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Applying Principles of Aseptic Surgery to Rodents

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The 1985 revision of the Public Health Service Guide for the Care and Use of Laboratory Animals (‘PHS Guide’) (Committee, 1985) and 1985 amendments to the Federal Animal Welfare Act (9 CFR, 1992) both contain provisions requiring aseptic technique for rodent survival surgery. The ‘PHS Guide’ applies to all live vertebrate animals used in research and, thus, includes laboratory rats and mice. Regulations of the Animal Welfare Act apply to hamsters, guinea pigs, and unusual laboratory rodents, but currently exclude rats of the genus Rattus and mice of the genus Mus.

Rodents are widely used in biomedical research, as evidenced by 55,074 citations for 1990 and 46,519 citations for 1991 under the Medline (online database of the National Library of Medicine) heading "Rodentia". However, only approximately 1.2 percent of the Rodentia citations (741 citations in 1990 and 548 citations in 1991) reported surgical procedures. When Rodentia citations with surgical procedures were subdivided by species of rodent, rats were first with the most listings, mice were second, and guinea pigs were third. Hamsters, gerbils, and other rodents were a distant fourth.

Occasionally, the argument is still made that aseptic technique is not necessary for rodent surgery because mice or rats often survive surgical procedures performed using less than aseptic technique. However, survival alone is not a valid criterion for judgment of the acceptability of a rodent surgical technique. The criterion for acceptability should be the absence of untoward, unplanned alteration of physiological functions or behavior due to perioperative infection. Post-surgical adhesions and subclinical infection can complicate analysis or observation of tissues. Failure to utilize aseptic surgical technique increases the potential for introducing bacteria and activating immune responses in reaction to the bacteria. Recently, responses of rats subjected to aseptic or septic surgical procedure were compared. Although there were no obvious clinical signs in either group of rats, differences were observed in open field behavior, "freezing" behavior, plasma fibrinogen, serum glucose, total white cell count, and wound histology scores (Bradfield, Schachtman et al. 1992). Activation of macrophages in response to intraperitoneal inoculation of bacteria (Bancroft, Schreiber et al. 1989), stimulation of cytokines and activation of B cells by bacterial endotoxins (lipopolysaccharides) (Abbas, Lichtman et al. 1991), and alterations of other physiological processes by subclinical viral, mycoplasmal, bacterial or parasitological infections (Committee on Infectious Diseases of...
Laboratory Rats and Mice 1992), are well documented in the literature. It has been documented that use of aseptic surgical technique has increased the success of ovarian transplants in mice and speeded the return to normal following other surgical procedures in mice (Cunliffe-Beamer 1972-73; Cunliffe-Beamer 1990).

A further argument for aseptic surgical technique in rodents is the fact that hamsters and guinea pigs are intolerant to many antibiotics. In these species, antibiotics can selectively destroy gram positive intestinal flora resulting in overgrowth of gram negative organisms and endotoxemia (Wagner 1976; Small 1987). Administration of antibiotics to "protect" against the consequences of poor aseptic technique could increase morbidity and mortality in hamsters and guinea pigs.

Development of protocols for aseptic rodent surgery can challenge the attending veterinarian, principal investigator, and Institutional Animal Care and Use Committee. The challenges arise from several sources. First, the same person often serves as surgeon, anesthetist, surgical technician, and scrub nurse when surgical procedures are performed on rodents. Careful planning is required to assure that all supplies and equipment required to complete the surgical procedure are not only ready for use, but are also placed exactly where they are needed before surgery begins. Second, experimental design frequently requires repetitive surgery, that is, performing the same surgical procedure on individual members of a group of rodents during a single sitting. In repetitive rodent surgery, it may not be feasible to have a new sterile pack of instruments for each rodent. Procedures to decontaminate instruments between each rodent must be developed. Third, the small body size of many laboratory rodents mandates dissecting microscopes and delicate microsurgical or ophthalmic instruments for many otherwise routine surgical procedures.

The `PHS Guide' defines major survival surgery as "any surgical intervention that penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover." The standards of the Animal Welfare Act in part 1.1 similarly define a major operative procedure as "any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions." Minor surgeries, by default, are all surgical procedures that do not penetrate a body cavity or produce a permanent impairment of function. However, one should remember that a relatively minor surgical procedure, such as vascular catheterization, can have life-threatening complications if bacteria are introduced into the blood stream.

The `PHS Guide' states that "survival surgery on rodents... should be performed using sterile instruments, surgical gloves, and aseptic procedures to prevent clinical infections." The standards of the Animal Welfare Act in part 2, state "... survival surgery will be performed using aseptic procedures including surgical gloves, masks, sterile instruments, and aseptic technique." However, neither document further defines aseptic surgical technique in detail. The primary objective of aseptic surgical technique is to reduce microbial contamination of the incision and exposed tissues to the lowest possible practical level. Items to address during development of aseptic technique for repetitive rodent surgery include (1) selection and sanitation of surgical table and associated equipment, e.g., microscopes, (2) preparation and sterilization of surgical instruments, (3) maintenance of sterility between rodents, (4) decontamination of skin surrounding the incision site, (5) use of surgical drapes, and (6) preparation of the surgeon.

When major survival surgical procedures are performed on non-rodents, `PHS Guide' and standards of the Animal Welfare Act require a dedicated surgical facility. In this facility, the `PHS Guide' requires separate areas for performing the surgery, storing supplies and preparing surgical instruments, preparing the animal for surgery, preparing the personnel, and providing intensive care and supportive treatment of post-operative animals. A dedicated surgical facility is not
required for major survival rodent surgery by either the `PHS Guide' or the Animal Welfare Act. A
rodent surgical area can be a room or part of a room that is easily sanitized and not used for other
activities when rodent surgery is in progress. The area should be subdivided so that there are specific
places for cages of rodents awaiting or recovering from surgery, preparing rodents for surgery, and
performing the surgery. This approach reduces the potential for contamination of the surgical field
by fur, feces and bedding. Before beginning rodent surgery, the laboratory bench or table where the
surgery will be performed should be cleaned and disinfected. Quaternary ammonium disinfectants or
70% alcohol are good choices for disinfecting laboratory benches prior to rodent surgery.
Laboratory benches in front of open windows, next to doors, or similar locations where air currents
and dust are difficult to control should be avoided as rodent surgery tables. Likewise, rodent surgery
should not be performed in or in front of an exhaust hood because air and particulates from
throughout the laboratory are drawn over the surgical field. A high efficiency particulate absorbent
(HEPA) filtered hood can be used as a rodent surgical area if the air flow within the hood does not
desiccate exposed tissues. A glove box or plastic bubble can be used to create an isolated "rodent
surgical suite" within a laboratory or animal treatment room.

Surgical instruments used in rodent surgery usually have delicate tips that are easily damaged.
Autoclavable tip guards are commercially available and should be used to protect tips of instruments.
Special instrument trays with rows of soft plastic fingers can be used instead of flat trays to store
delicate instruments. The plastic fingers prevent instruments from sliding into each other if the tray is
tilted. After use, instruments should be soaked in lukewarm water to remove blood and tissue,
washed with a free rinsing neutral pH detergent, rinsed thoroughly, and air dried. A toothbrush can be
used to scrub delicate surgical instruments. Before delicate instruments are returned to storage, the
tips should be examined, preferably under a microscope, to be certain that the ends meet properly,
and grooves should be examined to verify that no blood or tissue remains in grooves. The cutting
edge of microdissecting scissors should be examined under a microscope and be tested by cutting a
single thread in a gauze sponge or piece of fine suture. Instruments with damaged tips or dull blades
should not be used because their use can increase the amount of trauma associated with the
surgical procedure.

Methods to sterilize surgical instruments include steam, dry heat, ethylene oxide, chemical sterilants,
and radiation (Block 1991). By definition, sterilization means the absence of microbial life, including
viable bacterial spores. Steam or dry heat are preferred methods to sterilize surgical instruments.
Sterilization should be verified through periodic use of biological indicators manufactured for this
purpose. Glass bead sterilizers are a fast way to sterilize unwrapped surgical instruments (Callahan,
Fiorillo et al. 1992). However, instruments must be allowed to cool on a sterile surface before use to
avoid thermal injury (burning tissues). Instrument packs sterilized by ethylene oxide must be aerated
to remove residual gas. Some chemical sterilants, e. g., chlorine dioxide, are corrosive to metals as
well as irritating to tissues. Even noncorrosive chemical sterilants can be irritating to tissues. If
chemical sterilants are used on surgical instruments, sufficient time must be allowed to achieve
sterilization and instruments must be
rinsed with sterile water or sterile saline before use. Contact time varies with the chemical sterilant
and manufacturer's instructions should be consulted for contact time required to achieve
sterilization. Rinse solutions should be changed frequently to prevent contamination by the sterilant.
Quaternary ammonium, iodophor and phenolic disinfectants used to sanitize animal facilities should
not be used on surgical instruments. These disinfectants are not sterilants. Alcohol, contrary to
popular belief, is neither a sterilant nor a high- level disinfectant (Block 1991; Rutala 1990).
Recommendations for selection of disinfectant based on the physical make- up of the instrument
and its use have been published (Rutala 1990).
Maintaining sterile instruments when performing repetitive rodent surgery is a challenge. Contamination can be reduced by segregating surgical instruments according to function. Surgical instruments used to incise the skin are placed at one end of the tray. Instruments used in subcutaneous tissues are placed next to the skin instruments. Instruments used within internal cavities are placed next to instruments used in subcutaneous tissues and so on. The tips of the instruments are placed toward the top of the tray. This arrangement places instruments used in deep body tissues "off to the side" and minimizes reaching over them to reach other instruments (Cunliffe-Beamer 1983; Cunliffe-Beamer 1990).

Contamination of instruments by aerobic bacterial skin contaminants in repetitive rodent surgery can be reduced by wiping tips of instruments with 70% alcohol and a sterile swab between rodents. Alternatively, a glass bead dry heat sterilizer could be used after the tips of instruments are wiped with sterile saline or water to remove blood or tissue residue. Use of a sterile instrument holder with pockets also reduces potential for contamination because tips of instruments can be tucked in the pocket and covered while the next rodent is prepared for surgery. Even with alcohol wipe between rodents and holder with pockets, a new sterile instrument pack should be used after 4 or 5 individual rodents.

A surgical drape is a sterile cover that is draped over all or part of the rodent. The drape protects against accidental contamination of surgical instruments by providing a sterile "buffer zone" and provides a sterile surface on which to lay exteriorized organs. Surgical drapes for rodents can be made from a variety of materials. Lightweight, clear plastic drapes manufactured for larger animals can be cut in small pieces and steam sterilized between two paper towels. This type of drape conforms to the rodent's body and makes it easy to observe respiration. Opaque disposable paper or cloth drapes make it difficult to monitor respiratory rate of small rodents. In some circumstances, a sterile non-woven surgical sponge can be used to "drape" a small rodent.

Preparation of the incision site is an important part of aseptic technique. If fur is not removed over the incision site and skin is not decontaminated, hair and associated skin bacteria can be carried into deeper tissues. Alternatives for removing fur from rodents include plucking, clipping, shaving, or in selected instances, depilatories. Plucking the fur from an anesthetized mouse or similar-size rodent has many advantages. It is fast and easy and does not leave a stubble. Hair follicles in adult mice are usually in the telogen (resting) phase, and the hair can be removed manually with minimal injury (Sundberg 1993). If fur is removed with clippers, pressing a piece of adhesive tape over the clipped area picks up loose hair that would otherwise migrate into the incision. Use of depilatories should be reserved for situations where complete removal of fur from a very large area of skin is required. If the depilatory remains in contact with the skin for too long, a chemical burn could result. After the fur is removed from the area where the incision will be made, the skin needs to be cleansed and disinfected. In large rodents, e.g., rats or guinea pigs, skin can be washed with soap, rinsed with water, and disinfected with 70% alcohol or a surgical iodine. In small rodents, three applications of 70% alcohol, or two applications of 70% alcohol and one application of surgical iodine are often used to disinfect rodent skin. Sterile gauze sponges or sterile cotton swabs, depending on the size of the rodent can be used to disinfect the skin. Begin at the incision site and work outward in circles of increasing diameter (Bennett, Brown et al. 1990).

It is difficult to generalize about rodent surgery because the "patient" can vary in body weight, from a 1.5 or 2.0 gram newborn mouse to a 500-700 gram rat or guinea pig. The magnitude of this difference on a percent-body-weight basis is equivalent to comparing a 2 or 3 kg cat and a 765 kg horse. Even among rodents, surgical instruments must be matched to the size of the patient. Surgical procedures in small rodents, e.g., young mice, require delicate instruments such as those
designed for micro or ophthalmic surgery in order to minimize surgical trauma. Several books contain detailed descriptions of rodent surgical procedures (Waynforth 1980; Cunliffe- Beamer 1983).

Water is not usually withheld from small rodents prior to surgery. The inability of mice and rats to vomit prevents regurgitation of stomach content. The nibbling nocturnal feeding behavior of most small rodents and rapid intestinal transit times combine to eliminate distended digestive tracts as a problem for most laboratory rodent surgery. Thus, withholding food is not common practice prior to many rodents surgical procedures, although guinea pigs are often fasted prior to surgery (Harkness and Wagner 1989).

Hypothermia from anesthesia, wetting a significant portion of the body during preparation for surgery, or cooling of exposed body cavities is a potential problem during any rodent surgery. Decontamination of the skin should be accomplished without soaking the body of the rodent. The degree of hypothermia is influenced by the type and duration of anesthesia (Gardner, Davis et al. 1992) and environmental factors. Heat transfer should be considered when selecting the surgical table. Stainless steel is easy to sanitize, but it conducts heat away from the body. A temperature-controlled small water ‘blanket’ should be placed under the rodent during prolonged surgical procedures. A cork board, a plastic tray, or a few paper towels can be placed under the rodent to minimize heat transfer during short procedures. Post-operative care should include an external heat source while the rodents recover from anesthesia. The heat source should be positioned so that the rodents can move away from it as they recover from anesthesia. An electric light (50- 75 W bulb) suspended over one end of the cage is a very simple heat source for rodents recovering from anesthesia.

In summary, when aseptic surgical technique is not practiced, infection can be expected. These infections are often subclinical in rodents; nevertheless, adverse physiological effects have been demonstrated. Preventing post-surgical infection by using aseptic technique improves the quality of life for the rodent and eliminates a source of uncontrolled variation in research data.

References:


Cunliffe- Beamer, T. (1972- 73). Pathological changes associated with ovarian transplantation. The
Aseptic Surgical Procedures: Surgery performed using procedures that limit microbial contamination (i.e.,
aesthetic technique) so that infection or suppuration does not occur. Sanitation: The establishment of
conditions favorable to good health, especially with respect to infectious diseases or to make physically
clean and to remove, to a practical minimum, agents injurious to health. Applying principles of aseptic