Removal of blood from laboratory mammals and birds

FIRST REPORT OF THE BVA/FRAME/RSPCA/UFAW JOINT WORKING GROUP ON REFINEMENT

Contents *Pages*

Preface

Whenever animals are used in laboratories, minimizing any pain and distress they suffer should be as important an objective as achieving the experimental results. The refinement of procedures to make them more humane should now be an integral part of all scientific research. This is important both from humanitarian concerns and in order to satisfy the requirements of the Animals (Scientific Procedures) Act 1986.

In recent years, more attention has been focused on the need to recognize and control adverse effects of scientific procedures on animals. Similarly, attention is being paid to the need to improve and enrich the environment in which laboratory animals spend their lives. There is thus a great deal of scope for improving current laboratory practice for the benefit of animal welfare. Such improvements can also improve the quality of scientific research, since suffering and distress in animals can result in physiological changes which are likely to add another variable to experimental results.

Significant improvements in laboratory techniques and animal husbandry can be made immediately in several ways. In order to do this, clear, unequivocal and up to date information on all aspects of laboratory animal care and use must be readily available. Concern over the need to provide such information led to the RSPCA, FRAME, UFAW and the BVA establishing a Joint Working Group in 1989. The aim of the group was to set up a series of workshops to discuss ways that common laboratory procedures could be refined to minimize any pain or distress caused to laboratory animals. The members of each working party were drawn from the scientific community, from industry, academia and from animal welfare organizations. An observer from the Home Office Inspectorate was represented on the Working Party.

Each workshop was intended to address a single topic, the proceedings being published in the scientific press. This report, entitled 'The Removal of Blood from Laboratory Mammals and Birds', is the first of the series. It aims to describe in detail the most humane methods for taking blood samples from the common

laboratory animal species. A second report on improving housing systems for rabbits is nearly completed.

Some of those involved in these workshops are opposed to the use of animals in experiments that may cause the animals pain, suffering or distress. However, they share with many in science the common aim of reducing animal suffering wherever it occurs. The reports of these refinement workshops are intended to help achieve that aim, particularly if they are read in conjunction with other recent reports on the recognition, measurement, and alleviation of pain or distress in animals.

It is hoped that this, and subsequent reports, will be widely circulated within establishments designated under the Animals (Scientific Procedures) Act 1986, and that the recommendations contained therein will be adopted as 'Best Laboratory Practice'.

1 AIM OF THE REPORT

This report is the first in a series on refinement of scientific procedures and laboratory animal husbandry. Its aim is to help those removing blood from animals to do so in the most humane and efficient way so that any pain, distress or discomfort for the animals is kept to a minimum. The report should also be useful in training courses, or as basic reading before applying for a personal or project licence under the UK Animals (Scientific Procedures) Act 1986. The emphasis throughout is on practical detail. It is hoped that publications on refinement of experimental procedures such as these will lead to 'best practice becoming common practice' in all laboratories.

2 INTRODUCTION

Blood is removed from animals for a variety of scientific purposes. Scientists should be aware that the process may well be unnecessarily stressful for an animal, simply because of the handling, the type of anaesthetic, or the

discomfort associated with a particular technique. The physiological changes associated with increased stress may even invalidate results (Ajika *et al.*, 1972; O'Neill & Kaufmann, 1990; Sarlis, 1991). Comparison of 'normal' blood obtained through chronic indwelling cannulae in unrestrained animals with blood obtained by more conventional methods has shown significant differences, for example in the levels of prolactin, cortisol, corticosterone and glucose, as well as in counts for red and white cells and platelets, and packed cell volume. Since stress may cause physiological reactions which may affect the research, the method of blood sampling used should be checked for any associated changes, e.g. in blood corticosteroid levels. It would then be possible to see if an animal adapted to a procedure and becomes less stressed as a result. **It is obviously in the interests of good science, as well as of animal welfare, that stress should be kept to a minimum.**

It is worth noting that when collecting blood for antibody production, it may be desirable to collect blood into an anticoagulant and process the plasma. The yield of serum from blood is relatively poor and the yield of antibody can be 20–50% higher from plasma than from serum.

The report is divided into sections which describe the removal of blood from veins (Sections 3 & 4), arteries (Section 5), and by cardiac puncture (Section 6). Where the route for blood removal may also be used for the administration of substances, or for measuring blood pressure, further details are given. Each section is subdivided to describe possible techniques and their advantages and disadvantages. The potential adverse effects of each are discussed together with recommendations on how these can be minimized. The report concludes with a guide to the severity banding of blood sampling techniques for project licence applications, together with recommendations for training of personal licensees.

3 VENOUS ACCESS

3.1 Introduction

The volume of blood removed from an animal will normally be determined by the scientific

protocol which, in turn, will depend on aspects such as the sensitivity of assays to be used subsequently. Section 3.2 deals with removal of small volumes of blood of less than 0·1 ml. Volumes over 0·1 ml are dealt with in Section 3.3. Methods of venepuncture of particular concern are described in Section 3.4.

It is important that scientific techniques are continually refined so that only small sample volumes are needed. However, there may still be occasions when the small size of the animal is critical because of the volume or frequency of blood samples needed (e.g. in mice). In such cases, the welfare of an individual animal should not be jeopardized and either more animals should be used, or some form of compensatory blood transfusion or replacement considered as appropriate. Frequent sampling increases the stress for the animal and, if this is necessary, the scientist should consider cannulation. Even in the short term, this technique is a favourable alternative to repeated venepuncture. It is addressed as a separate topic in Section 4.

3.2 Volumes of the order of 0·1 ml (see Table 1)

A superficial vein can be punctured to obtain blood for haematological or chemical estimations requiring only 50–200 μl (approximately equivalent to 1–4 drops). Anaesthesia is normally not necessary since the associated stress would probably be greater than the discomfort of a needle prick or of a puncture with a small sharp sterile object.

3.2.1 Equipment

A sterile needle or lancet should be used to puncture the skin and underlying blood vessel. A scalpel blade is not recommended as its use is imprecise, and may lead to accidental mutilation of the animal, or operator if the animal is not adequately restrained.

3.2.2 Site

The recommended sites for venepuncture in a variety of species are given in Table 1.

To familiarize oneself with the location of a vein, it is strongly recommended that the

Table 1. Sites for venepuncture and venesection in small mammals

	Ceph/sph	Alar	Ear	tt	Coc	Ovs	Jug	Fem	Card	Mamm
Cats	+ + +	−	−	−	−	−	+ + +	−	−	−
Cattle	+	−	+	−	+ +	−	+ + +	−	−	+ +
Chickens	−	+ + +	−	−	−	−	+	−	+	−
Dogs	+ + +	−	−	−	−	−	+ + +	+	−	−
Ferret	+ +	−	−	−	+	−	+ + +	−	+ +	−
Gerbil*	−	−	−	+ +	+ +	+ +	+	−	+ +	−
Goats	+	−	−	−	−	−	+ + +	−	−	+
Guineapig	−	−	+	−	−	−	+	−	+ + +	−
Hamster*	−	−	−	−	−	+	+	−	+ +	−
Horses	−	−	−	−	−	−	+ + +	−	−	−
Marmosets	+	−	−	−	+ + +	−	−	+ + +	−	−
Mouse	−	−	+	+ + +	+ +	+	−	±	−	−
Pigs†	−	−	+	−	+	−	(cvc) + + +	−	−	−
Rhesus	+ + +	−	−	−	−	−	+ +	+ + +	−	−
Rabbit	−	−	+ + +	−	−	−	+	−	±	−
Rat	−	−	−	+	+ + +	−	+ +	−	+	−
Sheep	+	−	−	−	−	−	+ + +	+	−	−

− Not recommended.
+ Possible alternative.
+ + Acceptable route.
+ + + Preferred route.
*No preferred route has been found for the gerbil or the hamster.
†The cranial vena cava and not the jugular is used in the pig.
Routes such as the penile and the sub-lingual veins are not acceptable because of side effects or preferable practical alternatives. Needle sizes should be in the order of 15–50 mm long and 14 to 26G depending on the diameter of the vein and the volume of blood required (see Section 3.2.1). An injection of local anaesthetic under the skin may reduce the discomfort during the insertion of the larger bore needles (14–18G).

Alar   = Wing or brachial vein.
Card   = Heart — anaesthesia/analgesia should be given.
Ceph   = Cephalic
Coc   = Coccygeal vein.
cvc   = Cranial vena cava.
Ear   = Ear vein.
Fem   = Femoral.
Jug   = Jugular vein.
Mamm = Mammary vein.
Ovs   = Orbital venous sinus — anaesthesia/analgesia should be given.
sph   = Saphenous vein indicated by an 's' after the rating of ± .
tt   = Amputation tail tip (artery and vein) — anaesthesia/analgesia should be given.

relevant anatomy first be studied on dead animals to avoid having to make repeated unsuccessful entries when trying to locate a blood vessel.

A common site for venepuncture in a small animal is the coccygeal or tail vein. In small rodents the tip of the tail may be removed and in mice — unlike rats — this seems not to involve the removal of any coccygeal vertebrae. Tail cutting should be carried out only once or a maximum of twice. (In naked mole rats, nicks can be made at the end of the tail which regrows in 4–6 weeks and can then be used again.)

For small, tail-less animals, such as guineapigs and hamsters, the ear or jugular veins may be used, but this requires considerable skill; in these mammals cardiac puncture under general anaesthesia may be the preferred method. In large animals it is more likely that a small sample will be taken directly from a superficial vein (see Section 3.3). In birds, puncture of the wattles, combs or snoods can be used.

The use of the footpad for obtaining blood is unacceptable because of the sensitivity of the area and the risk of infection, since laboratory animals are usually kept near, or on, bedding

contaminated with urine and faeces. Infection can result in lameness and unnecessary suffering.

3.2.3 Preparation of site

It is important to maintain antisepsis throughout sampling so hair and superficial skin debris over the vein should first be removed. The method for removal of hair will depend on the site of the vein and the species of animal. Hair can be removed by plucking, or with curved scissors or clippers. Plucking out the hair can be carried out easily in cats and rabbits without causing the animal distress. Chemical depilatory creams can be used in awkward areas, but generally they are not recommended since they can cause skin reactions and contaminate samples. [N.B. Some animals, such as cats, may be disturbed by the noise made by electric clippers and some animal skins, for example of rabbits, are thin and sensitive.] Shaving with a razor and soap and water is not recommended as it will cause more skin damage than clipping. Exceptions will depend on the experience of the operator, the ease of clipping and the effect it may have on the animals, and on the quality of the sample required.

The clipped or plucked area should be cleaned with warm water, possibly with the addition of a detergent or disinfectant such as cetrimide. These agents should be subsequently removed with plain water to avoid contaminating the sample. Alcohol (70% ethanol in water) will defat the skin effectively in those species in which there is marked sebaceous gland secretion (e.g. sheep), but might contaminate a blood sample if not left to evaporate. It is almost impossible to produce a 'sterile' surface, and excessive cleaning will disturb the natural defensive bacterial ecosystem of the skin. Preparation can then have the after effect of causing irritation, skin dehydration and discomfort to the animal.

In order to reduce any discomfort associated with venepuncture some workers have recently investigated the use of local anaesthetic creams applied to the skin some 30–60 min before taking the sample. These appear to be beneficial in reducing discomfort in species such as rabbits, dogs and cats but are not so effective in rats (Flecknell *et al.*, 1990).

3.2.4 Taking the sample

The animal should be gently restrained by an experienced handler who, whenever possible, should be known to the animals. **The key role played by the person holding the animal and raising the vein cannot be over-emphasized.**

The vein must be clearly located (if in doubt do not do it — seek assistance) and the puncture carried out positively rather than hesitantly. The animal may well show signs of discomfort (as we might!), for example, it may squeak, but it should be reassured by gentle handling and 'talking'. Some pressure applied proximal to the site to occlude the venous return may be required to obtain sufficient volume of blood. The globule of blood formed can then be removed by a capillary tube or by a micropipette and plastic tip. After the blood has been removed, gentle but firm continuous pressure on the site for 30 s or so will quickly stop any bleeding. There are also several haemostatic preparations of calcium alginate, collagen fibrils and gelatin sponge available which may be of help if bleeding persists.

3.2.5 Potential adverse effects

There are four main adverse effects: haemorrhage, bruising, thrombosis and stress caused by inappropriate handling. The appropriate treatment depends on the site, cause and individual animal. **Advice on treatment should be sought, for example, from the Named Veterinary Surgeon or an experienced licensee.**

Haemorrhage due to poor haemostasis is not a common problem, unless the animal has a clotting defect, and in some cases gentle continuous pressure applied for several minutes may be all that is needed to stop the bleeding.

'Bruising' is due to subcutaneous bleeding at the time of venepuncture or after the animal has been placed in its cage or pen, when the site might be aggravated by the animal itself through licking or rubbing. The animal should be checked after some 30 min and, if necessary, appropriate action taken (consult the Named Veterinary Surgeon).

Thrombosis (clotting) and phlebitis (inflammation of the vein) are usually caused by dirty technique or leaking of an irritant substance (e.g. alcohol-based chemicals) around the vein. Occasionally they may result from self-mutilation.

The correct level of restraint is that which allows a satisfactory sample to be taken at the

first attempt but which does not cause the animal to become unnecessarily distressed. If this occurs other animals in the vicinity may become alarmed which will lead to both scientific (e.g. raised blood glucose, blood pressure) and welfare complications (see Section 3.2.6).

3.2.6 Scientific problems

These include sample contamination with skin bacteria, secretions and debris (through inadequate site preparation) or by subcutaneous tissue components. The significance of these contaminants depends on the purpose of blood collection. Tissue fluid contamination will be exacerbated by squeezing tissue in order to obtain a spot of blood, as well as by multiple needle stabs, so both these actions should be avoided. If persistent bleeding is encountered in an individual or a colony then the presence of a possible clotting defect should be considered (e.g. deficiency of Factor VII in beagles and vitamin K in rodents).

Excessive force used in restraint will stress the animal and raise catecholamine and glucocorticoid levels in the blood. This in turn will alter blood parameters such as packed cell volume and blood glucose level.

3.3   Volumes greater than 0·1 ml (see Table 1 and Section 6)

The volume of blood removed and the frequency of sampling will be based upon the purpose of the scientific procedure and the total blood volume of the animal (see Section 7 for maximum volumes). Serious consideration must be given to the combined effect of sample size and frequency of blood removal. Multiple sampling can be carried out by repeated needle punctures but better alternatives exist such as using a butterfly needle or a percutaneous (over the needle) cannula taped in position (see Section 4.1).

3.3.1 Equipment and restraint

Large animals are commonly physically restrained for venepuncture without anaesthesia. Small animals are frequently given a short-acting anaesthetic for ease of handling. However, it must be remembered that such agents may affect

haematological and biochemical parameters (see Section 8). Some animals, such as dogs and some primates, may be trained to present a limb for sampling without recourse to anaesthesia or physical restraint. Firm, empathetic handling is very important, as is the time taken to withdraw the sample. Both these parameters can affect the stress on the animal and consequently the quality of the sample and accuracy of the research. Consideration should be given to offering rewards after each bleeding, depending on the species. (Methods of restraining animals for carrying out minor procedures will be the topic of another refinement workshop in this series.)

Size of needle   The size of needle — the length and the bore is very important. A needle that is too long is awkward to manipulate and may result in blood clotting in the needle and laceration of the vein. A large bore on the other hand, whilst minimizing clotting (and also blood haemolysis), tends to damage the vein more and may predispose to formation of haematomata. Interestingly, larger bore needles (20G) have been found not to affect mice and rats any more than small bore needles (25G), but this could be due to the shorter handling time and faster blood removal associated with the larger needles (see Barclay *et al.*, 1988).

Our recommendation is to use as large a bore as possible to ensure rapid blood withdrawal without collapsing the vein, within the constraint of avoiding haematomata, i.e. the bore should be just less than the diameter of the vein.
It is possible to remove blood from most species with a range of needles between 10–50 mm in length and between 17–27G bore. Thus, removal of blood from the jugular vein of large mammals, e.g. farm animals, dogs, cats and some primates, could be carried out with a range of needle sizes between 14–20G × 25–40 mm. For removal of blood from the tail vein of a rat or ear vein in a rabbit a needle in the range of 23–26G × 10–20 mm may be the most appropriate. When sampling repeatedly a new needle should be used each time. The direction of the needle is usually with the flow of blood, but this may vary depending on the accessibility of the site.

3.3.2 Site and localizing a vein

The sites for venepuncture in a variety of species are given in Table 1.

It is important to locate the vein accurately before taking a sample. Obstruction of the venous return — normally above the site of puncture when it is caudal (tail-end) to the heart, and below the site of puncture when it is cranial (head end) — may be required in order to distend the vein and to prevent excessive movement of the vein. This makes location and introduction of a needle (or cannula) much easier.

Percussion may help to determine the course of a vein. The vein is first obstructed with the fingers of one hand and the vessel located by tapping with the fingers of the other hand. The fingers blocking the vein will detect the percussions and an imaginary line between the two will delineate the course of the vein. (This is particularly useful for the jugular vein.)

It is important that time be spent making an accurate location and good dilation of the vein *before* **puncturing the vessel.** Observing experienced operators will help in learning this technique. In some animals it is often quite easy to see the vein, particularly if the skin is not pigmented or the vein is large. Clipping or flattening the fur with a wetting agent (70% alcohol, or water with a detergent) may also help.

Another important point to consider is that the thickness of skin varies widely between species, as well as between sites on the same animal. (Dorsal and lateral skin surfaces tend to be thicker than ventral or medial aspects.) Furthermore, repeated sampling may lead to fibrosis of the vein and thickening of the skin.

Dilation of the vein   **Dilation of the vein (e.g. by gentle obstruction or by warming) will facilitate blood sampling.**

In anaesthetized animals, vasodilation may occur as a result of the anaesthetic. Anaesthetized animals may not, therefore, need warming and consideration should be given to bleeding under anaesthesia. In conscious animals, however, blood can be more easily obtained if the animal is warmed first. Animals can be placed in a *thermostatically* warmed box (e.g. made from perspex) at 30 °C for 10–15 min. It is essential that they should be kept under constant observation in order to prevent hyperthermia (indicated by breathing more rapidly, panting or salivating). The box size will depend on the species and the numbers of animals. If a light bulb is used as a heat source, care must be taken to avoid burning the ears, or blinding the animals. Rats and mice use their tails to help in thermoregulation and another common practice is therefore to dip the tails of these rodents into warm water (*measured* temperature of around 45 °C should be adequate) before bleeding. Although this is not nearly as effective as general body warming in older rats it may have the effect of softening the tail skin making needle passage easier and thus increasing accuracy.

The use of xylene (xylol, dimethylbenzene) to dilate the vein is not recommended as it causes skin rashes and is easily misused. The same effect can be obtained by warming the animal as described above. The effect of xylene is that of an irritant, causing the blood vessels to dilate. If it is used regularly and not adequately removed, tissue damage including thickening and even sloughing may result (e.g. of part of the ear in rabbits).

It is worth noting that some chemical agents used to sedate or anaesthetize an animal (for example, fentanyl/fluanisone ('Hypnorm'), acetyl promazine in rabbits) may cause peripheral vasodilation as well as having an effect on haematological and biochemical parameters (see Section 8).

3.3.3 Preparation of the site
See Section 3.2.3.

3.3.4 Taking the sample
Having located and tracked the course of the vein, and dilated and immobilized it, the next stage is to pierce the skin with a needle — sometimes with a syringe attached. Aim to pierce the skin and vein in one movement by directing the tip of the needle (bevel up) a little way up the vein so that the angle of penetration is almost parallel to the vein. (The angle of penetration should not be steep since the steeper the angle of needle entry the more likely it is to pass through the vein.) In small or nervous animals it may be preferable to put the needle in first and then to attach the syringe. It may be even better to use a butterfly needle with a flexible tube attached.

It is worth remembering that **a vein will collapse if the sample is taken too quickly, and so care should be taken to ensure that it is taken at an appropriate rate.** Gentle manipulation (e.g. bending, light pressure) may increase blood flow but if too vigorous will affect the quality of blood collected.

Vacutainers[1] may be used and this is common for the larger species. The correct volumes, sizes and venous flow rates must be considered. Haemolysis of the sample may prove to be a problem (for example, coloured serum or plasma resulting from haemolysis of red blood cells may interfere in chromatographic analyses such as ELISAs). However, this depends on the species, in terms of the size and fragility of red blood cells and on the strength of the vacuum applied. Haemolysis can be minimized by allowing blood to flow out of a needle rather than applying negative pressure.

Withdrawal of the needle   **After the needle (or cannula) has been withdrawn, continuous pressure should be applied immediately to the puncture site for at least 30–60 s.** The site should then be observed for a further 30 s to ensure that bleeding does not recur. The animal should be returned to its cage and checked again after 10–15 min. If there is any chance that bleeding may restart, the animal must be isolated at once so that it can be closely monitored. This will also avoid cannibalistic damage from other animals. Care must always be taken in handling the animals after blood withdrawal, since bad handling can stimulate bleeding due to physical trauma and/or raised blood pressure. The use of thermocautery and artificial skin sealants for haemostasis is not recommended and caustic pencils and other astringents should not be used because they cause discomfort. **If the bleeding cannot be arrested by haemostatic preparations a veterinary surgeon should be called.**

3.3.5   Potential adverse effects
Some of the problems that can arise after venepuncture have been mentioned above (3.2.5), i.e. haemorrhage, bruising, thrombosis and stress from inappropriate handling. Other problems

[1]Blood collection into evacuated vials attached to a special needle adaptor.

include infection of the site of needle entry which may then spread systematically. Repeated bleeding can lead to phlebitis and scarring which can also occur as a result of repeated attempts at venepuncture. The likelihood of this occurring can be reduced by improving technique (using indwelling cannulae) and by **rotating sample sites.** One recommendation is to begin sampling at one end of a length of vein, normally starting at the end furthest away from the heart. Haematomas which occur will gradually be resorbed but they provide, in the meantime, a temporary focus for infection and may cause inappetance and a rise in body temperature.

It is also possible to damage nerves which accompany a vein when a needle is misdirected, e.g. when sampling from the femoral and jugular veins. Venous occlusion can result from thrombophlebitis and this can occur after a substance has been administered perivascularly instead of intravenously. More rarely, embolisms due to dislodging a thrombus in a needle, or as a result of an accidental injection of a small amount of air can occur. Normally, these seem to have little effect on the animal, although such emboli have the potential to cause death.

3.3.6   Scientific problems
In some species blood can clot very quickly when withdrawn into a syringe. This problem can be overcome by drawing the blood straight into an anticoagulant solution such as heparin or citrate. The syringe should be loaded with the required volume of anticoagulant for a given final blood volume. Heparin should be used at a final concentration of 5–25 IU/ml. Citrate is usually used at a concentration of one part 3·8% sodium citrate plus three parts of blood, but be aware of the dilution effect. (N.B. Blood tends to clot more quickly in glass than plastic syringes.)

Snipping off the tip of the tail of rats and mice and 'milking' it can result in haemolysis of the blood sample and contamination with other tissue fluids. Other causes of haemolysis are collection through too fine a needle, application of too high a vacuum, and too vigorous mixing of sample with anticoagulant in a tube or syringe.

As described for the collection of small amounts of blood, excessive restraint or any other form of stress to the animal, will lead

to the release of various hormones with the side effects described in Section 3.2.6.

3.4 Methods of venepuncture not recommended and requiring special justification

Many traditional methods of blood sampling cannot be endorsed today because of their crudity and because there are now methods that cause less pain to the animal and give a more acceptable blood sample. It is now possible to obtain needles and cannulae of the right size for nearly all species such that it is unnecessary to have to resort to lacerating a vein along its course. Methods which involve cutting ear veins, repeatedly nicking tails (or the tail vein of any animal) and clipping claws below the quick must therefore be avoided. In rats, repeated cuts of the tail can lead to granulomas, resulting in a large mass of tissue at the end of the tail. If the samples are taken regularly at short intervals (e.g. once a week) the granuloma does not have time to develop. Repeated amputations of the tail in any species, but particularly rats, may remove the animal's natural ability to control its body temperature and balance and cause granulomata. This practice is, therefore, unacceptable as a method of repeated sampling. However, there are occasions when, for a single sample, snipping the end of the tail may still be the least invasive method.

Cutting across the metatarsal vein over the hock joint (this vein is often too awkward, too mobile and too narrow to use a needle) is not recommended because of problems with arresting the bleeding, damage to associated structures, and the high chance of infection—as with the footpad.

The use of the penile or pubic vein or the lingual vein is not recommended as this carries considerable potential for adverse effects, for example, if thrombosis occurs it can lead to a temporary blockage of the urethra or swelling of the tongue and will undoubtedly cause extreme discomfort and probably substantial pain.

3.4.1 Bleeding from the orbital venous sinus
This technique involves puncturing the venous sinus behind the globe of the eye and is variously known as retro-orbital, peri-orbital, posterior-orbital, and orbital venous plexus bleeding. In experienced hands, orbital venous sinus bleeding can be a useful method of obtaining good samples from tail-less animals such as hamsters, or from mice where volumes greater than those which can readily be collected from the tail vein are required. However, it is a technique that can have severe consequences for the animal and, therefore, *we do not recommend retro-orbital bleeding for use with recovery* other than in exceptional circumstances when there is no other method available. It must always be carried out under anaesthesia and only one orbit should be used. Because the technique carries with it considerable potential for inadvertent damage and consequential adverse effects, it should only be carried out by competent persons. **This technique is only acceptable as a terminal procedure under anaesthesia**. It should also be acknowledged that some people find this procedure distasteful and therefore should not be asked to perform it.

4 VENOUS CANNULAE AND CANNULATION (see Table 2)

4.1 Introduction

Cannulation is an important technique for removal of blood because it reduces the stress of multiple sampling associated with, for example, restraint and the discomfort of repeated needle pricks.

Cannulae can be implanted (cannulation) and used in place of multiple needle entries at any one site, or indeed in place of single sampling from several sites within a relatively short time period. If sampling over a few hours is required a temporary cannula such as a butterfly needle or a plastic cannula held in place with tape or some form of bandage could be used. Long-term cannulation is very suitable for repeated short-term multiple sampling.

There are a number of potential problems with the technique which must be addressed. Whatever the method, surgical skills are essential

Table 2. Common sites for the positioning of long term venous cannulae

	Ear	Fem	Coc	Cph	Jug	Mamm
Cats	−	+	−	+	+ + +	−
Cattle	−	−	−	−	+ + +	+ +
Chickens	−	−	−	−	+ + +	− (Alar + +)
Dogs	−	+	−	+	+ + +	−
Ferret	−	+	−	−	+ + +	−
Gerbil	−	+	+	−	+ + +	−
Goats	−	+	−	−	+ + +	+ +
Guineapig	−	−	−	−	+	−
Hamster	−	+	−	−	+ +	−
Horses	−	−	−	−	+ + +	−
Marmosets	−	+ +	+	−	+ +	−
Mouse	−	±		−	±	−
Pigs	+	+	−	−	+ + +	−
Rhesus	−	+ +	−	−	+ +	−
Rabbit	−	+	−	−	+ + +	−
Rat	−	+ + +	+	−	+ +	−
Sheep	−	+	−	−	+ + +	+

− Not recommended.
+ Possible alternative.
+ + Acceptable route.
+ + + Preferred route.
Alar   = Wing vein on inner aspect.
Ear   = Ear vein.
Fem   = Femoral vein.
Coc = Coccygeal vein.
Cph = Cephalic vein.
Jug   = Jugular vein often into the right atrium.
Mamm = Mammary vein.

to position and fix the cannula (see Gellai & Valtin, 1979; Desjardins, 1986; Dennis *et al.*, 1986; Van Dongen *et al.*, 1990). In both short- and long-term cannulation it may be necessary to restrain the animal in some way to stop it removing the cannula, but this depends very much on the species. It is not always possible to leave animals with complete freedom as they may bite at the cannula. Many of the larger species seem to adapt well to long- and short-term cannulation, particularly after a period of training (acclimatization) to the restraint and some can be housed in groups with appropriate bandaging and protection for the cannula. Smaller mammals, such as rats, on the other hand are often restrained by some form of harness, swivel and tether along which the cannula runs. (This arrangement is sometimes referred to as an umbilicus — see Section 4.9.) But even small mammals should be conditioned (acclimatized) to the harness before cannulation, and even then stress may still occur. Cannulae can be maintained in dogs, pigs, rats and rabbits without the use of harnesses or jackets.

When dealing with social animals, every effort should be made to keep them in groups, rather than singly housed. Pigs, cats and marmosets have been group housed successfully with cannulae in place. Primates generally tend to play with harnesses and destroy them and both primates and dogs are inclined to chew at the apparatus on others in the same pen. They may, therefore, have to be kept singly. An alternative which permits separation but preserves social contact is to have mesh rather than solid dividers between cages or pens although animals can sometimes catch their cannulae in the netting if they are not well protected.

The researcher has to carefully consider the balance between the potential adverse effects and the benefits of either multiple blood sampling or cannulation. The technique chosen will doubtless depend on the expertise available, and the frequency and volume of samples required.

The methods described in the following sections have the potential of causing a great deal of discomfort to the animal and they therefore warrant pre-operative administration of analgesics and careful post-operative care and monitoring for the duration of the cannulation period.

4.2 Short-term cannulations (normally less than a day)

This has been successful in most species except mice.

Using aseptic precautions, a butterfly needle[2] can be inserted into a vein and left *in situ*. A similar device but with the needle replaced by **a plastic cannula is however, preferable.** This has no metal tip to damage the wall of the vein and so movements of the animal's limbs or neck do not lead to the sharp point of a needle rubbing against the side of the vessel wall. Thus, endothelial lacerations, perforations and thrombi are less likely to occur. These flexible cannulae are inserted over a needle into the vein and then the needle, trocar or guidewire is removed, and the flexible cannula taped or sutured in place.

Heparinized saline (e.g. 30 IU/ml) or some other anticoagulant should be left in the cannula or needle between sampling. A multiple entry port on the end of an indwelling cannula is extremely useful as it tends to keep the anticoagulant in place and makes access for sampling very easy with minimum animal restraint. Huber point needles prolong the life of these ports as they tend not to destroy the silastic septum.

Longer cannular patency may be achieved in rats through cannulation of the femoral artery for the removal of blood, rather than using the jugular vein or carotid artery. However, even when using the femoral artery the disadvantage remains that dislodged thrombi can cause lameness of one or both hind limbs.

4.3 Long-term cannulation (i.e. 2 days or more)

One of the major problems with long-term cannulation is that blockages due to thrombi are

more likely to occur. Surgical skills are essential to position and fix the cannula and this must be carried out aseptically in order to achieve maximum performance. **If the animal is young, a sufficient length of cannula should be left to allow for growth.** (This is especially important in pigs — at least 24 cm may be needed for animals between 15 and 150 kg.) **Loops of flexible plastic or rubber material left in strategic positions to allow for movement can be important in preserving cannula patency.**

4.4 Performance of cannulae

In small animals cannulae can reasonably be expected to remain patent for at least 2 to 3 days and some workers achieve patency for 3 to 4 weeks or longer. In larger animals it is far easier to maintain cannula function for periods of several months — even years. Continuous infusion, for example, by minipumps held in a jacket and a fluid-filled swivel, helps to keep a cannula open but this can only be implemented with some form of tethered restraint. However, in general it is better to avoid long periods of restraint and with any system animals should go through a training period before the experiment in order to adapt to wearing a jacket, or even to being restrained without being unduly stressed. Animals can then be selected for the experiment on the basis of jacket or restraint tolerance.

It is also necessary, when considering cannulation, to differentiate between infusion and the administration of substances and the removal of blood. It is easier to administer compounds than to remove blood long term, as thrombi attached to the end of a cannula can act as a one-way valve — permitting infusion but restricting withdrawal.

4.5 Equipment and material

Polypropylene, polyethylene, nylon and rubber cannulae are commonly used but silicone rubber (or silastic) and Tygon™ cannulae appear to be the most biocompatible and can be obtained in a sufficient range of sizes appropriate to all species. Non-silastic materials tend to cause fibrotic reactions with time, whereas silastic

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[2]A needle attached to a length of polythene tube with two flattened plastic wings to provide a surface to tape it to the skin.

cannulae seem to cause little reaction, even after 18 months. Vinyl tubing is no longer commonly used.

The problem with silastic is that it is too flexible and, therefore, predisposed to kinking, especially with the smaller sizes. It is also easy to obstruct the lumen by overtightening anchoring ligatures and care should be taken to check patency at the time of operation. Large sizes of silastic cannulae in which the ratio of the outer to the internal diameter is greater than 2 : 1 usually cause no problems, but internal diameters of 1–2 mm can be difficult to use. In general, the larger the internal diameter of the cannula the more successful it is likely to be and the easier it will be to unblock it, if necessary. To avoid the problem of a small cannula kinking, a polypropylene cannula inside a larger silastic cannula can be used, or a polypropylene cannula can be coated with silastic paint. This will have the effect of combining the greater rigidity or polypropylene with the biocompatibility of silastic. This type of cannula is less easily kinked, easier to flush and will also give truer pressure measurements than silastic alone.

4.6 Placement of cannulae

Two main mechanisms exist for securing a cannula. First, by placing it in the lumen of the vein and tying it in place to the vein itself. Second, the cannula can be inserted into a tributary of the target vein with the tip in the lumen of the major vein. The former method involves tying off a vein and destroying its patency but usually this has little effect on the animal. With a jugular cannula the tip may be left very close to the right atrium in the cranial (anterior/superior) or caudal (posterior/inferior) vena cava. Care should be taken not to place it within the right atrium since cardiac arrhythmias may be induced and lead to death by atrial fibrillation.

When cannulae have to be advanced significant distances contrast radiography or fluoroscopy techniques should be used to ensure the final location of the cannula is correct. If radiographic techniques are not available, **initial measurements have to be very carefully taken to ensure the correct length of cannula is inserted**. When any

vein (i.e. the distal part of the main vein or a minor tributary) is tied off, it will thrombose, and so the cannula tip must be placed well away from the site of anchorage. The cannula may be placed with or against the blood flow, but it seems preferable to locate it with the flow.

Trials should be conducted at the time of placing the cannula to ensure that blood can be removed in the position the animal will be in when it regains consciousness or when it is on experiment.

4.7 'Sealing' the cannula

The cannula can end in a multiple entry point, for example, a silicone rubber stopper that can be attached to the end of the cannula and which is capable of being pierced several times. This has the advantage that it is self-sealing and will better protect against microbial contamination of the fluid column within the cannula. Such multiple entry ports are often found on a saline drip bag or the plunger seal from a 1 ml disposal syringe and can be inserted into a Luer mount of a needle which in turn is inserted into the end of the cannula. Alternatively, the mount can be filled with silastic glue. Care should always be taken when using a needle to withdraw blood, to penetrate only the silastic bung otherwise the needle may pierce the side of the cannula. It may be safer to use Huber needles (with side exits).

The end of the cannula may also be flushed, clamped and then plugged with a spigot of solid plastic or metal. Providing this is done aseptically, it is perfectly satisfactory. The disadvantage of this method is that blood may re-enter the lower end of the cannula before sealing has been affected. The likelihood of this happening can be minimized by clamping the cannula. Another alternative is to heat-seal the end of the tubing, making sure that a little saline runs in before sealing.

A multiple entry point can be left under the skin or allowed to protrude like a 'button'. A spigotted cannula can be sited under a simple harness, but if left protruding too far from the skin it is liable to catch or be chewed by an animal.

Care should always be taken not to introduce air bubbles into the circulation at the time of

flushing, although with small volumes few adverse effects are likely.

4.8   Channelling the cannula to an exit site

The cannula can be taken under the skin to an exit site, usually on the back of the neck or between the shoulder-blades. Other exit sites are possible but those over the back tend to be safer from interference by the animal. Sometimes a jugular cannula may be passed directly between the muscles to the back of the neck rather than channelled subcutaneously. Whichever route is chosen, care should be taken to ensure that the cannula does not kink when the animal moves. Care must also be taken in selecting the correct length of cannula. If it is too short the cannula may pull out, whereas too great a surplus of tubing may lead to flexion or kinking.

There are several methods of fixing the cannula to the skin. The cannula can simply exit through the skin and be taped or bandaged in some way around the neck of the animal, e.g. with elastoplast. Alternatively, it can be left subcutaneously with a multiple entry point, or brought to the surface and mounted in some form of solid securing block. A specially engineered plastic or metal mount — a skin button — can be used for this purpose.

Cannulae may also be buried over the neck by way of superficial skin sutures holding the cannula in a channel between 2 folds of skin. It is important to remember that sutures or tethering buttons may cause discomfort. This has been overcome in rats by using small half dumb-bells on the end of the cannula which can avoid the necessity for suturing (see Gellai & Valtin, 1979).

The smaller the exit site, the less irritation is likely to be caused to the animal. However, anything that breaks through the integrity of the skin is a potential source of infection and so the site should be carefully and regularly checked and, if necessary, treated with a mild antiseptic (e.g. Hibitane, Dermisol, wound dressing powder) or the animal should be given a course of antibiotics under the direction of a veterinary surgeon.

4.9   Tethering

Tethering apparatus consists of a flexible spring attached to a swivel at one end and to the animal at the other by means of a harness. The apparatus may be necessary to prevent the animal (or its cage mates) interfering with cannulae. Tethering, however, undoubtedly restricts normal movements of animals, such as rolling over and lying on their backs, and tethered animals are often housed singly, thus adding to the stress and severity of the procedure (Brodie *et al.*, 1966).

4.10   Taking a sample and keeping the cannula patent

When collecting blood from a cannula, the anticoagulant mixture (see below) in the cannula should first be removed by drawing into a syringe until fresh blood appears. The blood sample can then be taken. **The 'dead space' in the cannula is then replaced by a carefully calculated amount of fresh anticoagulant.** This will help prevent thrombosis.

Appropriate anticoagulants include heparin saline (10–1000 IU/ml) or sodium citrate (0·05% w/v), or either of these in a dense solution such as 25–50% glucose, 5–40% polyvinyl-pyrrolidone. Other dense solutions include plasma expanders such as 'Haemaccel' and mixtures with glycerol. Antibiotics may also be included (see Section 4.12.3).

To maintain cannula patency, the cannula should be flushed with saline at least twice a week, if not daily. There is some dispute over the frequency of this operation as some workers maintain repeated flushing should be avoided.

Strict aseptic precautions should be followed to prevent infection.

4.11   Removal of cannula at the end of the experiment

Cannulae should be removed when no longer required to avoid continuing discomfort to the animal and infection and thrombus formation. If the animal is to be used again after

cannulation, or it has to be re-cannulated, the old cannula should be removed and the vein tied off.

4.12   Potential adverse effects

4.12.1   Blockages

The major problem in cannulation is not the placement of the cannula, but its subsequent blockage. Whilst biocompatible compounds such as silicone rubber have improved the situation, thrombi can still form at the end of a cannula either completely blocking it, or forming a valve such that infusion is possible but not blood withdrawal. Filling the cannula dead space with anticoagulants and flushing will help prevent such thrombi forming.

Blockage of the cannula can also occur if the tip of the cannula rests against the wall of the vein. Applying a vacuum will then cause the wall to be sucked in blocking the cannula. Patency may be restored by repositioning the animal or by repositioning the tip of the cannula. It follows that at the time of placing the cannula it is necessary to ensure that blood can be removed in the position the animal will be in when conscious or when on experiment.

4.12.2   Unblocking a cannula

Dislodging a thrombus can be extremely difficult and it is best to avoid blockage in the first place with good surgical skills and aseptic protocols. If blockage does occur, a gentle suction should first be applied (if substantial pressure is used the walls of the silastic cannula collapse). Unfortunately, this is rarely successful and attempts to displace the thrombus by forcing fluid through the cannula may have to be made. This is more often successful and the resultant embolus rarely seems to cause a problem to the animal. If it does, the animal may have to be killed. If the thrombus is very firmly fixed then forced flushing with fluids simply distends the silastic tubing, and may even 'blow it off' a connection. With larger cannulae it may be possible to pass a second smaller cannula (e.g. a Fogerty catheter) or a small diameter wire through the original tubing to physically displace the clot. Other measures include flushing with streptokinase, but care should be taken to ensure the animal is not allergic to streptokinase or

plasmin. Urokinase, or a similar thrombus dissolving enzyme can also be used. [N.B. Streptokinase is *active* in primates, dogs, cats and rabbits, and *inactive* in rodents, sheep, cattle, horses and birds.]

4.12.3   Infections

Cannula infection can be avoided through the use of sterile equipment and solutions, and by employing aseptic technique.

Should infection occur this may lead to thrombus formation manifest by raised body temperatures, sometimes accompanied by inappetence and lethargy. Adding antibiotics to the anticoagulant solution may help to limit infections. A broad spectrum water soluble antibiotic such as ampicillin, or a potentiated sulphonamide can be used (consult your Named Veterinary Surgeon). However, if there is a persistent infection, it is wise to abandon the procedure, or at least remove the cannula and allow the animal to recover fully before inserting another.

4.12.4   Accidental removal

Another major problem in cannulation is the accidental removal of a cannula if the animal catches it on the cage or pen, or if it is deliberately removed by the animal or its cage mates. The problem of the end of the cannula catching is avoided by good harnessing. Removal of a cannula by cage mates is avoided by isolating the animal, although this, in turn, may stress the animal and lead to spurious results (see Section 4.1). **If an animal persistently scratches at its cannula, consult the Named Veterinary Surgeon.**

4.12.5   Gastric ulcers

There is some evidence from studies in rats that gastric ulcers can result from long-term restraint and tethering (see Brodie *et al.*, 1966).

5   BLOOD COLLECTION FROM ARTERIES

5.1   Introduction

The main reason for collecting blood from arteries is that large samples can be obtained

rapidly and relatively easily. Usually the carotid artery is used. Other examples of this technique include bleeding from the central ear artery in rabbits, and occasionally the femoral artery in small mammals, e.g. rats, marmosets. The vessel does not have to be occluded, but if the animal is stressed vasoconstriction will occur and it will prove difficult to bleed.

Sections 5.2 to 5.4 deal with needle puncture, cannulation and carotid loops. Many of the caveats and practices described for venepuncture also apply to removing blood from arteries.

5.2 Needle puncture (particularly in rabbits)

When bleeding rabbits, it is best to restrain them in a towel to stop any inadvertent movement which could damage the artery on puncture. It may be appropriate to give a sedative or general anaesthetic. The area over the artery is depilated, treated with a local anaesthetic cream (see Flecknell, 1990) and then cleaned with a suitable agent before the vessel is punctured. A 20G × 2·5 cm needle should be used and the blood collected into an attached appropriately sized syringe (the arterial pressure will usually force the syringe plunger back). **After the procedure a sterile swab should be held tightly on the artery for at least 2 min (5 min may be necessary) before release.** Failure to prevent continued bleeding will result in a large haematoma over the artery which can result in permanent damage to the ear.

For other animals, the procedure is essentially the same except that the appropriate needle size for the species will have to be selected.

5.3 Cannulation

The two main differences between venous and arterial cannulae are in their placement and the consequences of dislodging a thrombus. Arterial cannulation may be a more reliable technique for the removal of blood over a prolonged period than using a vein.

The high blood pressure has to be taken into account when introducing an arterial cannula since any unsecured hole in an artery will bleed

profusely. Small non-traumatic haemostats or vascular clamps (bulldogs) should be used proximally (nearest the heart) whilst introducing the cannula to occlude the flow, or the vessel should be rested on artery or tissue forceps. Where possible the cannula should be placed with the flow of blood.

A dislodged thrombus in the arterial circulation may subsequently block the circulation to an organ by lodging in the artery that supplies it. Examples include blockage of the renal artery — particularly the right — or lumbar or femoral arteries. Thrombi have even been known to lodge in the ovarian or testicular arteries. Blockage of the mesenteric artery can lead to blood in the faeces. Femoral thrombi cause temporary lameness for a few days until a collateral circulation has been established or the thrombus resorbed. Femoral artery thrombi are very painful, causing raised body temperature. In such cases the administration of analgesics is strongly advocated or the experiment should be terminated.

5.4 Carotid loops

This is a well-established technique for gaining access to the arterial circulation but is only useful in the larger mammals such as dogs, sheep and cattle. The procedure is technically difficult (Davey & Reinert, 1965) and should be learned under direct supervision with an experienced licensee.

6 CARDIAC PUNCTURE

Cardiac puncture should always be carried out under general anaesthesia in all laboratory animals.

Normally the left ventricle is punctured with the animal laid on its right-hand side. (Alternatively the right ventricle can be penetrated and the animal laid on its left side for venous sampling.) Another equally acceptable method is to lay the animal on its back and introduce a long needle just underneath the sternum into the heart. It is common to bleed out animals terminally using this method, but it is also useful for obtaining *single* blood samples from guineapigs

and hamsters and occasionally ferrets. (N.B. In rats, where recovery from the procedure was intended, death rates of up to 12% have been recorded after heart puncture (Stuhlman *et al.*, 1972) and it is not without danger in rabbits.) Where the procedure is intended as terminal, death after exsanguination should be ensured by the administration of an overdose of an anaesthetic or by incising the heart.

Repeated cardiac puncture will have to be carefully justified in a project licence because of the potential for adverse effects and is not recommended for any species — long-term cannulation is preferable.

6.1 Equipment

Needles for cardiac puncture should be sufficiently long to penetrate the ventricle. For a single bleed (with subsequent recovery) in small animals, a 25 mm × 21–23G needle will suffice. For other animals the size of the needle will have to be increased in relation to the size of the animal. Cardiac puncture for exsanguination in larger animals such as rabbits is probably best carried out with a 25–50 mm × 18G needle attached to a 10 or 20 ml syringe. It is also helpful to have one or two extra 20 ml syringes ready which can be attached rapidly to the needle (see Section 6.2 below). Alternatively, natural blood flow may be sufficient without the use of a syringe if the needle is attached to a length of wide-bore tubing leading into a collecting vessel.

6.2 Taking the sample

A general anaesthetic is required. The heart is located normally on the thorax at the point of the elbow in most species and can be felt or heard with a stethoscope. If the procedure is not intended to be terminal for the animal (for example, when bleeding a tail-less mammal) then aseptic precautions must be observed. Any dirt or debris that might contaminate the sample should first be removed. It may be sufficient to simply part the fur before inserting the needle (see Section 3.2.3). However, if recovery of the animal is intended, asepsis may be better maintained by clipping. The needle should be

sufficiently long to penetrate the ventricle, and a vacuum in the syringe should be maintained during needle entry. Aim for the loudest noise on the stethoscope, or the area that feels to have maximum heart rebound. Successful entry is seen by the appearance of blood into the syringe. The needle should be fixed at this point with the fingers so that it is not inadvertently moved out of the ventricle, either during withdrawal or when the syringe is changed. Filling and changing syringes is easiest done with the help of an assistant; i.e. the assistant can loosen the full syringe on the 'fixed' needle and remove it, the first person can then attach a fresh syringe.

6.3 Subsequent care of animal and potential adverse effects

Any animal that is to recover should be separated from other animals until fully conscious. It should be kept warm, watched carefully and killed if found in unalleviable distress. In repeated cardiac punctures there is a risk of subsequent bleeding into the pericardium leading to heart failure and death. Ventricular fibrillation may occur during sampling but premedication with atropine may help avoid this complication.

6.4 Other problems

Frequent problems are: failure to enter the ventricle, bleeding into the pericardium with cardiac arrest (often due to multiple entries), bleeding into the thorax due to perforation of a blood vessel. (N.B. the clots can be retrieved post-mortem and processed for serum as with blood.) Contamination of the sample with lung constituents (air, fluid or even pus if the animal has pneumonia) can occur.

7 VOLUME OF BLOOD TO BE REMOVED (see Tables 3 and 4)

If too much blood is withdrawn too rapidly, or too frequently without replacement, an animal may go into short-term hypovolaemic shock and

Table 3. Circulating blood volume in laboratory animals

Species	Blood volume (ml/kg)*
Cat	47–66
Cattle	60
Chicken	60
Dog	79–90
Ferret	75
Gerbil	67
Goat	70
Guineapig	67–92
Hamster	78
Horse	75
Marmoset	—
Mouse	78–80
Pig	65
Rhesus	54
Rabbit	44–70
Rat	50–70
Sheep	60

*Assuming the animal is mature, healthy and on an adequate plane of nutrition.

in the longer term suffer from anaemia. Removal of around 10% of the circulating blood volume will initiate homeostatic cholinergic mechanisms. If 15–20% volume is removed, cardiac output and blood pressure will be reduced. Removal of 30–40% can induce haemorrhagic shock, and 40% loss can cause 50% mortality in rats (see McGuill & Rowan, 1989). Smaller volumes removed at too frequent an interval will cause

anaemia. These effects should happen rarely and be avoided whenever possible. However, it is essential to be able to recognize the signs and symptoms of shock and anaemia and to be able to take appropriate action.

7.1 Recognition of signs of hypovolaemic shock and anaemia

Hypovolaemic shock manifests as a fast and thready pulse, pale dry mucous membranes, cold skin and extremities, restlessness, hyperventilation, and a subnormal body temperature. Plasma expanders may have to be used in cases of hypovolaemia and **the Named Veterinary Surgeon should be consulted if shock occurs inadvertently.** In animals that have had more than 10% of their circulating blood volume removed, a routine replacement of the same volume of warm (30–35 °C) normal saline would constitute good animal care.

Signs of anaemia include pale mucous membranes of the conjunctiva or inside the mouth, pale tongue, gums, ears or footpads (if non-pigmented), intolerance to exercise, and at a more extreme level an increased respiratory rate when at rest. Monitoring of the individual animal is very important and the surest method is to use each animal as its own baseline. Packed cell

Table 4. Normal haematological values

Species	PCV (%)	RBC (10^{12}/l)	Hb (g/dl)	Reticulocyte (%RBC)	Wbc (10^9/l)	Clotting times (seconds)
Cat	30–50	6·0–10·0	8–14	0–1·0	5·5–19·5	—
Cattle	24–46	5–10	8–15	—	4–12	—
Chicken	23–55	1·25–4·5	7·0–18·6	—	9–31	—
Dog	38–53	4·5–8·0	11–18	0–1·5	6·0–17·0	180
Ferret	35–51	11·3	12–17·4	—	2·5–15·4	—
Gerbil	48	7·0–10·0	12–17	—	4·3–21	—
Goat	29–38	13·0–18·0	8–14	—	5·0–14·0	60–300
Guineapig	35–42	4·5–7·0	11–17	1·8–6·1	10·0	—
Hamster	39–59	4·0–10·0	2–30	—	7·6	55
Marmoset	40–55	5·3–8·1	12–19	1·0–10·0	3·0–13·0	—
Mouse	35–45	7·7–12·5	10–20	3·3–13·3	8·0	120–600
Pig	30–50	5·0–9·0	10–16	—	7·0–20·0	60–300
Rhesus	36–43	5·6–13·4	11–13	0·5–3·0	5·6–15·4	—
Rat	35–45	7·2–9·6	12–18	1·7–21·1	14·0	20
Rabbit	30–50	4·5–7·0	8–15	2·9–8·0	9·0	60–360
Sheep	29–38	8·0–14·0	10–12	—	4·0–12·0	60–300

Note there are considerable influences of strain (breed), sex and age on these figures and contemporary healthy animals should be used as controls.

volume, haemoglobin level, red blood cell and reticulocyte counts can be monitored throughout a series of bleeds where there might be concern about the development of anaemia.

7.2   Volume

As a rough guide, up to 10% of the circulating blood volume can be taken on a single occasion from normal healthy animals on an adequate plane of nutrition with minimal adverse effect. This does not mean the animal does not experience any after effects — merely that it does not show any. This volume may be repeated after 3–4 weeks. For repeat bleeds at shorter intervals a maximum of 1·0% of an animal's circulating blood volume can be removed every 24 h: i.e.

0·01 × circulating blood volume (ml/day) (roughly = 0·6 ml/kg/day)

The circulating blood volume can generally be estimated as 55–70 ml/kg body weight (see Table 3). However, care should be taken in these calculations as the percentage of circulating blood will be lower (−15%) in obese and older animals.

8   EFFECTS OF ANAESTHETIC AND SEDATIVE DRUGS ON BLOOD COUNTS

Unless an animal is unusually well adjusted to having a blood sample taken, it will be stressed by the procedures involved (e.g. approach of operator, capture, restraint, administration of immobilizing agent(s), obtaining the sample). As part of the stress reaction, catecholamines (adrenaline, noradrenaline) are produced. One of the actions of these hormones is to cause the spleen to contract. The spleen is a storage site for red cells and, in some species, it can hold up to 25% of the red cells in the body. When it contracts, red cells will be released into the circulation. This is not an abnormal reaction but provides the blood with a greater oxygen carrying

capacity to cope with the normal 'fight or flight' reaction. Because of it, however, the red blood cell count (RBC), packed cell volume (PCV) and haemoglobin (Hb) levels will be artificially high in blood samples obtained from stressed conscious animals. Animals with a large reactive spleen include cats, dogs, sheep and goats. Animals with unreactive spleens include primates, birds and reptiles.

Some commonly used general anaesthetics and sedative agents affect the red cell count and other related measurements by contracting or relaxing the splenic capsule (see Yale & Torhorst, 1972; Mattheij & van Pijkeren, 1977). Many sedative drugs can produce a progressive relaxation of the spleen and red cells will then accumulate there. In cats, this occurs over a 30–40 min period and during this time, the number of red blood cells in the circulation falls. When splenic relaxation is complete, the red blood cell count reaches a steady low level and the animal may even show a pseudoanaemia. As consciousness returns, splenic smooth muscle tone returns to normal and so the red cell count increases. The following drugs cause splenic relaxation: xylazine HCl (Rompun), promazine HCl, barbiturates, alphaxalone-alphadolone (Althesin, Saffan), acetylpromazine, and diazepam (Valium).

In experiments where red cell values are important, care must be taken to standardize the interval between giving the sedative (at a standard dose in mg/kg body weight) and collecting the blood sample. Knowledge of the spleen physiology of the species being used and of the actions of the drugs being administered can be used to advantage. For example, if a high yield of plasma or serum is required from a sheep, use a sedative such as xylazine and wait 40 min before collecting the blood sample.

9   GUIDE TO SEVERITY LIMIT BANDING FOR A PROJECT LICENCE APPLICATION IN THE UK

In the UK, removal of blood is considered a scientific procedure which may have adverse

effects on an animal and consequently requires the authority of a project licence. The site, volume and frequency of sampling should be detailed in Section 19b of the project licence. Each procedure in the licence has a predicted severity limit (in Section 19a) and the following classifications may be of help in this assessment.

A one-off removal of blood (except for orbital venous sinus sampling or tail amputation) should be considered as having only a 'mild' effect on the animal. Repeated blood sampling at weekly intervals, or single sampling requiring more than simple venepuncture (e.g. cardiac puncture), should be considered as 'moderate'.

Any form of cannulation (including carotid loops) should be considered as 'moderate' because of the surgery required to prepare the animal. However, the overall severity banding of all these techniques would be considered mild because of the minor long-term effects. Regular sampling through an indwelling cannula however would normally be classed as 'mild', though this might depend on the volume of blood and frequency of blood removal. Simple removal of blood should never go into the 'substantial' category unless some aspect of trauma or haemostatic defect is being investigated.

10   TRAINING

Blood collection procedures can only be observed and not practised before applying for a personal licence. Such observation is an absolutely essential part of training. Inexperienced persons must examine dead animals to learn the relevant anatomy. Use should also be made of demonstration and instruction videos, as well as inanimate objects (such as oranges) to gain familiarity in handling and using needles and syringes. After a personal licence has been granted the work has to be carried out under the supervision of a suitably qualified licence holder who will have experience of that technique. We recommend that the new licensee first assists and then does the procedure under direct supervision (i.e. the supervisor should be present and not just be available if required — the supervisor should prevent as well as rectify anything going wrong). Such supervision requirements should also be

discussed with the Home Office Inspector. **However, before carrying out any work on live animals, such persons should also be familiar with handling those animals and, if possible, the animals involved should be known to the licensee. On occasions it may be necessary and useful to train the animals to a procedure by carrying out several dummy runs and giving rewards.**

11   SUMMARY OF GOOD PRACTICE RULES

There are several general rules which apply to any procedure carried out on an animal. If these are not observed it is likely the procedures will not be successful, both from the point of view of animal welfare and of scientific validity. Furthermore, these rules should be followed in order to meet the conditions attached to the personal licence.

(i) Make sure that you hold the necessary personal licence. The work must also be covered by a project licence and the operator must be familiar with all the relevant details in Section 19b.

(ii) Know what you are doing and how you are going to do it.

(iii) If in doubt about a procedure or technique ask for advice.

(iv) Assemble all equipment before collecting and preparing the animal, pre-label all containers.

(v) Enlist the help of an assistant familiar with the individual animal, such as an animal technician, wherever necessary.

(vi) Be gentle and firm with the animal.

(vii) Never leave an animal unattended.

(viii) If there are any complications which may compromise the health of an animal ask for advice from the Named Veterinary Surgeon, the Named Person in Day to Day Care, or an experienced licensee.

(ix) If there is an accident and the procedure goes badly wrong, know how to dispatch an animal humanely and have the means to do so available.

(x) Be able to recognize adverse effects in the species you are dealing with and know how to alleviate them.

(xi) Always confirm that the animal has recovered satisfactorily from the procedure.

As the personal licensee, the welfare of the animal is ultimately your responsibility at all times.

GLOSSARY OF TERMS

Acclimatization Accustomizing an animal to the procedures, the people and places involved.

Acute Short-term (may also denote non-recovery (AC) work).

Cannula A length of hollow tubing for insertion into an organ or (blood) vessel. (Contrast with *'catheter'* in which entry is by means of a natural orifice and channel.)

Cannulation The act of placing a cannula into a blood vessel.

Canthus The corner of the eye, usually defined as inner (medial) or outer (lateral).

Caudal The part nearer the tail (opposite of cephalic).

Cephalic The part nearer the head (opposite of caudal).

Chronic Long-term.

Coccygeal The name relating to the vertebrae in the tail.

Dorsal Relating to the back of the animal.

Embolus (pl-i) (embolism) In this context a dislodged thrombus from the end of a cannula released into the circulation by e.g. flushing.

Femoral (artery) The major artery to the back legs located in the groin.

Granuloma A reaction resulting in excessive production of inflammatory tissue.

Harness An apparatus designed to enable an animal to carry a cannulation device with it.

Haematoma A collection of blood outside the heart and blood vessels which may form a clot.

Hypovolaemic A low blood volume.

Intravenous Inside a vein.

Jugular The main vein running down the neck.

Keratitis An inflammation of the cornea.

Lateral On the outer side.

Medial On the inner side.

Microphthalmia Used to denote a small eye after trauma or infection resulting in the eye shrivelling.

Occasional Defined here as sampling at intervals of more than one week.

Percussion Gentle tapping on a blood vessel to discover its course.

Perivascular Outside and around a vein as might occur after fluid has been misinjected intravenously.

Phlebitis An inflammation of a vein.

Saphenous The superficial vein draining the hindleg which can be located laterally over the hock or tarsus.

Swivel A device whereby tubing attached to a cannulae can be connected to the cage or some other solid support without unduly restricting movements of the animal.

Tether The support attaching the cannula and tubing to the animal's harness.

Thrombophlebitis Combined inflammation and clotting in a vein.

Thrombosis The process of the formation of blood clots in the sense of blocking a vein or cannula.

Thrombus (pl-i) A clot normally formed at the end of a cannula.

Trauma Physical damage to tissue, e.g. through rough handling.

Umbilicus A common name for a harness and tether.

Venesection Cutting through the vein as with a needle.

Venepuncture Penetrating into a vein as in a stab or prick.

APPENDIX A

Retro-orbital bleeding

The animal is anaesthetized and held gently but firmly by the scruff of the neck on a solid surface so that the eye protrudes. This may help occlude the venous return from the head and neck. Care must be taken not to obstruct the breathing. The orbital sinus is then penetrated with a sterile micro-pipette, a Pasteur pipette or micro-capillary tube (e.g. 100 μl size). This is pushed through the conjunctiva laterally (outer side), dorsally (above) or medially (inner side), to the back wall of the orbit where it punctures the venous sinus and so fills with blood. On withdrawal of the pipette or tube, blood exudes from the canthus where it can be collected. (N.B. There may be contamination of the sample with surface debris using this technique.) The scruff of the neck should be released momentarily before withdrawal of the pipette to minimize haemorrhage from the puncture site. Care must also be taken not to abrade the cornea whilst putting pressure on the globe to limit haemorrhage after the sample has been obtained. In a mouse 100–200 μl can be collected by this method.

There is some debate as to whether the inner (medial or nasal side) or outer (lateral) canthus is the optimal place to penetrate the conjunctiva. Some consider damage is minimized by using the lateral route. In the

rat it has been suggested that the conjunctiva be penetrated at the dorsal or upper aspect of the eye. This is because in this species there is a dorsal venous plexus rather than a retro-orbital venous sinus (Timm, 1989).

There are many potential adverse effects to this technique and these should be given full consideration before it is used. There does not seem to be any published work on the after-effects, except for a recent study on histological changes in the orbital region of rats after orbital puncture (Van Herck et al., 1992). It is possible that the disturbance index (Barclay et al., 1988) could be used to determine the severity of this procedure. Haemorrhage may occur after blood collection, resulting in a retro-orbital haematoma and excessive pressure on the eye. The pressure is almost certainly painful for the animal and damage to the optic nerve and other intra-orbital structures can lead to deficits in vision and even blindness. Pressure from a haematoma may also lead to the animal not being able to close its eye, which in turn can lead to corneal ulceration and rupture. Other adverse factors include the chance of the micro-pipette passing through the bones of the orbit and causing neural damage, the penetration of the globe itself with a loss of vitreous humour, and infection leading initially to swelling and then to degeneration of the eye. Keratitis (inflammation of the cornea) with pannus formation (blood vessel proliferation) is also a common adverse effect and may be caused by the operator rubbing the corneas whilst putting pressure on the globe to limit haemorrhage after the sample has been obtained. The common sequelae of some of these adverse effects is a shrunken eye (microphthalmia) which is non-functional and the animal is likely to have been in considerable pain in the interim period.

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Videos

Note