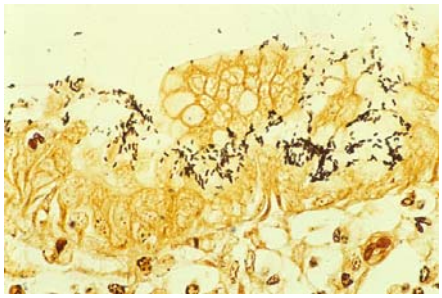
Spring-Summer 2013
Volume 6, Issue 2

WEILL CORNELL MEDICAL COLLEGE EDITION

In Focus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology

Murine Rectal Prolapse



H. pylori **colonized the surface of re-generative epithelium (Warthin-Starry's silver stain)**

Image credit: http://en.wikipedia.org/wiki/Helicobacter_pylori

In the mouse, a rectal prolapse is a protrusion of rectal tissue from the anus, presenting as a small red mass[1-6]. Mice are particularly susceptible to this condition because they have a very short rectum.[5] Rectal prolapse can occur when mice strain to defecate or give birth[4]. Other factors may also make mice more susceptible to rectal prolapses. These can include diarrhea, intestinal tumors or masses, and inflammatory typhlitis or colitis which may be associated with Helicobacter spp, *Citrobacter rodentium*, *C. freundii* and pinworm infections[4,7]. Some transgenic strains such as IL-2 and IL-10 deficient mice, which are models for inflammatory bowel disease and develop spontaneous bowel inflammation, are known to be predisposed to rectal prolapse[4]. However, frequently there is no discernible cause[1].

Rectal prolapses can be classified as mild, moderate or severe[3]. When the moist rectal mucosa is barely visible (≤ 1mm) beneath the tail, it is considered mild[3]. A moderate prolapse is when

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Positive Reinforcement Training: Worth the Effort

Positive reinforcement training (PRT) is a form of operant conditioning in which the trainer reinforces desired behaviors with a reward after the completion of a target behavior. Studies have shown that PRT in the research setting is beneficial to both the animals and their handlers and it also reduces stress associated variability in research data. [2,3,4,5]. Initial costs involved in developing and launching a PRT program in a research facility are modest compared to the benefits, such as improved animal and personnel welfare and increased data reliability.

Current literature provides numerous examples demonstrating that many species can be trained to allow treatments to be administered and/or procedures performed, which would otherwise require sedation[2,3]. The resulting positive interaction that the animals have with their handlers is also beneficial to the handlers as it improves the quality of their experience and job satisfaction. Although PRT is predominantly used with larger animals, its use is expanding to laboratory

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Inside:

The Use of Anesthetics and Analgesics When Performing Murine Tail Biopsy (Tail Tipping)

Mouse tail measurement for tail biopsy

Image credit: http://www.theodora.com/rodent_laboratory/genotyping.html

With the advent of genetically engineered mice, genotyping (evaluating genetic make-up) has become an essential technique in animal research. Although multiple tissues, including hair, blood, saliva, ear, digits, and tail can be sampled and used successfully, amputation of the distal end of the mouse's tail remains one of, if not, the most common procedure for genotyping. Refinement of tissue sampling, genotyping methodology, and the effect of tail biopsy on animals has been a focused area of investigation in recent years. Studies have investigated the most appropriate age of animals at the time of tissue sampling, the type of tissue to harvest, the quantity of tissue to collect, and whether general anesthesia or topical anesthesia ameliorate discomfort associated with the procedure.

Until recently revised, MSKCC's/WCMC's tail tipping guidelines stated that anesthesia or analgesia was unnecessary before 21 days of age, with the presumption that tail biopsy should be conducted before coccygeal vertebrae are completely ossified. However, vertebral development has been shown to differ by mouse strain. Hankenson *et al*, found that mature vertebrae are detectable by microCT in the distal 5mm of tail as early as 17 days of age in some mouse strains, including C57BL/6 and C3H[2]. In the same study, as age increased so did acute behavioral responses to tail biopsy (based on a pain-scoring scale). Mice 31-42 days of age had an increased response to tail biopsy up to 60 minutes post-biopsy, which is expected given the presence of greater numbers of mature vertebrae in older animals.

Recently, several studies examined the effects of anesthetics (both local and general) and analgesics on tail biopsy in mice[3,4,5]. Isoflurane, one of the most commonly used anesthetics in laboratory rodents, was evaluated for use in tail biopsy. It was selected for evaluation due to its ease of administration, ability to manipulate the depth of anesthesia,

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Please Welcome Two New System/Financial Analysts to the CCMP

Yuliya Zorkina

Yuliya, as System/Financial Analyst for the Administration, Finance and Information Systems (AFIS) group, brings a depth of experience in billing and financial analysis in both academic and clinical setting. At CCMP she will be handling day-to-day finances and billing operations at CCMP. She can be reached at zorkinay@mskcc.org or 646-888-2408.

Jason A. North

Jason brings broad experience (15+ years) in research, operations, finance and information technology to the post. Jason will concentrate on matters primarily related to administrative operations and information systems (EnCCoMPass implementation and support) for the CCMP and can be reached at northj@mskcc.org or 646-888-3544.

EnCCoMPass Protocol Module Launched at WCMC!

The CCMP and the IACUC announced the release of the EnCCoMPass Protocol module. EnCCoMPass Protocol is a protocol development and review system which is easier to use and is more robust than eSirius. New protocols and 3-year renewals have to be be submitted through EnCCoMPass Protocol as of July 1st.

There are several key differences between EnCCoMPass Protocol and eSirius.

1. EnCCoMPass Protocol is a dynamic system, therefore only questions applicable to your proposal will be generated.
2. The availability of pre-approved techniques (PATs) will reduce the amount of time needed to develop your proposal and will facilitate approval.
3. There is a separate 'Anesthetics' section, with PATs describing the common regimens.
4. The 'Hazardous Agents' section is automatically generated based on responses to specific questions.

EnCCoMPass is accessible from the internet without using VPN. IACUC and RARC's EQA staff are available to help you with EnCCoMPass Protocol or you can email questions to EnCCoMPassProtocol@med.cornell.edu. An online tutorial is available which reviews the application and its features.

Importantly, to prepare for the release of EnCCoMPass Animal, the animal ordering module coming this summer, all Principal Investigators need to provide RARC with a list of protocol members authorized to order animals. An email link specific for this purpose is available on the EnCCoMPass Protocol home page.

EnCCoMPass can be accessed at http://EnCCoMPass.weill.cornell.edu.

Positive Reinforcement Training, *Cont. from pg. 1*

odents and other species. It has long been nown that rats that are handled gently nd with positive rewards for a period of ime before they are asked to tolerate an ncomfortable procedure have lower stress levels during these procedures and better tolerate human handling in general. In 2012, Rygula *et al* showed that rats that are given a positive reinforcement during handling known as "tickling", where the handler administers manual stimulation of the rats' abdomen by holding the animal on its back in one hand and performing rapid right-hand finger movements across the ventral body surface of the animal, produce high frequency (50kHz) ultrasonic vocalizations known as "laughter calls" or "chirping"[6]. According to Rygula, "These vocalizations in response to tickling have been postulated to directly index positive emotions akin to human joy"[6]. The rats that were given this type of positive reinforcement during handling would come toward the handlers when approached. Tickling reinforcement as part of a basic habituation procedure for rats would likely improve the lives of the rats by decreasing stress and stress variables.

In another study, Coleman *et al* found that "PRT increases the animal's control over its environment. PRT also desensitizes the animal to stressful stimuli (such as injections), thereby increasing overall well-being and creating a better experimental subject"[2]. They also noted that 'threat behaviors' in primates being trained decreased dramatically after the initiation of a PRT program[2]. Alliger *et al* reported that providing rats the ability to make choices was more relevant to the animals' well-being and decreased stress than the choices themselves[1]. Rat subjects that were given more autonomy and choice, specifically in the amount of light in their cage, not only showed individual preferences for particular lighting levels but performed better in cognitive tasks as well. She also pointed out that "Animals in laboratory settings lack such control potentially leading to frustration and subsequently abnormal behaviors"[1]. Evidence in support of the benefits of PRT includes significantly lower mean total white blood cell and neutrophil counts as well as serum glucose levels when samples were collected by PRT rather than under anesthesia[4].

Current literature describes the use of PRT for the purpose of chemical-free sample collection, desensitization, target training, and enrichment in many different species housed in lab animal facilities and represents a clear effort toward meeting the goal of refinement as defined by Russell and Burch in their 3R's principles[7]. PRT as an activity on its own with no performance goals provides the animal with mental and physical stimulation through learning and successfully performing a task (enrichment), a benefit in its own right. Often the motivation behind initiating PRT programs begins with the animal technicians and scientists who handle animals frequently as a principal

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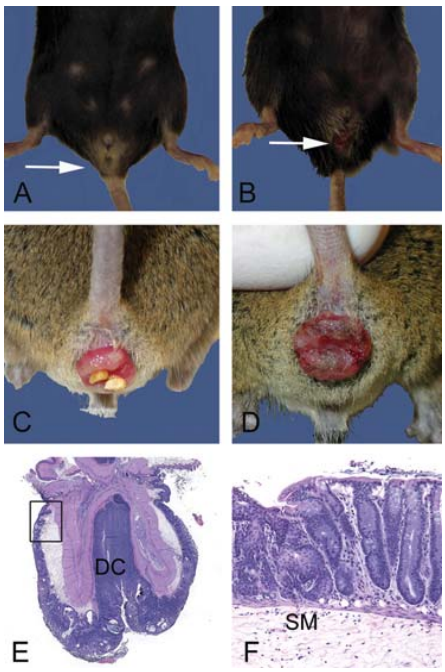
Rectal Prolapse in the Mouse, *Cont. from pg. 1*



A. Normal mouse with the anus indicated (arrow).

B. Mouse with a mild rectal prolapse.

C. Moderate rectal prolapsed.

D. Severe rectal prolapse

E. Photomicrograph of prolapsed mucosa covered by a serocellular crust. Distal colon (DC) and rectoanal junction (box) are indicated.

Image and caption credit:
Pettan-Brewer C and Treuting PM. 2011. Practical Pathology of Aging Mice. Pathobiology of Aging & Age-related Diseases, North America 1.
<http://www.pathobiologyofaging.net/index.php/pba/article/view/7202>./7202>.

Image credit: http://journalstar.com/news/local/positive-reinforcement-leads-to-a-record-setting-rat/article_127c21a8-e2cb-11de-b6c4-001cc4c002e0.html

≤ 3mm of moist, inflamed tissue is protruding from the anus[3]. In these cases, the mouse is otherwise healthy and normal, and the mucosa is not bleeding or necrotic[3]. A severe prolapse occurs when any of the following conditions are noted: there is ≥ 3 mm of tissue protruding from the anus, the mucosa is bleeding, necrotic or there is evidence of dried blood, or the mucosa is dry and the mouse has lost greater than 20% of its body weight, is hunched, lethargic or has a ruffled fur coat[1,3-5].

When Veterinary Services' staff identifies cages with affected mice and contacts laboratory staff, various management options may be presented depending on the condition's severity. Treatment is generally unrewarding unless it is conclusively linked to a pathogenic organism. For mild to moderate rectal prolapses, the mouse may be provided with a gel diet or moistened feed to soften the feces and minimize straining. Topical antibiotic ointment must be applied to the prolapse at least three times a week to prevent desiccation and tissue necrosis. Paper bedding may also be used to minimize damage to the exposed mucosa as wood chip bedding may become attached[3].

If the mouse is a nursing dam, she may be allowed to continue nursing her pups until weaning, after which time she should ideally be sacrificed, as this condition could be heritable[3,5].

In larger species, rectal prolapses are typically reduced by applying hypertonic saline or sugar solution to the exposed tissue to reduce edema and then manually reinserting the tissue ad applying a purse string suture to the anus to prevent the tissue from prolapsing again. Due to the mouse's small size, this option is not feasible[4].

Affected mice should be monitored daily for signs of weight loss, hunching, ruffled fur coat and lethargy[1]. Euthanasia is required within 24 hours when the rectal prolapse becomes severe or the animal becomes hunched, lethargic and/or has ruffled fur[1,5].

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PRT, *Cont. from pg. 2*

job responsibility and they sometimes face a stiff challenge convincing their supervisors that PRT is worth the effort. Prescott puts it best, "Generally speaking the largest cost of training is the initial time investment in educating staff and implementing the behavior modification process. However, there is every indication that this investment will be recouped within a short period, and that it is more than outweighed by the benefits to [animals], staff and science in terms of improved animal welfare, facilitated and reduction in the variability of research data[5]."

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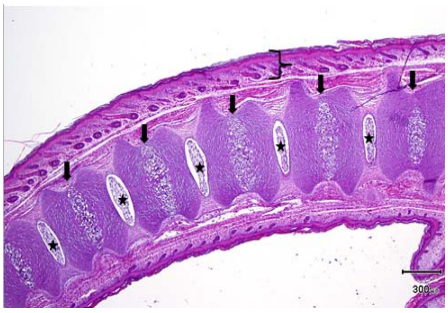
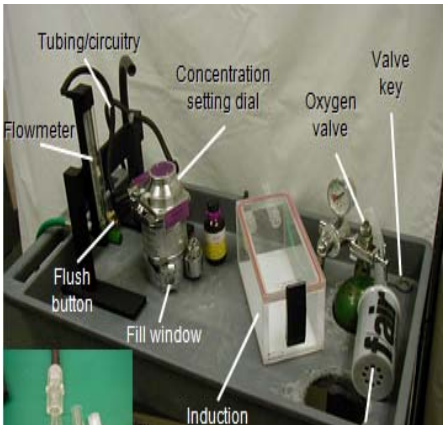
Murine Tail Biopsy, *Cont. from pg. 2*



Longitudinal section of tail, 2-3 day-old C57/BL6 mouse, H & E stain. The bracket indicates dermis and epidermis. Arrows indicate vertebral bodies, which are still composed of cartilage at this age. Stars indicate developing intervertebral discs. Photo credit: Julie White, DVM,CCMP's Laboratory of Comparative Pathology

Photo credit: http://www.acuteaday.com/blog/tag/newborn-mice/



Isoflurane anesthesia machine configured for small rodents.

limited physiologic effects, rapid achievement of muscle relaxation for surgical manipulations, and its elimination by exhalation without metabolism by the liver. Isoflurane reduced undesirable behavioral responses in mice older than 1 month of age who received a tail biopsy[1]. A recent study also showed that a two minute exposure to isoflurane anesthesia provided extended analgesia as demonstrated by increased tail flick latency for 30 to 90 minutes after anesthetic recovery as measured by a hot-water immersion test[5]. It is important to note that isoflurane has been shown to decrease locomotor activity for up to 5 hours post-exposure in both BALB/c and C57BL/6 mouse strains.

Administration of a topical local anesthetic is a common method of controlling post-procedural pain in mice undergoing tail biopsy. However, there is currently no published data that supports the use of local anesthetics for tail biopsy. Indeed, studies have shown that some methods of anesthesia and analgesia, such as vapocoolents (e.g. topical ethyl chloride spray) have significant inflammatory effects on tail tissue and show no appreciable reduction in pain-related behaviors in mice[3,4]. Similar findings have been noted with other topical anesthetics such as cetacaine, benzocaine, and lidocaine. The exception is a single study which found that a 30 second immersion in bupivicaine does appear to relieve pain associated with the tail biopsy procedure in pre-weaning mice but not in mice after weaning.

Based on these findings, MSKCC's and WCMC's guidelines were revised to recommend tail biopsy be performed on mice between 12 to 17 days of age. This upper limit was established based on the fact that in most strains of mice, ossification of vertebrae is complete by 17 days of age[1]. Biopsying the tail at a younger age should reduce the severity of pain experienced both during and after amputation. These guidelines recommend the use of isoflurane for anesthesia in mice 17 days old or younger prior to and during tail biopsy. In mice 18 days and older, general anesthesia is required during the tail biopsy procedure; preferably an inhalant anesthetic such as isoflurane. Tail biopsies should be avoided in mice older than 28 days, as complete ossification of vertebrae and mature nerve endings are present[2]. If tail biopsy is performed in mice older than 28 days, the IACUC recommends the use of a single injection of a pre-emptive analgesic such as carprofen or buprenorphine. Only 2mm of tissue from the distal tail is necessary to obtain sufficient DNA for PCR and Southern blot. However, 5mm is typically collected, but no more than 1cm should be amputated. The procedure should be performed on a non-porous hard surface, using a clean, sharp instrument such as a surgical blade or sharpened scissors (see image below). Wiping the tail with an alcohol pad prior to biopsy is recommended. The blade or instrument should be disinfected between animals with 70% ethanol or a glass bead sterilizer. Hemostasis can be achieved by applying 30 seconds of pressure at the site of biopsy to stop bleeding. Cautery, tissue adhesives, coagulation powder or sticks may also be used, but simple manual pressure is the least traumatic. As with any procedure, gentle animal handling will reduce the stress on the animal associated with the procedure.

In summary, tail biopsy should be performed in the youngest mice feasible. Use of the general anesthetic isoflurane reduces post-biopsy pain. Adherence to the IACUC guidelines for murine tail biopsy will reduce pain and distress associated with this technique.

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Appropriate surface for tail biopsy.