Production and Use of Plastinated Rat Models for Teaching/Learning Methods for Bleeding

Maria L. Streber 1, Teresa Davila 2, Gabriel Solano 3, Lilly Esquivel 1, and Robert Henry 4

1Experimental Research and Lab. Animal Unit, INCMNSZ, Mexico City, Mexico; 2ENCB, IPN, Mexico City, Mexico; 3Lab. Animal Facility, School of Psychology, UNAM, Mexico City, Mexico; 4Department Biomedical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, USA

Summary
Severe injury may occur when an inexperienced person attempts to draw blood from a rodent in an inappropriate manner. To help solve this problem, aids for teaching blood collection techniques from rodents were planned and made. These teaching aids for collection of venous blood from the retro-orbital, facial, and tail veins have been used successfully to train students for bleeding rats. Various preservation techniques were utilized: maceration and drying for osteology; plastination for wet biological specimens; and glycerin for rat puppets. The plastination process slowly replaces tissue fluids with a dehydrant (acetone). Then the acetone is replaced with a polymer under vacuum. The resultant plastinates are real biological specimens which are clean, dry, odorless, and durable. The puppets used the cutaneous structures (skin with head, limbs and tail) which were dehydrated and soaked in glycerin. All specimens were beneficial for blood collection training in rats.

Keywords: rat model, plastination, bleeding techniques

1 Introduction

Blood collection from rodents is necessary for many experimental or diagnostic procedures. Good training is important before collecting blood samples from the retro-orbital, facial, or lateral tail veins in rats or mice, which can provide moderate to large amounts of blood. Severe injury may occur to animals if the procedure is not done properly. These collection techniques require training accomplished on anesthetized animals (Van Herck et al., 1998). Plastination is a useful technique for preservation of biological specimens. Plastination methodology consists of slowly replacing tissue fluids with a dehydrant (acetone) and then the acetone is replaced with a polymer, under vacuum. The results are clean, dry, odorless, and durable real biological specimens (von Hagens et al., 1987; Steinke et al., 2008; de Jong and Henry, 2007). Rat puppets provide an alternative to live animal usage for the experience and sensation of holding the animal, for eye-hand coordination, and to decrease student anxiety when handling live animals. Production of these puppets uses glycerin application to skin, limbs, and tails that renders the tissue soft and pliable. These models incorporate the Three Rs for acquisition of knowledge and skills through reducing the number of animals used for demonstration and practicing techniques, replacement of live animals, and refinement of bleeding techniques.

Objective
The purpose of this project was to prepare and use teaching/learning models from skulls, plastinated heads, and glycerin prepared skin and body structures (puppets) from a group of rats for repetitive training of multiple students. This will help reduce the need for sacrificing additional animals for training purposes since these prepared specimens will be reused.

2 Materials and methods

Twenty healthy adult Wistar rats (10 males, 500 g and 10 females, 350 g), no longer used for research projects, were donated for this project by the UNAM animal facility. The rats were euthanized using CO2. Heads and/or the entire cutaneous structures were collected.

1. To prepare skulls, the skin from fresh samples was removed and frozen. The soft tissues were macerated by boiling in hot water with soap for 1 h, and then cooled to room temperature. Mechanical removal of soft tissues was done by hand. Each cleaned skull was bleached with 30% hydrogen peroxide (Fig. 1).

2. Other heads were left with the skin intact or the skin was removed to show the underlying anatomy. After removal of brains, these heads were fixed by immersion in 10% buffered formalin for one week. After fixation, the specimens
were flushed with running tap water for 24 h to remove excess formalin. The fixed specimens were dehydrated in three weekly changes of 100% acetone to remove excess water and adipose tissue. The dehydrated specimens were submerged in a silicone-catalyst mixture and impregnated by decreasing the vacuum one atmosphere for a week period at -15°C (von Hagens et al., 1987; de Jong and Henry, 2007) (Fig. 2).

3. From fresh samples, the entire cutaneous covering was removed and cleaned of subcutaneous adipose tissue, muscle, and brain, while keeping the head, limbs and tail intact. Each cleaned skin was filled with gauze sponges and cotton. The skins were sutured to hold the stuffing and to avoid shrinking. They were fixed by immersion in 10% buffered formalin for one week. After fixation, the specimens were flushed with running tap water for 24 h to remove excess formalin. To begin dehydration, the fixed specimens were placed on a grid over acetone inside a covered glass dessicator chamber. After two weekly changes of 100% acetone in the dessicator, the specimens were submerged in glycerine baths. The first bath was a mixture of 50% glycerine and 50% acetone and then two weekly baths of 100% glycerine. After the glycerine soaks, the skins were allowed to dry at room temperature with many changes of paper to absorb excess glycerin. For final natural appearance and support, rolled wires with cotton and gauze sponges were used to stuff the glycerinated skins (Fig. 3, 4).
3 Results

Rat heads and whole cutaneous preps were produced with and without soft tissues to provide models to demonstrate anatomy and to provide training models for simulation of blood collection from the retro-orbital plexus, as well as the facial and lateral tail.
veins (Fig. 5-11). Models were used by instructors and students to demonstrate anatomy and practice placement of blood collection tubes, lancets, and needles with syringes.

These real models also were helpful to the student in gaining confidence on how to hold the animal and determine where to insert the pipette. This new sensation was comforting to inexperienced students. These plastinates and glycerin specimen models also were useful for developing eye-hand coordination.

4 Discussion

In teaching/learning, a discussion of the pros and cons of various bleeding techniques is the most important issue. This will enable the student not only to understand techniques but, more importantly, to make educated decisions as to which site to draw blood from. Details like number of samples, the amount of blood to be taken, and post-procedural care, etc. also are very important (Diehl et al., 2001).

Regarding retro-orbital bleeding, discussion of potential severe injuries that may occur to the animal, causing pain and tissue damage to the eye, should address the following:

- Retro-orbital hemorrhage resulting in hematoma and excessive pressure on the eye;
- Corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia caused by proptosis of the globe;
- Damage to the optic nerve and other intra-orbital structures, which can lead to deficits in vision and even blindness;
- Fracture of the delicate bones of the orbit and neural damage by the micro-pipette;
- Penetration of the eye globe itself with a loss of vitreous humor;
- Necrotic dacryoadenitis of the Harderian gland.

Since rats have a plexus rather than the sinus found in mice, the retro-orbital bleeding procedure in rats may result in greater tissue damage than in mice. In fact, it is advocated that retro-orbital bleeding should only be done under anesthesia, because of the severity of adverse effects that may occur (Van Herck et al., 2001). The retro-orbital sinus bleeding technique is still being used for rat pharmacokinetic and toxicokinetic evaluations by some academic institutions, pharmaceutical companies, and contract research organizations (CROs), even though some argue that this is not a humane method and should no longer be recommended (Hui et al., 2007).

Regarding tail vein sampling, it is quick and simple to perform. However, this technique may require the rats to be warmed in order to dilate the blood vessel prior to taking the sample. We recommend the distal end of the rat’s tail to be used, since the structures of the musculoskeletal system gradually diminish towards the end of the tail, while the sizes of the blood vessels do not decrease in proportion, and the blood vessels become most prominent near the tail’s tip (Staszyk et al., 2003).

The facial/mandibular technique permits a good volume of blood to be collected in a rapid manner with fewer risks or complications, and it is easy to learn.

Prepared models helped to build confidence for the potential phlebotomist. Since the models are reusable, one could practice on them repeatedly and review the salient landmarks at leisure.

The plastination technique provided useful real anatomic models for sample collection practice. These plastinated specimens could be enhanced by filling the vessels prior to plastination with colored latex or RTV silicone, e.g., red for arteries and blue for veins. In addition, anatomic corrosion vascular casts, isolated or intact on the macerated skulls, would be a helpful resource for demonstration of vessels.

The rat puppets were a really valuable model, as they allowed the trainee to experience the sensation of holding the animal. It aided in developing dexterity and eye-hand coordination when the student was determining where to draw blood and finally insert the pipette. This led to a decrease in anxiety when the students first started to handle live animals.

5 Conclusions

Advantages of this model include reduction of the use of live animals. Initial use of anatomically real models may reduce student anxiety, as well as trauma induced to animals during subsequent learning attempts in live animals. Rat heads and puppets are an excellent model for education, skill development and refinement of bleeding technique. This kind of teaching material can improve the teaching/learning process.

References


1 http://www.nc3rs.org.uk/bloodsamplingmicrosite/page.asp?id=388


**Acknowledgement**

We thank INCMNSZ for the financial support.

**Correspondence to**

Maria L. Streber  
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán  
Experimental Research and Laboratory Animal Unit  
Vasco de Quiroga No. 15 esq. San Fernando  
Sección XVI, Tlalpan C.P. 14000, México D.F.  
Mexico  
Phone: +52 55 5487 0900  
e-mail: mstreberj@gmail.com