm to the swivel, infusing the neomycin/polymixin B solution. A Y-connector can be placed in this line (Fig. 2A) for introduction of additional solutions. A slit in the cage lid (Fig. 2A) facilitates transfer of the animal and swivel unit to the home cage (Fig 2B.) A bracket on the wall of the home cage supports the swivel assembly. To accommodate the tether length, it was necessary to increase the wall
height of the home cage by adding the upper portion of a second cage. Some mice climbed the tether in the home cage and gnawed on exposed tubing segments. To prevent this, a protective cylinder was fabricated from the body of a 20cc plastic syringe by cutting off the Luer hub and shortening the barrel of the syringe to 4.5 cm. A stainless shaft collar with a knurled setscrew was cemented to the base of the syringe cylinder to hold it in place below the swivel. The setscrew tightened against a short length of silicone tubing to prevent crushing the spring.

**Cystograms** Under halothane anesthesia, diatrizoate meglumine -diatrizoate sodium mixture (Hypaque 60, Nycomed Inc., Princeton NJ) was instilled into the catheter until slight resistance or meatal drainage was appreciated. Plain radiographs were obtained in antero-posterior and lateral planes. Catheters were subsequently flushed.

**Urine cultures** Urine samples for culture were obtained by two methods. After cleansing the catheter with an isopropyl alcohol prep pad, a sterile 25 Ga needle and syringe were used to draw fluid from the catheter and transfer it to a sterile Culturette™ swab (Becton Dickinson Microbiology Systems, Sparks, MD.) Samples were drawn on 4 consecutive weeks, each after at least 60 hours without animal study or catheter irrigation. As culture volume was variable and potentially represented irritant remaining in the catheter, an alternate means of obtaining fluid was pursued. After attempting to acquire a catheter sample, the meatus and surrounding skin were cleansed with chlorhexidine solution. The first fluid to emerge from the meatus after flushing the catheter with sterile normal saline was captured directly onto the Culturette™ swab. Swabs were sent for culture.
**Catheter patency assessment** Wound healing and catheter patency were assessed under anesthesia both at the beginning and end of study weeks as well as randomly during times when the mice were not being actively studied. When instillation of the neomycin/polymixinB solution into the catheter readily yielded output at the meatus, the catheter was deemed patent. In mice with previously patent catheters, absence of meatal output with >1 cc of irrigant was indicative of loss of access to the bladder and the animal was necropsied. Necropsy was also done when mesh exposure resulted in catheter exposure.

**Results**

All 15 mice tolerated the procedure well and displayed stable micturition patterns when their bladders were perfused at 1 to 3 ml/hr. This stability was evident both within single study sessions (Fig. 3A) and several weeks after catheter implantation (Fig. 3B.) Mice were without obvious signs of discomfort the day following the procedure, exhibiting normal nesting and grooming behavior. Mean patent catheterization was 46 days for the 15 mice (median 40 days; range 1 to 16 weeks.)

Instillation of fluid via the catheter produces drainage at the meatus in animals with patent catheters (Figure 4, top and middle). A leak was suspected in an otherwise healthy mouse during the first evaluation after surgery; fluid instillation failed to generate drainage at the meatus. After a cystogram demonstrated intraperitoneal contrast (Fig 4, bottom), the animal was killed one week after surgery. One mouse died during a brief exam under anesthesia 8 days post procedure; her catheter remained patent and in proper position; the abdominal and back wounds appeared healthy. All other mice were killed upon either loss of catheter access to the bladder or exposure of the
catheter with mesh elevation. With the above exceptions, mice survived a minimum of 5 weeks with patent catheters.

The onset of mesh exposure did not necessitate immediate euthanasia. Animals were terminated when mesh exposure and loss of anchoring resulted in catheter exposure. Three of the four animals thus terminated maintained functional catheters for 7, 28, and 45 days after exposure was first noted. The fourth was terminated on the day of exposure when a scab elevated the button and anchors so that torque was not transmitted to the swivel.

The first 5 of the 15 mice had a single layer of Dacron mesh at the polysulfone button; the mesh's rigidity, however, seemed to irritate the overlying skin. Subsequent implantations included the Vicryl mesh described in Methods. Of the 4 mice killed for mesh failure, all had the combination Dacron/Vicryl mesh. Of these, 2 had progressive skin irritation and erosion; although infection was suspected, cultures and analysis for bacteria and parasites were negative. The remaining 2 had scabbing at the mesh site but no erythema, purulent drainage, or other skin abnormalities; the reason for mesh exposure in these 2 is not known.

Urine culture results were inconclusive. Cultures were obtained in a total of 9 mice. All cultures obtained by the flush method were positive, predominately for non-fermenting gram-negative bacilli, not Pseudomonas aeruginosa. When concomitant cultures were successfully drawn from the catheter, they showed no growth in 3 mice, growth of different bacteria in 1 mouse, or growth of similar species in 2 mice. Bacteria cultured from the catheter draws included primarily Pseudomonas species and Stenotrophomonas maltophilia. All organisms were sensitive to enrofloxacin (Baytril,
Bayer Pharmaceuticals), with which animals having positive cultures were treated. Regardless of culture results, voiding patterns remained unaltered.

No bladder stones were detected in this series of animals.

**Discussion**

Historically, studies of murine voiding patterns have been performed by hand, counting and estimating the size of urine spots accumulated on paper drawn beneath the cage at a known rate \(^2\) or by performing cystometrics in anesthetized mice \(^3,4\). In the awake, unrestrained mouse, minute daily fluid consumption and resultant low urine output demand significant time and effort to obtain an adequate number of voids for evaluation. When fluid consumption is augmented with the use of sweetened solutions, fluid intake can vary between mice and across strains; furthermore, it is dependent on diurnal rhythms \(^7,3,8\). On the other hand, while cystometry may be more precise, cystometry under anesthesia is not necessarily representative of findings derived in the unanesthetized state \(^5,6\).

Indwelling bladder catheters in awake rats have been used for cystometry \(^6\). However, the apparatus prevented free movement of the animal within the cage. Continuous urine collection in unrestrained rats has been achieved by implanting a bladder catheter that tunnels subcutaneously to exit at the posterior neck; the urethra is simultaneously surgically occluded\(^9\). Intravenous jugular catheterization for studying rodent drug self-administration were developed in the 1960s\(^10-13\) and have been adapted recently for the mouse \(^14\). Commercial systems permit infusion through such catheters while maintaining free movement of the mouse around the cage; a swivel and tether system protects the infusion lines. By combining these methods with our
electronic balance technology\textsuperscript{9}, we have developed a system that facilitates measurement of void volume and frequency while controlling bladder filling rate in the awake, minimally-restrained mouse. In addition, the indwelling catheter could be used for cystometry or to administer agents intravesically for the evaluation of their pharmacologic effects \textsuperscript{15,16} or of bladder permeability \textsuperscript{17}.

The data from these 15 mice represent an ongoing evolution of surgical technique aimed at prolonging the viability of this small animal model. Pilot work in our lab began with 3 Fr catheters tapering to 1 Fr in the bladder (Fig 4, top); subsequent addition of a retention bead at the catheter’s end helped prevent catheter withdrawal from the bladder. Furthermore, since the 1 Fr diameter impeded flow from our infusion pumps, and since mice seemed to tolerate a 3 Fr catheter well, tapering was discontinued. Despite the benefit offered by the retention bead, catheter withdrawal remained a limiting factor; therefore, additional measures were taken to minimize further the pulling effects generated by prolonged or frequent attachment to the tethering system. Two more retention beads were added, one just deep to the mesh and a second intra-abdominally at the point where the catheter exits the peritoneum. Moreover, a small amount of slack is now intentionally left within the abdomen.

Additionally, the coarse edges of the cut Dacron mesh included with the tethering system seemed to irritate the overlying skin, sometimes gradually emerging without obvious signs of infection. The rigidity of the Dacron is necessary to transmit torque to the coil and swivel mechanism as the mouse moves. Therefore, a layer of fine Vicryl mesh was placed superficial to the Dacron to provide a less irritating surface. While mesh exposure eventually occurred in a total of 9 mice, it was felt to be responsible for
catheter failure in only 4 cases. Mobility of the mesh and button unit may have limited the durability of the catheterization in earlier animals when anchoring sutures were placed only in the subcutaneous fat pads. Anchoring to the underlying muscle mechanically strengthens the unit responsible for transmitting torque to the swivel. The animals do not display evidence of pain, discomfort, or restricted mobility.

The series of animals in this study were maintained in their home cages with obturators in place approximately 5 cm above the polysulfone button. The intent was to evaluate the ability of the mice to tolerate the presence of the catheter per se. In subsequent experiments (not reported here), animals were maintained in the home cage with the counterbalanced arm and swivel attached with an obturator at the end of the entire assembly, >20 cm of catheter length. Several animals climbed the tether and severed the catheter loop at the swivel; this was prevented with the protective cylinder described in methods. It is necessary to maintain fluid in the swivel.

The importance of the positive urine cultures has not been demonstrated. All cultures obtained by flush through the catheter and bladder were positive but did not necessarily correlate with culture results from the catheter aspirate. As behavior and voiding parameters did not seem to be altered in animals having positive culture results, it seems that these cultures reflect either contamination or colonization and not active infections. Furthermore, there does not seem to be an increased incidence of bladder stone formation, a phenomenon that has been noted in rats with either bladder sutures or xenografts 18 or with surgically implanted urethral catheters 19. While gross examination of the bladders at necropsy has not revealed erosions or a marked
inflammatory response, the bladder’s histologic response to the presence of the catheter remains to be assessed.

**Conclusions**

Indwelling bladder catheter and swivel technologies used in combination with automated balance enables long-term, non-invasive experiments on murine voiding behavior. Stable voiding patterns were achieved with infusion pumps in ambulatory mice exhibiting normal rodent behavior. This method enables reliable continuous intravesical delivery of medications, control of fill rate, and measurement of intravesical pressure, void frequency, and volume for at least 5 weeks.

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References


8. Wood R, Eichel L, Messing EM and Schwarz E: Automated noninvasive measurement of cyclophosphamide-induced changes in murine voiding


Figure 1. Catheter implantation technique for chronic bladder access in ambulatory mice. The bladder was exposed through a lower midline incision (A.) A stay suture of 5.0 Vicryl (Ethicon, City, NJ) was placed along the lateral wall of the peritoneal cavity. A small nick was made with a fine iris scissor just beneath the suture, and a 14 gauge hollow tunneling trocar was passed through the abdominal musculature, coursing subcutaneously to a 5mm exit site at the intrascapular region (B.) A mouse bladder catheter was fashioned prior to surgery from a piece of 3 French polyurethane tubing (Instech Labs, Plymouth Meeting, PA), with three 0.5 mm lengths of the 3 Fr polyurethane tubing having been sequentially passed over the catheter to serve as retention beads. The beads were positioned such that one remained in the bladder, one at the peritoneal exit site, and one at the mesh beneath the polysulfone button. Placement of the second bead allowed for a small amount of catheter slack within the peritoneal cavity. Catheters were soaked and flushed in 70% ethanol and then in a neomycin/polymixin B solution (40ug/200units/ml saline) before use; they were subsequently plugged with a 23 gauge stainless steel obturator. A 5.0 Vicryl (Ethicon, City, NJ) purse-string suture was placed at the dome of the bladder before making a small cystotomy within the suture bounds. The tip of the catheter with a retention bead was positioned in the bladder before closing the cystotomy by tying the purse string suture snugly around the catheter (C.) The opposite end was directed to the exit site by insertion into the hollow trocar and subsequent trocar withdrawal (D and E.) The pre-placed Vicryl stay suture on the lateral abdominal wall was also tied around the
catheter, keeping the second retention bead intraperitoneal (F.) The peritoneum and skin were closed sequentially with running 5.0 Vicryl. A subcutaneous pocket was created beneath the intrascapular incision; the exposed catheter was then passed through a polysulfone button tether (G) (LW62; Instech Labs, Plymouth Meeting, PA) before subcutaneous placement. The button was anchored previously to a dual layer of mesh, Vicryl superficial to Dacron, using interrupted 5.0 Prolene (Ethicon, City, NJ). A spring tethered the button to a swivel (375/22 for mouse; Instech) suspended from a counter-balanced arm (SMCLA; Instech) attached to the diuresis cage lid (Fig 2A) (Nalgene.) The button and mesh base were sutured to the underlying fat pad and musculature with 5.0 Prolene (H.) The skin on either side of the subcutaneous button was closed with interrupted 5.0 Vicryl. The tip of the catheter was protected by 3 cm of stainless spring, used later as part of the tethering system. Acetaminophen (1 mg/ml drinking water) was given for 24 to 48 hours post procedure. Mice recovered in their home cages (1 mouse/cage) a minimum of three days prior to experimental perfusions at 1 to 3ml/hr with the antibiotic saline solution. Note the absence of a sterile drape for illustrative purposes.

Figure 2. The diuresis cage (A) and home cage (B) for study of chronically catheterized mice. The bladder catheter exits from the mouse's back and is protected by a metal coil tether. The tether is secured by a clamp at the base of the swivel. The counter-balanced arm carries the weight of the catheter assembly (details in text.) The diuresis cage is positioned above an electronic balance.
Figure 3. Infusion via an indwelling bladder catheter generates stable voiding patterns irrespective of fluid intake. Mouse W163 (above) was perfused at 1 ml/hr and displayed relatively stable void volumes and rates during single study sessions (panel A) and across one month (panel B). The asterisk (*) in panel B denotes the 8-hour session from panel A.

Figure 4. Antero-posterior (top) and lateral (middle) cystogram views during a cystogram in one of our earlier studies. Note the normal bladder contour, overflow of contrast from the meatus, and the absence of an intraperitoneal leak. The bottom panel confirms the intraperitoneal leak suspected in another mouse.
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