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REVIEW ARTICLE

ANAESTHESIA OF ANIMALS FOR BIOMEDICAL RESEARCH

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Anaesthetists trained to deal with human patients may be required to anaesthetize animal species as part of their involvement in biomedical research. Although the skills developed in clinical anaesthesia are of obvious relevance, several problems may be encountered when attempting to anaesthetize an unfamiliar species. This article reviews those areas which are of greatest practical importance to medical anaesthetists and discusses the ethical and legal aspects of research which involves the use of live animals.

ETHICAL AND LEGAL CONSIDERATIONS

The use of animals in biomedical research attracts considerable criticism from animal welfare groups. It is important that all those involved in such research projects are aware of the sensitive nature of this issue, and it is helpful to become familiar with the moral and ethical arguments which have been advanced on both sides of this debate. In Western Europe and North America, a broad consensus amongst research workers appears to have emerged, which accepts the need to use live animals in medical research, but seeks to reduce to a minimum any pain, suffering or distress which these animals may experience. This consensus is reflected in legislation such as the Animals (Scientific Procedures) Act, 1986 in the U.K., the Animal Welfare Act in the U.S.A., and the Council of Europe Directive within the E.C.

The Animals (Scientific Procedures) Act, 1986

Within the U.K., all research work which involves the use of living vertebrate animals is regulated by the Animals (Scientific Procedures) Act, (A(SP) Act), 1986, administered by the Home Office. The Act controls "regulated procedures" performed on "protected animals". A "regulated procedure" is any experimental or other scientific procedure which may cause the animal pain, suffering, distress or lasting harm. The "protected animals" are all vertebrates other than man, and the Act also includes protection for immature forms (fetuses and embryos) in addition to adult vertebrates. The Home Office definition of "pain, suffering, distress or lasting harm" is very broad, and includes physiological and psychological stress, disease, injury, disturbance to normal health and any significant discomfort. It is concerned both with short-term and long-term effects. Although death is also considered to be causing lasting harm, killing an animal by one of a number of specified techniques may remove the work from the control of the Act.

Personal Licences

The Act sets out to control "regulated procedures" in several ways. It controls the competence of individual research workers by means of "Personal Licences". A Personal Licence lists the techniques that may be carried out by the individual research worker and states the species of animal and whether anaesthesia is to be used for all, part or none of the techniques. The techniques listed on a Personal Licence are very specific and it is important not to carry out any procedure which is not specifically listed. The most important aspect of acting as the Personal Licence holder on a research project is that the licensee carries the primary responsibility for the care of the animals. These licences are granted to people who are believed to be competent to perform the procedures listed on the licence. Before a Personal Licence may be granted, the applicant will be required to attend a recognized training course, and will need to present evidence of his or her competence and relevant experience. Initially, the licence holder is unlikely to be competent to carry out all of the procedures without supervision, and a supervision clause will be added to the licence. When the licence holder is considered competent, the supervisor may request that the supervision condition is removed. When applying for a licence, the applicant is asked to state that they have read and understood the A(SP) Act, the Guidance Notes which are provided by the Home Office, and any relevant Codes of Practice.

Project Licences

Before a research worker who holds a Personal Licence can begin work which involves the use of animals, the research project must also have been authorized, by means of a "Project Licence". Full details of Project Licences are given in the Home Office Guidance Notes on the Act, but the following points are particularly important.

Project licensing is central to the operation of the A(SP) Act. The philosophical basis of the Act is to

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perform a cost–benefit analysis of research work. The cost to the animals in terms of adverse effects is weighed against the likely benefits to man or other animals. In order to perform this judgement, the Home Office require the Project Licence application to give details of the purpose of the work and provide a scientific justification for the proposed study. The Project Licence must also provide a detailed description of the procedures which are involved, an estimate of the number of animals that may be used, and an assessment of the potential adverse effects on the animals. This last assessment is referred to as the overall "severity" of the Project and it may be judged to be mild, moderate or substantial. A fourth category, unclassified, is reserved for studies which are carried out entirely under terminal general anaesthesia.

In many instances, the overall severity of a procedure can be reduced by applying carefully defined humane end-points. For example, rather than waiting until an experimentally induced condition results in the death of an animal, the animal could be humanely killed early in the course of the condition. In other circumstances, for example procedures involving surgical techniques, the severity of the procedure can be reduced by using analgesics and by providing a high standard of postoperative care. It is most important that a research worker becomes familiar with the detailed descriptions of the permitted procedures on the relevant Project Licence, and with the degree of pain, suffering or distress that the animals are permitted to undergo. Project Licences have a "severity band" which indicates this, and the licence will also detail what steps must be taken to minimize the adverse effects on the animal.

If it seems likely that the severity limit of a Project will be exceeded, the animal must either be killed, or the Home Office Inspector must be contacted.

The Personal Licensees involved with a Project should keep a record of all the procedures they undertake. Although it is the Project Licence holder's responsibility to report to the Home Office the details of all of the animals used on a Project, the records maintained by the Personal Licence holder will assist them in completing an accurate report.

Research workers may wish to visit another research institute in the U.K. to carry out a new technique, or to take part in a collaborative project. Before the visit, application must be made to the Home Office for permission to undertake work at the establishment. It is also important to ensure that the Personal Licence of the research worker is revised to incorporate any new procedures which may be undertaken and that the host institution's Project Licence covers the work that will be undertaken.

"Designated Premises" and the Certificate of Designation

Even after Project and Personal Licences have been granted, further controls are provided by the requirement that the work must only be carried out at a "designated place" which is specified on the Project and Personal Licences. The research institute is granted a Certificate of Designation which is held by some person in authority at the institute.

The Certificate holder is ultimately responsible for ensuring that all work carried out at the institute complies with the requirements of the A(SP) Act. To assist in fulfilling these statutory duties, the Certificate holder is required to appoint two "named persons": a "named person in day to day care" who is responsible for the health and welfare of all animals housed in the institute, and a "named veterinary surgeon" who is responsible for advising on animal health and welfare.

The implementation of the Act is also influenced by Home Office Codes of Practice. At present, only one of these, which sets out standards for housing and care of animals, has been published. This Code of Practice details the cage sizes, stocking densities, room temperatures, relative humidity and ventilation, staffing requirements and standards of building maintenance and design required for research animal units.

The Home Office Inspectorate

The administration and enforcement of the A(SP) Act is the responsibility of the Home Office Inspectors. Their role is to advise the Secretary of State on applications for Personal and Project Licences and Certificates of Designation. In effect, their advice is almost always accepted, hence they make the initial decision concerning the granting of licences. The Home Office Inspectors make unannounced visits to research laboratories to ensure that all of the conditions on Personal and Project Licences and on the Certificate of Designation are being fulfilled. The Inspectorate has immediate right of access to all areas of the premises. If an Inspector believes that an animal is undergoing excessive suffering, he may insist that it is killed immediately.

From the above description of the 1986 Act, its implementation may sound somewhat cumbersome and bureaucratic, and initial attempts at completing a licence application may seem difficult. However, in practice the legislation functions efficiently and advice on the drafting of licence applications is usually available within a research institute. Applicants are also normally advised to contact their Home Office Inspector at an early stage, to discuss any proposed research project.

Problems may arise when the anaesthetist is not directing the research project, but has been asked to provide specialized assistance with respect to the anaesthetic management of the research animals. It is important to realize that administering an anaesthetic is regarded as a research procedure, and the anaesthetist must have a Personal Licence to authorize their work. In addition, it is vital that the anaesthetist inspects carefully the Project Licence which authorizes the work, to determine the techniques for which authority has been granted. Certain procedures which may be regarded as routine clinical practice in a hospital setting require specific authority to be granted before they can be carried out; for example, the use of neuromuscular blocking agents is the subject of special controls in the U.K.

TABLE I. *Basic physiological data for laboratory animals*

Species	Ventilatory frequency (b.p.m.)	Tidal volume (ml)	Heart rate (beat min^{-1})	Blood volume (ml kg^{-1})	Body temp. (°C)	Body weight (kg)
Cat	26	30	150	85	38.6	3.0
Dog (Beagle)	25	150	100	80	38.3	15
Gerbil	90	—	260–600	—	39.0	0.09
Goat	20	325	80	70	39.4	50
Guineapig	120	2.5	155	75	38	0.5
Hamster	80	0.8	350	72	37.4	0.08
Mouse	180	0.15	570	75	37.4	0.030
Pig						
20 kg	18	420	80	70	39.0	20
200 kg	12	3800	65	65	39.0	200
Primate (Baboon)	35	50	150	75	39.0	10
Rabbit	50	20	220	70	38.0	3
Rat	90	1.6	350	58	38.0	0.2
Sheep	20	300	75	60	39.1	45

In general, it is advisable to discuss the proposed anaesthetic methodology with the local Home Office Inspector before commencing work. It is also good practice to prepare a written programme, both to assist in compliance with any legal requirements and to enable the methodology to be duplicated in the absence of the anaesthetist.

The Animal Welfare Act

In North America, the methods of regulation of research involving live animals are somewhat different, but are based on similar principles. In the U.S.A., the Animal Welfare Act sets out national standards. Within each research institute a local Ethics Committee reviews individual research proposals and determines if they comply with local and national requirements. In many institutes, the detail required in a submission to the Ethics Committee (usually referred to as the Animal Care and Use Committee) is similar to that of a U.K. Project Licence application. Although no separate Personal Licence system exists, most Ethics Committees require details of the relevant training and experience of the personnel who are involved in the project. In addition, many institutes now require new research workers to attend a training course before they are permitted to start their research work.

COMPARATIVE ANAESTHESIA

The most obvious practical problem that may be encountered by an anaesthetist who routinely deals with human subjects arises as a result of the small body size of some of the animals that are used in research laboratories (table I). Body size influences the type of equipment and the anaesthetic techniques that may be used, and the overall management of the animal during the perioperative period.

Anaesthetic equipment

When dealing with animals of sizes comparable to those of adult humans and infants, standard anaesthetic apparatus can be utilized. Human paediatric breathing systems are particularly useful for animals weighing 1–10 kg and a Bain's circuit or Ayre's T-piece is the apparatus used most frequently. In species with a body weight less than 1 kg, the majority of anaesthetic breathing circuits, tracheal tubes and laryngoscopes designed for use in humans are unsuitable. Some purpose-made equipment can be purchased (see below), but in many instances apparatus must be constructed by the anaesthetist. Suitable circuits can be constructed from plastic or silastic tubing, and tracheal tubes from flexible "over-the-needle" catheters. Table II lists the approximate dimensions of tracheal tubes and laryngoscopes suitable for intubating the trachea of common laboratory animals. Techniques for intubation are described in several texts [9, 15, 25]. A particularly useful description of small rodent intubation and construction of a purpose-made

TABLE II. *Equipment required for tracheal intubation in laboratory animals*

Species	Body weight	Tube diameter (o.d.) (mm)	Laryngoscope
Mouse	25–35 g	1.0	Purpose made laryngoscope [4]
Hamster	120 g	1.5	Purpose made laryngoscope [4]
Rat	200–400 g	1.8	Purpose made (12–16-gauge plastic cannula) laryngoscope [4]
Guineapig	400–100 g	1.5–2.5	Purpose made laryngoscope [4]
Rabbit	1–3 kg	2–3	Wisconsin size 0–1
	3–7 kg	3–6	
Cat	0.5–1 5 kg	2.0–3	MacIntosh size 1
	> 1.5 kg	3–4.5	
Dog	0.5–5 kg	2–5	MacIntosh size 1–4
	> 5 kg	4.0–15	
Primate	< 0.5 kg	—	Not reported
	0.5–20 kg	2–8	MacIntosh size 1–3
Sheep	10–90 kg	5–15	MacIntosh size 2–4
Pig	1–10 kg	2–6	Soper or Wisconsin
	10–200 kg	6–15	size 1–4

TABLE III. *Sedative/immobilizing agents for animals (dose (route)). †Fentanyl/fluanisone*

	Mouse	Rat	Hamster	Gerbil	Guineapig	Rabbit
Diazepam (mg kg^{-1})	5 (i.p.)	2.5 (i.p.)	5 (i.p.)	5 (i.p.)	5 (i.p.)	2 (i.v.)
Acepromazine (mg kg^{-1})	2.5 (s.c.)	2.5 (s.c.)	5 (s.c.)	5 (s.c.)	2 5 (s.c.)	1 (s.c.)
Hypnorm† (ml kg^{-1})	0.3 (i.p.)	0.4 (i.p.)	0.5 (i.p.)	0 5 (i.p.)	1 (i.p.)	0.5 (i.m.)
Xylazine (mg kg^{-1})	10 (i.p.)	10 (i.p.)	10 (i.p.)	5 (i.p.)	5 (i.p.)	5 (i.m.)
	Dog	Cat	Sheep/Goat	Pig	Primate	Ferret
Diazepam (mg kg^{-1})	—	—	2 (i.m.) 1 (i.v.)	1 (i.m.)	1 (i.m.)	2 (i.m.)
Acepromazine (mg kg^{-1})	0.1 (i.m.)	0 1 (i.m.)	0.1 (i.m.)	0.2 (i.m.)	0.2 (i.m.)	0.2 (i.m.)
Hypnorm† (ml kg^{-1})	0.2 (i.m.)	—	—	—	0.3 (i.m.)	0.5 (i.m.)
Xylazine (mg kg^{-1})	1–2 (i.m.)	1–3 (i.m.)	1 (i.m.) (sheep) 0.05 (i.m.) (goat)	—	—	—

laryngoscope is given by Costa and colleagues [4] and an alternative technique for the rat is illustrated clearly by Remie and colleagues [24].

After intubation, the animal may be allowed to breathe spontaneously, but mechanical ventilation allows more reliable and reproducible blood-gas variables to be maintained and this is required for many studies. Ventilators designed for human paediatric use are suitable for animals of 1–2 kg body weight and greater. Small animals require purpose-made apparatus, or adjustment of the anaesthetic breathing system to provide a controlled leak of ventilatory gas. Suitable ventilators are available from Harvard Apparatus Ltd (Fircroft Way, Edenbridge, Kent, U.K.; 22 Pleasant Street, South Natick, MA 01760, U.S.A.). Tidal volumes of approximately 10–15 ml/kg body weight and ventilatory frequencies from 80–100 b.p.m. (mice) to 10–20 b.p.m. (dog, sheep and pig) are usually required to maintain normocapnia. Monitoring of respiratory function presents several problems (see below).

Because of the technical difficulties associated with intubation, volatile anaesthetics are administered to small rodents usually via a face mask. This causes some problems with regard to scavenging of waste anaesthetic gases, and it is recommended that the concentric face mask system described by Hunter, Glen and Butcher [18] is purchased (Fluo-vac, International Market Supply, Dane Mill, Congleton, Cheshire, U.K.). Anaesthetic induction with volatile anaesthetics in small rodents is best carried out using a purpose-made induction chamber connected to a standard anaesthetic vaporizer and suitable gas scavenging equipment. In larger species such as rabbits, cats and dogs, anaesthesia may be induced using a face mask, but the patient may prove uncooperative. To prevent unnecessary stress, either induction should be achieved using injectable agents, or a potent sedative/tranquillizer should be administered as preanaesthetic medication (table III). The MAC value of the majority of volatile anaesthetics is similar in man and animals, and little difficulty will be experienced in judging depth of anaesthesia. An exception relates to nitrous oxide, which does not induce loss of consciousness in animals. It is therefore unacceptable to use an anaesthetic regimen consisting of nitrous oxide and a neuromuscular blocking agent alone.

Anaesthetic techniques

In most larger animals, superficial veins are readily accessible for drug administration and anaesthetic induction; venepuncture techniques are described by Tuffrey [27]. Catheters designed for use in man are suitable for most species, including small rodents. Skin thickness varies in different species, and in some animals it may be necessary to make a small scalpel incision before inserting a large over-the-needle type percutaneous catheter into a peripheral blood vessel. As in humans, prior application of EMLA cream facilitates venepuncture in conscious patients [12].

Because of the technical difficulties associated with venepuncture in small rodents, many injectable anaesthetic regimens have been developed which are administered by i.p. or i.m. injection. In general, the i.p. route is preferred to i.m. administration, as the irritant nature of some anaesthetics, coupled with the small muscle mass present in these animals, can lead to myositis [26]. A major disadvantage of both routes is that it is not possible to adjust the dose of anaesthetic according to the individual animal's response. This can lead to high anaesthetic mortality, particularly when agents with a narrow anaesthetic index are used, such as pentobarbitone. There is also a considerable variation in dose responses between animals of different strains, with sleep times varying by as much as 300% in different strains of mice [20]. Published dose rates of anaesthetic agents, such as those listed in table IV, should therefore be used only as a general guide and, whenever possible, a single animal should be anaesthetized and the

response assessed, before larger numbers of animals are used.

A second problem associated with the administration of relatively large dose rates of anaesthetics by the i.p. route is the very prolonged recovery times which result. For example, in the rat, several regimens produce 30–45 min of anaesthesia, but sleep times may extend to 120–180 min. During this period, the animal remains susceptible to hypothermia, respiratory failure or injury from cage mates if recovering in a group. To avoid this problem it may be helpful to consider use of reversible anaesthetic regimens [13, 17].

Species variation in drug responses. In addition to the variation in drug responses between individuals, different species vary in their response to some of the commonly used anaesthetics. Of particular relevance to anaesthetists is the very great species variation in response to ketamine and other dissociative anaesthetics. In man, ketamine is reported to produce good analgesia, and in the majority of non-human primates a cataleptic state with analgesia and immobilization is attained. The efficacy of this agent decreases in species with less cortical development. In the cat it is moderately effective, but ketamine rarely immobilizes pigs and sheep completely. In rodents, it is remarkably ineffective until doses which produce profound respiratory depression have been administered; at these dose rates, mortality may exceed 50%, and for this reason ketamine should not be used as the sole immobilizing agent. Most frequently, it is combined with a sedative such as a benzodiazepine, or an alpha$_2$ adrenergic agonist such as xylazine. In contrast to the effects of ketamine used alone, these combinations may produce surgical anaesthesia in many species.

Other species-specific drug responses of note include the effects of morphine, which should be administered in small doses to cats to avoid producing excitement, and alphaxalone–alphadolone which should not be administered to dogs, as the solubilizing agent in the commercial preparation can promote extensive histamine release in this species.

The depth of anaesthesia that can be provided by an anaesthetic before significant respiratory or cardiac depression ensues also varies in different species. Propofol is an effective anaesthetic in rats, mice, dogs, cats, pigs and sheep, but in rabbits severe respiratory depression occurs before the onset of surgical anaesthesia. Other commonly used human anaesthetic regimens are ineffective in some animal species, for example midazolam and other benzodiazepines do not produce unconsciousness in dogs and cats, and may cause agitation and excitement when administered by i.v. injection. Large doses of potent opioids such as fentanyl are effective analgesics, but unconsciousness is not achieved readily, many species developing excitement or respiratory arrest before consciousness is lost. Combinations of opioids and tranquillizers or sedatives are widely used, however, and produce safe and effective anaesthesia (table IV). As mentioned earlier, nitrous oxide does not produce loss of consciousness, so regimens based on high-dose opioids, nitrous oxide and a neuromuscular blocking agent are not acceptable.

Further information on species responses to individual anaesthetics has been reported by Green [14], Flecknell [9], Short [25] and Hall and Clarke [15]. It is also important to note that virtually all drugs which are in current clinical use have been tested for safety and efficacy in laboratory animals. Examination of the relevant literature often provides information on appropriate dose rates of many compounds.

Anaesthetic management

Animal health status. Laboratory species are susceptible to a wide range of diseases and a pre-anaesthetic clinical examination should be carried out to ensure that the subject is free from clinical signs of disease. Before major surgical procedures, routine haematological and biochemical evaluations should be undertaken. Normal values for these variables can be obtained in Loeb and Quimby [19] and Mitruka and Rawnsley [21]. Subclinical disease, for example respiratory infection, represents a considerable problem and it is advisable to discuss the health status of the animals with veterinary staff in the research animal unit. Most units will be undertaking some type of disease surveillance programme or quality assurance scheme, and will be able to provide advice on this topic. Working with animals that are free from disease is often essential if meaningful research data are to be obtained. It is also important to appreciate that some infectious diseases are zoonoses, and may represent a health hazard to personnel. This is a particular problem when working with non-human primates. Again, advice from veterinary staff should be obtained and local safety codes of practice should be adhered to.

Preoperative preparations. In order to allow time for preanaesthetic clinical evaluation to be undertaken, it is essential that animals are obtained 1–2 weeks before commencing any study. Local guidelines may exist, and these should be adhered to. Transport of animals from a supplier to the research laboratory is stressful, and animals require a period of acclimatization to adapt to their new surroundings. This acclimatization period also enables recording of body weight and food and water consumption—information which is extremely useful for monitoring postoperative recovery (see below).

Food should be withheld for 12–16 h before anaesthesia of dogs, cats, primates, ferrets, pigs and sheep. Fasting is unnecessary in small rodents and rabbits, unless upper gastrointestinal tract surgery is to be undertaken. Sheep and goats present particular problems with regard to anaesthetic management. These species have a stomach that consists of several sections, one of which, the rumen, acts as a fermentation chamber for the digestion of cellulose. During anaesthesia, normal oesophageal activity is suppressed and the gas which is produced accumulates in the rumen, causing massive distension of the abdomen. It is often necessary to relieve this distension by passage of a stomach tube.

TABLE IV. *Dose rates of anaesthetic, analgesic and related compounds for laboratory animals. Animals grouped: mouse, rat, hamster and gerbil; guineapig, rabbit, dog and cat; primates, pig, sheep and goats, ferret. Drugs listed in alphabetical order. Dose rates based on published data [14, 15] and clinical experience at the Clinical Research Centre, Harrow and the Comparative Biology Centre.*
** Sedation; ** immobilization; *** anaesthesia; ! severe respiratory depression may occur*

Drug	Mouse	Rat	Hamster	Gerbil
Alphaxalone/ alphadolone	10–15 mg kg^{-1} i.v.***	10–15 mg kg^{-1} i.v.***	150 mg kg^{-1} i.p.**/***!	80–120 mg kg^{-1} i.p.**/***!
Atropine	0.04 mg kg^{-1} s.c., i.m.	0.05 mg kg^{-1} s.c., i.m.	0.04 mg kg^{-1} s.c., i.m.	0.04 mg kg^{-1} s.c., i.m.
Buprenorphine	0.05–0.1 mg kg^{-1} s.c. 12 hourly	0.01–0.05 mg kg^{-1} s.c., i.v. 8–12 hourly	—	—
Butorphanol	1–5 mg kg^{-1} s.c. 4 hourly	2 mg kg^{-1} s.c. 4 hourly	—	—
Doxapram	5–10 mg kg^{-1} i.v.	5–10 mg kg^{-1} i.v.	5–10 mg kg^{-1} i.v.	5–10 mg kg^{-1} i.v.
Flunixin	2.5 mg kg^{-1} s.c., i.m. ?12 hourly	2.5 mg kg^{-1} s.c., i.m. ?12 hourly	—	—
Hypnorm (fentanyl + fluanisone)	0.5 ml kg^{-1} i.p. */**	0.4 ml kg^{-1} i.m. or i.p. **	0.5 ml kg^{-1} i.m. or i.p. **	0.5 ml kg^{-1} i.m. or i.p. **
Hypnorm + diazepam	0.4 ml kg^{-1} i.p. 5 mg kg^{-1} i.p. ***	0.3 ml kg^{-1} i.m. 2.5 mg kg^{-1} i.p. ***	1 ml kg^{-1} i.m. 5 mg kg^{-1} i.p. or i.v. ***	0.3 ml kg^{-1} 5 mg kg^{-1} i.p. ***
Hypnorm + midazolam (2 parts water for injection + 1 part Hyp. + 1 part midaz.) (5 mg ml^{-1})	10 ml kg^{-1} i.p. ***	2.7 ml kg^{-1} i.p. ***	4 ml kg^{-1} i.p. ***	8 ml kg^{-1} i.p. ***
Ketamine + medetomidine	75 mg kg^{-1} i.p. 1 mg kg^{-1} i.p.	75 mg kg^{-1} i.p. 0.5 mg kg^{-1} i.p.	—	—
Ketamine + xylazine	150 mg kg^{-1} i.p. 10 mg kg^{-1} i.p. **/***!	90 mg kg^{-1} i.p. 10 mg kg^{-1} i.p. **/***	200 mg kg^{-1} i.p. 10 mg kg^{-1} i.p. ***	50 mg kg^{-1} i.p. 2 mg kg i.p. **
Morphine	2.5 mg kg^{-1} s.c. 2–4 hourly	2.5 mg kg^{-1} s.c. 2–4 hourly	—	—
Nalbuphine	4–8 mg kg^{-1} i.m. ?4 hourly	1–2 mg kg^{-1} i.m. 3 hourly	—	—
Naloxone	0.01–0.1 mg kg^{-1} i.p., i.v.	0.01–0.1 mg kg^{-1} i.p., i.v.	0.01–0.1 mg kg^{-1} i.p., i.v.	0.01–0.1 mg kg^{-1} i.p., i.v.
Pentobarbitone	45 mg kg^{-1} i.p. **/***!	45 mg kg^{-1} i.p. **/***!	50–90 mg kg^{-1} i.p. **/***!	60–80 mg kg^{-1} i.p. **/***!
Pethidine	10–20 mg kg^{-1} s.c. or i.m. 2–3 hourly	10–20 mg kg^{-1} s.c. or i.m. 2–3 hourly	—	—
Propofol	26 mg kg^{-1} i.v.	10 mg kg^{-1} i.v.	—	—
Thiopentone	30–40 mg kg^{-1} i.v. ***	30 mg kg^{-1} i.v. ***	—	—

Drug	Guineapig	Rabbit	Dog	Cat
Alphaxalone/ alphadolone	40 mg kg^{-1} i.v. **	6–9 mg kg^{-1} i.v. ***!	—	9–12 mg kg^{-1} i.v. *** 12–18 mg kg^{-1} i.m.
Atropine	0.05 mg kg^{-1} s.c., i.m.	0.05 mg kg^{-1} i.m.	0.05 mg kg^{-1} s.c., i.m.	0.05 mg kg^{-1} s.c., i.m.
Buprenorphine	0.05 mg kg^{-1} s.c. 8–12 hourly	0.01–0.05 mg kg^{-1} s.c. or i.v. 8–12 hourly	0.01–0.02 mg kg^{-1} i.m., s.c., i.v. 8–12 hourly	0.005–0.01 mg kg^{-1} s.c. or i.v. 8–12 hourly
Butorphanol	—	0.1–0.5 mg kg^{-1} i.v. 4 hourly	0.4 mg kg^{-1} s.c. or i.m. 3–4 hourly	0.4 mg kg^{-1} s.c. 3–4 hourly
Doxapram	5 mg kg^{-1} i.v.	2–5 mg kg^{-1} i.v.	2–5 mg kg^{-1} i.v.	2–5 mg kg^{-1} i.v.
Flunixin	—	1.1 mg kg^{-1} s.c., i.m. ?12 hourly	1 mg kg^{-1} per os daily	1 mg kg^{-1} s.c., daily for up to 5 days
Ketamine	100 mg kg^{-1} i.p. **	50 mg kg^{-1} i.m. **	—	20 mg kg^{-1} i.m. **
Ketamine + medetomidine	40 mg kg^{-1} i.p. 0.5 mg kg^{-1} i.p.	25 mg kg^{-1} i.m. 0.5 mg kg^{-1} i.m.	—	—
Ketamine + xylazine	40 mg kg^{-1} i.p. 5 mg kg^{-1} i.m. **/***	25 mg kg^{-1} i.m. 5 mg kg^{-1} i.m. ***	5 mg kg^{-1} i.v. 1–2 mg kg^{-1} i.v., i.m. ***	15 mg kg^{-1} i.m. 1 mg kg^{-1} s.c., i.m. ***
Morphine	2–5 mg kg^{-1} s.c. or i.m. 4 hourly	2–5 mg kg^{-1} s.c. or i.m. 2–4 hourly	0.5–5 mg kg^{-1} s.c. or i.m. 4 hourly	0.1 mg kg^{-1} s.c. 4 hourly
Nalbuphine	—	1–2 mg kg^{-1} i.v. 4–5 hourly	0.5–2.0 mg kg^{-1} s.c., i.m. 3–8 hourly	1.5–3.0 mg kg^{-1} i.v. 3 hourly
Naloxone	0.01–0.1 mg kg^{-1} i.p., i.v.	0.01–0.1 mg kg^{-1} i.m., i.v.	0.01–0.05 mg kg^{-1} i.m., i.v.	0.01–0.05 mg kg^{-1} i.m., i.v.

Drug	Primates	Pig	Sheep/goats	Ferret	Drug	Primates	Pig	Sheep/goats	Ferret
Hypnorm (fentanyl/ fluanisone)	0.65 ml kg^{-1} i.m. **	0.22 ml kg^{-1} i.m. *	0.1–0.2 ml kg^{-1} i.m. **	—	Pentobarbitone	37 mg kg^{-1} i.p. **/***!	30–45 mg kg^{-1} i.v. ***!	20–30 mg kg^{-1} i.v. ***	25 mg kg^{-1} i.v. ***
Hypnorm + diazepam	1 ml kg^{-1} i.m. 2.5 mg kg^{-1} i.p. ***	0.3 ml kg^{-1} i.m. 2 mg kg^{-1} i.p. or i.v. ***	—	—	Pethidine	10–20 mg kg^{-1} s.c. or i.m. 2–3 hourly	10 mg kg^{-1} s.c. or i.m. 2–3 hourly	10 mg kg^{-1} i.m. 2–3 hourly	10 mg kg^{-1} s.c. or i.m. 2–3 hourly
Hypnorm + midazolam (2 parts H_2O + 1 part Hyp. + 1 part midaz.) (5 mg ml^{-1})	8 ml kg^{-1} i.p. ***	0.3 ml kg^{-1} i.m. + 2 mg kg^{-1} i.v. or i.p. ***	—	—	Propofol	—	10 mg kg^{-1} i.v.	5–7.5 mg kg^{-1} i.v. ***	7.5 mg kg^{-1} i.v. ***
Ibuprofen	10 mg kg^{-1} i.m. ?4 hourly	10 mg kg^{-1} i.v. ?4 hourly	5–10 mg kg^{-1} per os 24–48 hourly	—	Thiopentone	—	30 mg kg^{-1} i.v. ***	10–20 mg kg^{-1} i.v. ***	10–15 mg kg^{-1} i.v. ***
Alphaxalone/ alphadolone	12–18 mg kg^{-1} i.m. **/*** 10–12 mg kg^{-1} i.v.***	6 mg kg^{-1} i.m. ** then 2 mg kg^{-1} i.v. ***	2.2 mg kg^{-1} i.v. (adult) 6 mg kg^{-1} i.v. (lamb)***	12–15 mg kg^{-1} i.m.**/*** 8–12 mg kg^{-1} i.v.***	Ketamine + xylazine	10 mg kg^{-1} i.m. 0.5 mg kg^{-1} i.m. **/***	5 mg kg^{-1} i.m. 1 mg kg^{-1} i.m. */**	4 mg kg^{-1} i.v. 1 mg kg^{-1} (sheep) 0.05 mg kg^{-1} (goat)*/**	25 mg kg^{-1} i.m. 2 mg kg^{-1} i.m. ***
Atropine	0.05 mg kg^{-1} i.m.	0.05 mg kg^{-1} i.m.	0.05 mg kg^{-1} i.m.	0.05 mg kg^{-1} i.m.	Methohexitone	10 mg kg^{-1} i.v. **/***	5 mg kg^{-1} i.v. ***	4 mg kg^{-1} i.v. ***	—
Buprenorphine	0.01 mg kg^{-1} i.m. 8–12 hourly	0.01–0.05 mg kg^{-1} i.m. 8–12 hourly	0.005–0.01 mg kg^{-1} i.m. 4–6 hourly	—	Morphine	1–2 mg kg^{-1} s.c. 4 hourly	up to 20 mg total dose i.m. 4 hourly	10 mg total dose i.m., s.c. 4 hourly	—
Doxapram	2 mg kg^{-1} i.v.	1–2 mg kg^{-1} i.v.	2 mg kg^{-1} i.v.	1–2 mg kg^{-1} i.v.					
Flunixin	?2.5–10.0 mg kg^{-1} i.m. daily	?1 mg kg^{-1} s.c. daily	?1 mg kg^{-1} s.c. daily	—	Naloxone	0.01–0.05 mg kg^{-1} i.v., i.m.	0.01–0.05 mg kg^{-1} i.v., i.m.	0.01–0.05 mg kg^{-1} i.v., i.m.	—
Hypnorm (fentanyl/ fluanisone)	0.3 ml kg^{-1} i.m. **	—	— **	0.5 ml kg^{-1} i.m.	Pentobarbitone	5–15 mg kg^{-1} i.v. ***	30 mg kg^{-1} i.v. **/***	30 mg kg^{-1} i.v. ***	25–30 mg kg^{-1} i.v. ***
Ketamine	5–25 mg kg^{-1} i.m. */***	10 mg kg^{-1} i.m. **	20 mg kg^{-1} i.p. *	10–30 mg kg^{-1} **	Pethidine	2–4 mg kg^{-1} i.m. 3–4 hourly	2 mg kg^{-1} i.m. 4 hourly	200 mg i.m. total dose 4 hourly	—
Ketamine + diazepam	15 mg kg^{-1} i.m. 1 mg kg^{-1} i.m. **/***	10 mg kg^{-1} i.m. 2 mg kg^{-1} i.m. **	4 mg kg^{-1} i.v. 2 mg kg^{-1} i.m. or 1 mg kg^{-1} i.v.***	25 mg kg^{-1} i.m. 2 mg kg^{-1} i.m. ***	Propofol	—	2.5–3.5 mg kg^{-1} i.v.	3–4 mg kg^{-1} i.v.	
					Thiopentone	15–20 mg kg^{-1} i.v. **/***	6–9 mg kg^{-1} i.v. ***	15 mg kg^{-1} i.v. ***	—

It is also essential to pass a tracheal tube in these species, to prevent inhalation of liquid rumen contents which are regurgitated during anaesthesia.

If it is intended that the animal recovers from anaesthesia, then equipment required for post-operative care should be prepared for use before commencing the experimental procedure (see below).

Handling and restraint. Restraint of the subject to allow easy induction of anaesthesia frequently presents difficulties when working with animals. Parallels may be drawn with human paediatric practice, in which stormy inductions can result because of a distressed and agitated patient. To avoid this problem, it is strongly recommended that the assistance of an experienced animal technician is obtained. Training in correct animal handling techniques should be sought and, although practical instruction is essential, it may be helpful to consult publications such as those by Anderson and Edney [1] and Tuffrey [27]. To avoid the need for excessive physical restraint, use can be made of anaesthetic induction chambers or a tranquillizer or sedative can be administered before induction of anaesthesia.

Anaesthetic maintenance

The general principles of monitoring and supporting vital body functions during anaesthesia apply equally to animals and man, but maintenance of body temperature is of special importance when anaesthetizing small rodents. The techniques used when managing human paediatric patients may be used, and a range of purpose-made heating pads and blankets are available commercially. Body temperature can be monitored using standard human electronic thermometers, although the probe size of these devices is usually too large for mice, and specially designed probes are needed to monitor temperature in this species.

Monitoring of respiratory function is simple in large animals, and respiratory monitors, capnographs and respirometers designed for human use can be used successfully. Small animals (< 1 kg) present special problems; for example, the sampling volume of a capnograph frequently exceeds the minute volume of these animals. Similarly, the small tidal volumes may fail to trigger thermistor devices used to monitor ventilatory frequency. Capnograph probes and respiratory monitors that are provided with a mount for incorporation into a standard diameter anaesthetic circuit may introduce excessive equipment deadspace and should be modified before use in small animals. The most useful monitoring device is a pulse oximeter, which can be used for rats (positioned on the hind foot pad), rabbits (on the ear, tongue or nose) and in a variety of sites in larger species. It is important to assess a manufacturer's instrument before embarking on a study, as considerable variation in performance is encountered.

Monitoring of blood-gas variables in laboratory species is feasible, providing that an analyser capable of accepting small sample volumes is used. Percutaneous arterial cannulation is practicable in rabbits and larger species, but surgical exposure of vessels is required in rats and guineapigs. In small animals, it is advisable to replace the blood withdrawn for analysis with whole blood from a donor animal. Cross-matching is rarely practicable, but initial transfusion reactions are rare. As with man, blood should be collected from a donor animal into ACD. Information on appropriate storage times of blood from laboratory species is not available and it is advisable to obtain blood for transfusion shortly before it is required.

General principles of fluid therapy apply and replacement fluids such as plasma volume expanders and crystalloid solutions can be used in all species. Blood volume is generally 60–70 ml kg^{-1}, so there may be practical problems related to the very small infusion rates required by small animals.

Monitoring of cardiovascular function can be carried out using techniques similar to those used in man. Invasive arterial pressure monitoring is practicable in most species, although problems may be encountered when attempting arterial cannulation in very small animals such as mice. In larger species, percutaneous catheterization enables routine monitoring of arterial pressure. Non-invasive monitoring equipment may be used in cats, dogs and larger animals, but it is important to assess the performance of different manufacturers' equipment, as the ability to detect low amplitude signals varies considerably. Specially designed apparatus is available to monitor arterial pressure in small rodents via a cuff placed around the tail.

The ECG can be monitored using standard techniques, although some apparatus designed for use in man will not detect the low amplitude signals from small animals. A further complication arises from the rapid heart rates of some species. Monitoring devices for use in man often have a detection limit of 300 beat min^{-1}, and this rate is exceeded in small rodents and rabbits. A monitor specifically designed to overcome these problems is available (Silogic EC60, Silogic Design Ltd, Enterprise House, 181–189 Garth Rd, Morden, Surrey, U.K.).

Special problems

Some problems unique to the research environment may be encountered. One major issue concerns the potential interaction between the anaesthetic regimen used and the research design. When an anaesthetic regimen is formulated, it is important that the experimental design is considered in conjunction with the pharmacology of the anaesthetic agents, so that interactions may be minimized. This may require an extensive review of the relevant literature, but is essential if serious problems are to be avoided. It is important to appreciate that previous investigators may not have considered this problem adequately, and adoption of an anaesthetic regimen from a previously published study does not guarantee that it is appropriate for the present purpose.

A requirement of some research projects is the provision of stable, long lasting anaesthesia, with minimal depression of the circulatory or respiratory systems. Several compounds unfamiliar to human anaesthetists, such as urethane [7] and chloralose,

may be used for this purpose. Although the claims made for these agents are well established by historical precedence, it is advisable to review the existing literature critically before accepting them without question.

Anaesthetists may occasionally be asked to assist in studies involving non-mammalian species, such as fish, reptiles, birds and amphibia. These species often respond very differently to anaesthetic regimens that are satisfactory in mammals, and it is strongly advised that specialist advice is sought before anaesthetizing these animals.

Postoperative care

As in human anaesthetic practice, monitoring procedures and supportive therapy should be continued into the postanaesthetic recovery period. It is important to appreciate that the extensive nursing support available in human clinical practice may not be available in a research animal unit. Responsibility for the provision of postoperative care rests with the anaesthetist, and this may require the establishment of a specialized recovery area. For small animals, a human infant incubator can be used to provide a suitable environment. Assistance should be sought from staff in the facility to set up recovery pens for larger species. Although all animals require nursing attention during recovery, the response to physical contact varies considerably. Companion animal species which have become accustomed to handling, such as dogs and cats, respond positively to attention from their handlers, but other species such as mice and guineapigs may find such attention stressful.

It is important to monitor fluid and food intake and body weight in the postoperative period and provide supportive therapy as necessary. In small animals, supplementary fluids can be provided by administering 4% glucose–0.18% saline or 0.9% saline by the s.c. or i.p. routes, the initial dose being administered before recovery from anaesthesia. Larger species can receive i.v. therapy, but special measures may be required to prevent the animal interfering with infusion cannulae. If prolonged drug or fluid therapy is required, it may be appropriate to implant an indwelling catheter, which can be tunnelled to emerge between the shoulders at the base of the neck, so that the animal cannot interfere with it. Techniques for cannulation have been reviewed by Desjardins [6].

ASSESSMENT AND ALLEVIATION OF POSTOPERATIVE PAIN

The provision of adequate pain relief following surgical procedures is of special importance. As animals are unable to communicate directly with man, problems of pain assessment similar to those encountered with neonatal humans arise. Two approaches to the recognition of pain have been suggested: either anthropomorphic criteria may be used, based on a knowledge of the degree of pain experienced by humans after comparable surgical procedures, or methods of assessment based on observations of the animal's behaviour can be developed. An anthropomorphic approach has serious limitations, as it requires uncritical assumptions to be made, not only that pain occurs in the same circumstances in animals and man, but also that pain intensity, duration and analgesic requirements are identical. Knowledge of the individual variations which occur between human patients indicates that such assumptions are untenable. Another limitation of this approach is that it precludes any comparative assessment of different types of analgesic regimens.

The second approach of attempting to assess changes in behaviour that may be pain related has many similarities to techniques used for pain assessment in human neonates and infants. Several schemes for pain assessment and pain scoring have been published [2, 22], but attempts to use them to assess pain have had limited success [3]. Recently, however, studies in which trained observers completed a linear analogue pain score on behalf of their veterinary patients have proven encouraging [23]. All these scoring systems require the assessor to have a good knowledge of the normal behaviour of the animal species concerned, and preferably to be familiar with the normal behaviour of the individual animal. This requires considerable experience, and it is often advisable to seek the advice of a laboratory animal veterinary surgeon or experienced animal technician when attempting such an assessment. It is important to note that many laboratory rodents are most active at night, and an assessment carried out during the light phase of their photoperiod may not be particularly useful. As an alternative to the relatively subjective approach of behavioural observations and scoring of clinical appearance, the use of food and water intake and body weight as indices of postoperative pain have been evaluated. Initial investigations suggest that these variables are depressed following surgery, and that analgesic agents reduce the magnitude of this effect [11]. Body weight is a simple variable to monitor, and may provide a reasonably reliable indicator of the efficacy of analgesic therapy in small rodents.

Management of acute pain in animals

The methods available for the management of acute postoperative pain in animals are similar to those which are used in man. Centrally or peripherally acting analgesics may be administered systemically or techniques involving the use of local anaesthetics may be used. The application of supporting bandages or other means of protecting and immobilizing damaged tissues should also be considered. Selection of a particular regimen of treatment varies depending upon the nature of the pain, its cause and its estimated severity and duration.

The majority of animals which have undergone surgical procedures require opioids to produce effective pain relief. All the opioids available for clinical use in man have undergone testing for efficacy in laboratory animals and hence dose regimens can be suggested on the basis of these data [8]. The efficacy of these dose regimens in treating clinical pain remains uncertain, as the agents were assessed using experimental analgesiometry (for example the tail flick test). As our understanding of

postoperative pain in animals improves, it should be possible to revise the dose rates listed in table IV.

In research animal facilities, it is rarely possible to provide the level of nursing care available in human clinical practice. This creates problems in providing prolonged periods of pain relief, as most opioids require repeated administration at 3–4 h intervals. One approach is to use buprenorphine, which has been shown to have a long duration of action (6–12 h) in several species (rat [5], rabbit [10] and pig [16]). An alternative approach is to adapt the technique of continuous infusion of opioids which has been developed in humans. In animals, opioids can be administered continuously either via i.v. fluids administered via a giving-set, or by bandaging a portable, battery-operated syringe driver to the animal (e.g. Graseby Dynamics MS16). Prolonged analgesia can also be produced by extradural morphine, although at present this technique has been used clinically only in larger species such as the dog, pig, sheep and horse. The undesirable side effects of opioids that are of concern in man must also be considered when administering these compounds to animals, but in general they are of considerably less clinical significance. Respiratory depression is perhaps the most serious consequence of overdose with opioids in man. This side effect may be seen occasionally in animals if large doses of morphine or pethidine are administered, or if potent μ agonists such as fentanyl or alfentanil are used. Significant respiratory depression occurs rarely after the use of normal clinical doses of morphine and pethidine, and has yet to be reported as a problem after administration of mixed agonist–antagonist drugs such as buprenorphine, nalbuphine and butorphanol.

Non-steroidal anti-inflammatory drugs are of use in the management of mild to moderate pain in animals, and may be of particular value in circumstances in which the use of opioids is contraindicated because of the requirements of a particular experimental procedure. Some recently introduced NSAID, such as flunixin and carprofen, have an efficacy which may approach that of some opioids, and these compounds are currently undergoing clinical evaluation.

Local anaesthetics such as bupivacaine may be used in the same way as in man, to provide local nerve blocks, for example after thoracotomy.

As in man, the requirement for postoperative analgesics rarely exceeds 72 h, and in most instances provision of pain relief for 24–48 h appears adequate.

REFERENCES

1. Anderson RS, Edney ATB. *Practical Animal Handling*. Oxford: Pergamon Press, 1991.
2. Association of Veterinary Teachers and Research Workers. *Guidelines for the Recognition and Assessment of Pain in Animals*. South Mimms, Potters Bar: Universities Federation for Animal Welfare, 1989.
3. Beynen AC, Baumans V, Bertens APMG, Havenaar R, Hesp APM, Van Zutphen LFM. Assessment of discomfort in gallstone-bearing mice: a practical example of the problems encountered in an attempt to recognize discomfort in laboratory animals. *Laboratory Animals* 1987; **21**: 35–42.
4. Costa DL, Lehmann JR, Harold WM, Drew RT. Transoral tracheal intubation of rodents using a fibreoptic laryngoscope. *Laboratory Animal Science* 1986; **36**: 256–261.
5. Cowan A, Lewis JW, Mcfarlane IR. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *British Journal of Pharmacology* 1987; **60**: 537–545.
6. Desjardins C. Indwelling vascular cannulas for remote blood sampling, infusion, and long-term instrumentations of small laboratory animals. In: Gay WI, Heavner JE, eds. *Methods of Animal Experimentation*, Vol. VII, Part A. London: Academic Press, 1985; 143.
7. Field KJ, Lang CM. Hazards of urethane (ethyl carbamate): a review of the literature. *Laboratory Animals* 1988; **22**: 252–262.
8. Flecknell PA. The relief of pain in laboratory animals. *Laboratory Animals* 1984; **18**: 147–160.
9. Flecknell PA. *Laboratory Animal Anaesthesia*. London: Academic Press Ltd, 1987.
10. Flecknell PA, Liles JH. Assessment of the analgesic action of opioid agonist–antagonists in the rabbit. *Journal of the Association of Veterinary Anaesthetists* 1990; **17**: 24–29.
11. Flecknell PA, Liles JH. The effects of surgical procedures, halothane anaesthesia and nalbuphine on locomotor activity and food and water consumption in rats. *Laboratory Animals* 1991; **25**: 50–60.
12. Flecknell PA, Liles JH, Williamson HA. The use of lignocaine–prilocaine local anaesthetic cream for pain-free venepuncture in laboratory animals. *Laboratory Animals* 1990; **24**: 142–146.
13. Flecknell PA, Liles JH, Wootton R. Reversal of fentanyl/fluanisone neuroleptanalgesia in the rabbit using mixed agonist/antagonist opioids. *Laboratory Animals* 1989; **23**: 147–155.
14. Green CJ. *Animal Anaesthesia*, amended edition. Theydon Bois, Essex: Laboratory Animals Ltd, 1981.
15. Hall LW, Clarke KW. *Veterinary Anaesthesia*, 9th Edn. London: Baillière Tindall, 1991.
16. Hermansen K, Pedersen LE, Olesen HO. The analgesic effect of buprenorphine, etorphine and pethidine: a randomized double blind cross-over study. *Acta Pharmacologica et Toxicologica* 1986; **59**: 27–35.
17. Hu C, Flecknell PA, Liles JH. Fentanyl and medetomidine anaesthesia in the rat and its reversal with using atipamazole and either nalbuphine or butorphanol. *Laboratory Animals* 1992; **26**: 15–22.
18. Hunter SC, Glen JB, Butcher CJ. A modified anaesthetic vapour extraction system. *Laboratory Animals* 1984; **18**: 42–44.
19. Loeb WF, Quimby FW. *The Clinical Chemistry of Laboratory Animals*. New York: Pergamon Press, 1989.
20. Lovell DP. Variation in pentobarbitone sleep time in mice. 1. Strain and sex differences. *Laboratory Animals* 1986; **20**: 85–90.
21. Mitruka BM, Rawnsley HM. *Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals*. U.S.A.: Masson Publishing, 1977.
22. Morton DB, Griffiths PHM. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* 1985; **116**: 431–436.
23. Reid J, Nolan AM. A comparison of the postoperative analgesic and sedative effects of flunixin and papaveretum in the dog. *Journal of Small Animal Practice* 1991; **32**: 603–608.
24. Remie R, Bertens APMG, Van Dongen JJ, Rensema JW, Van Wunnik GHJ. Anaesthesia of the laboratory rat. In: Van Dongen JJ, Remie R, Rensema JW, Van Wunnik GH, eds. *Manual of Microsurgery on the Laboratory Rat*, Part 1. Amsterdam: Elsevier, 1990.
25. Short CE. *Principles and Practice of Veterinary Anaesthesia*. Baltimore: Williams and Wilkins, 1987.
26. Smiler KL, Stein S, Hrapkiewicz KL, Hiben JR. Tissue response to intramuscular and intraperitoneal injections of ketamine and xylazine in rats. *Laboratory Animal Science* 1990; **40**: 60.
27. Tuffrey AA. *Laboratory Animals: An Introduction for New Experimenters*. Chichester: John Wiley and Sons Ltd, 1987.