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The technique of inhalation anaesthesia in experimental investigation in the rat

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The rat is the most frequently used animal in scientific inquiry conducted for the purpose of advancing basic knowledge that may lead to an improvement in the results of treatment. Understanding of the pharmacological properties of inhalation anaesthetics, in combination with monitoring of their concentration in the inspired and end-tidal gas, together provide safe and precise control of the depth of the anaesthesia. However, accurate application of the inhalation method of anaesthesia requires special equipment for the delivery and effective scavenging of inhalation anaesthetics.

Key words: inhalational anaesthesia, desflurane, isoflurane, sevoflurane, rat

Rats have been well investigated, and a large amount of information about their biology has been established. Table 1 presents a list of the rat's basic biological data [1]. Experimental procedures on animals require their loss of consciousness and complete suppression of the perception of pain. This can be achieved by general anaesthesia, which can be induced by using a variety of agents and techniques. Injectable anaesthesia requires the application of one or more anaesthetic agents via intramuscular, intravenous, or intraperitoneal injection [8, 12, 21].

Inhalation anaesthesia, however, offers better control and the ability to change rapidly the level of anaesthesia and to take over breathing for the animal. Because the anaesthetics are eliminated from the blood by exhalation, with less reliance on drug metabolism to remove the drug from the body, there is less chance of drug-induced toxicity. The disadvantages of inhalation anaesthesia are the complexity and cost of the equipment needed to administer the anaesthesia and the potential hazards to personnel.

Table 1. Physiological variables of adult rat [1]

Body weight: male/female	300–400/250–300 g
Rectal temperature	38–39°C
Heart rate	330–480 bmp (beat per min)
Blood pressure: diastolic/systolic	58–145/88–184 mm Hg
Respiratory rate	85.5 (66–114) breaths/min
Tidal volume	0.86 (0.6–1.25) ml
Maximum volume of single bleeding	5 ml/kg body weight
Blood volume	5.6–7.1 ml/100 g body weight
Plasma volume	3.08–3.,67 ml/100 g body weight
Red blood cell count	7.2–9.6 $\times$ 10^6 cells/μl
Haemoglobin	11–19 g/dl
Packed cell volume	46%
Total leukocyte count	14 (5–25) $\times$ 10^3/μl
Neutrophils	22 (9–34)%
Lymphocytes	73 (65–84)%
Platelets	1240 (1100–1380) $\times$ 10^3/μl

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The frequent use of even a single agent produces all the required features of general anaesthesia: unconsciousness, analgesia, suppression of reflex activity and muscle relaxation. Newer inhalational anaesthetic agents may be applied such as desflurane, isoflurane and sevoflurane [8, 12–15].

Administration of the anaesthetic agents by inhalation requires a device that is designed to supply oxygen, air and volatile anaesthetic agents to the animal. The device should consist of sources of oxygen and air, a flowmeter and a vaporiser [8, 21]. The oxygen and air supply may be provided from a cylinder or from a central gas supply by means of a hose connection. The air and oxygen cylinders or pressurised hose connectors deliver the gases via a reducing valve to a flowmeter. The flowmeter enables the individual gas flow and the relative proportion of oxygen and air to be controlled (Fig. 1A). The most common type of flowmeter consists of a bobbin mounted in a glass tube. The gas flow is indicated by the position of the bobbin on the graduated scale on the tube. The gasses pass from the flowmeter and through a vaporiser, which is filled with an inhalational anaesthetic agent. Calibrated vaporisers are designed for use with particular volatile agents (Fig. 1A). When delivered by a calibrated vaporiser, the appropriate concentration of anaesthetic is not affected by changes in the gas flow or in the temperature of the gases [16].

Induction of the inhalational anaesthesia is achieved by means of an induction chamber (Fig. 1B). A partially transparent box enables the animal to be observed during induction. To attain rapid induction the chamber must be filled quickly. The time needed to fill the chamber completely with the respiratory mixture is dependant on the ratio of the chamber volume to the flow rate. The application of inhalational anaesthetics during surgical procedure requires the use of a face-mask or an endotracheal tube. The face-mask should fit snugly around the muzzle without obstructing the nose and mouth and providing minimal dead space (Fig. 1C). The placement of the endotracheal tube should be performed by intubation or tracheostomy. The face-mask or the endotracheal tube is connected to the source of the anaesthetic mixture by an anaesthetic breathing circuit. The idea of the T-piece breathing system as commonly used in paediatric anaesthesia is also applicable in laboratory anaesthesia (Fig. 1D) [8, 16, 21]. The main advantages of the T-piece circuit are its simplicity, low resistance and small dead space. The circuit is ideal for spontaneous breathing and its support. It consists of an expiratory limb, to which the anaesthetic gas mixture is supplied by a small side inlet tube. In order to prevent dilution with exhaled gas the capacity of the expiratory limb must be greater than the tidal volume of the animal and the fresh anaesthetic gas mixture twice the minute volume of the animal [8, 21]. One end of the T-piece system is connected to the face-mask or endotracheal tube, while the other is open. During the critical period ventilation can be controlled by intermittently occluding the end of the reservoir limb.

Volatile anaesthetic agents are assumed to be unaffected by passage through the body and to be eliminated unchanged from the lungs [16]. The potency of inhalation anaesthetics is expressed by its minimum alveolar concentration (MAC_{50}) value. MAC_{50} is the alveolar concentration of an anaesthetic agent which results in blocking the response to a specified painful stimulus in 50% of a group of animals [8, 16, 21]. This fact may act as a caution to ensure that practical anaesthesia reaches a concentration that exceeds MAC_{50} by 10% to 30% [8, 13–15].

The most important factor conditioning the speed of induction of anaesthesia and the rate of recovery is the blood/gas partition coefficient [16]. Lower solubilities indicate a more rapid increase and decrease in the alveolar concentration during induction of and recovery from anaesthesia than that observed with other potent inhaled agents that are more soluble. The partition coefficients of desflurane, isoflurane and sevoflurane are illustrated in Table 2.

These data suggest that induction of and recovery from anaesthesia may be more rapid with desflurane than with sevoflurane, and more rapid with sevoflurane than with isoflurane. Sevoflurane also benefits from an absence of pungency, which facilitates its use for an inhalational induction of anaesthesia [20].

It is well established that inhalation induction of the anaesthesia requires a concentration to values of approximately 2 MAC of the anaesthetic agent [21]. Depending on the grade of stress evoked by the

Table 2. Pharmacological properties of desflurane, isoflurane and sevoflurane in the rat [3, 4, 15, 19, 22, 23]

Parameter	Desflurane	Isoflurane	Sevoflurane
MAC_{50} (vol%)	7.1 ± 0.4	1.4 ± 0.2	2.3 ± 0.2
Blood/gas: partition coefficient	0.45	1.4	0.65

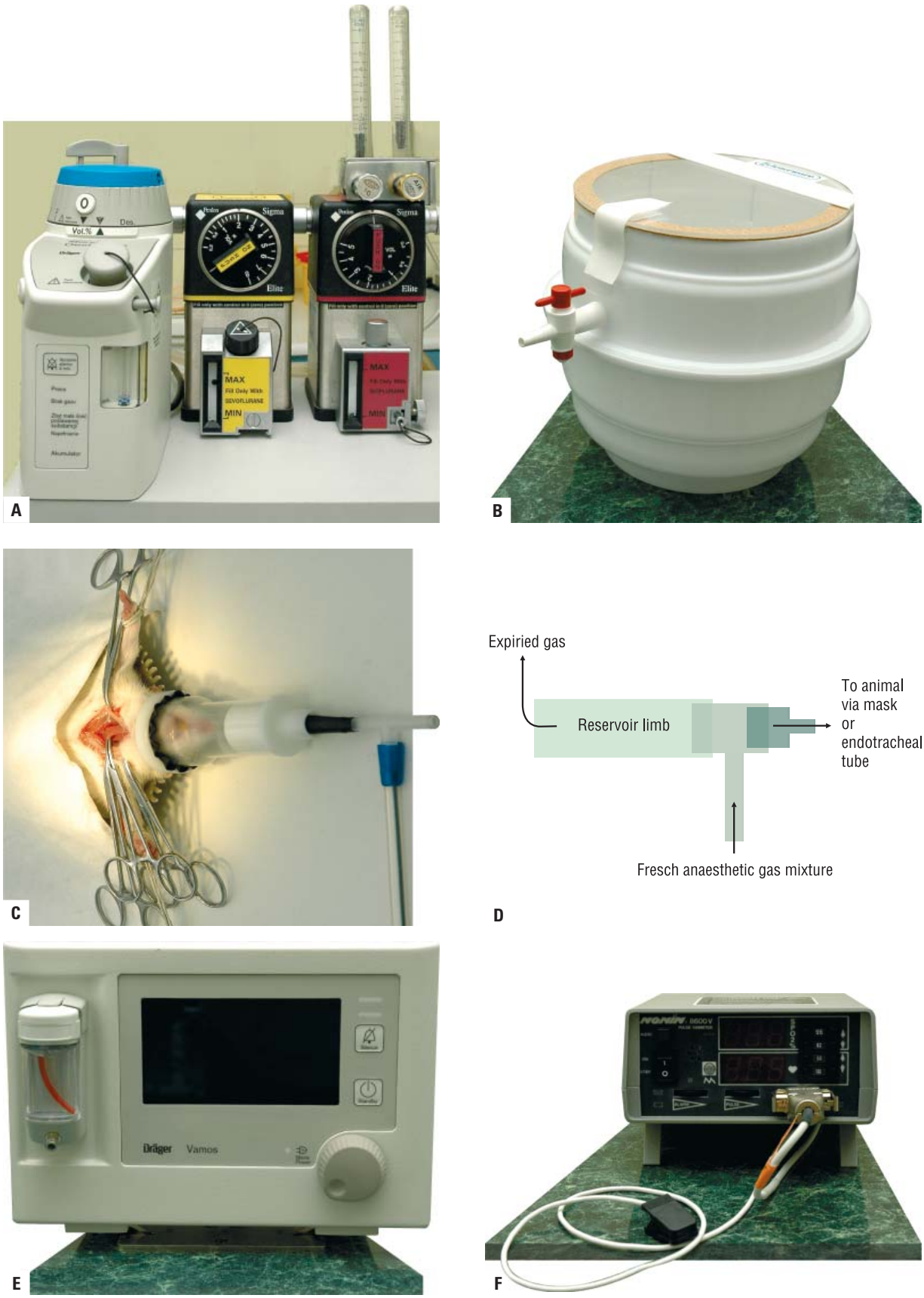

Figure 1. A. Equipment for inhalation anaesthesia (flowmeter for delivering air and oxygen, vaporisers for the use of sevoflurane, isoflurane and desflurane (Penlon, UK and Baxter, UK, respectively); **B.** Induction chamber for the rat; **C.** Rat ventilated by mask; **D.** Schema of T-piece circuit; **E.** CO_2 and anaesthetic gas analyser Vamos (Dräger, Germany); **F.** Pulse oximeter OX 8600 MV (Nonin Medical, Inc, USA).

laboratory procedure, maintenance of inhalation anaesthesia is normally accomplished by delivering approximately 1.2 MAC to an animal via a mask or directly into the lungs via an endotracheal tube [13, 14]. Intubation is recommended whenever possible, particularly when a procedure will be prolonged. Endotracheal access is essential to provide monitoring or/and support of ventilation. Regardless of the kind of ventilation, capnography has become a trusted aid during this process (Fig. 1E). Only the lungs can deliver enough CO_2 to generate one capnogram after another [10]. Intubation of a principal bronchus evokes lower end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) than tracheal intubation because the tidal volume is intended for ventilation of both lungs. Under physiological conditions the $P_{ET}CO_2$ mirrors the arterial partial pressure of CO_2 ($PaCO_2$) [10].

What has been said for capnography also applies to pulse oximetry. Pulse oximetry is able to discover the onset of hypoxaemia, and provide information on both pulse rate and peripheral perfusion (Fig. 1F) [10]. In order to maintain the comparability of the physiological condition during experiments in spontaneously ventilated animals capnography and pulse oximetry are absolutely required, allowing the appropriate level of mechanical ventilation to be maintained [13, 14].

The importance of monitoring the cardiovascular system and temperature in anaesthetised animals undergoing investigation was discussed in detail in our previous work [12].

In the last decade it has became common to monitor the concentration of inhalation anaesthetics in inspired and end-tidal gas (Fig. 1E). On the basis of investigations the concentration of inhalation anaesthetics required for general anaesthesia is well known [8, 13, 14, 21]. Regardless of the system and technique used, the data obtained from the anaesthetic gas analyser will be helpful in identifying the approach of equilibrium between inspired and end-tidal concentrations of anaesthetic vapour. During induction the concentration of the anaesthetic agent in the gas inspired should be much higher than needed to maintain anaesthesia. This procedure makes up for the time of induction. During maintenance of the anaesthesia the alveolar, tissue and venous concentrations of the anaesthetic sooner or later, depending on its solubility, reach equilibrium and the end-tidal partial pressure correctly reflects the partial pressure of the anaesthetic agent in the brain [10, 16].

All commonly used inhalation anaesthetics can safely be applied to small rodents, provided appropriate equipment is available. Desflurane, isoflurane and sevoflurane in a concentration of 1.5 MAC cause only a mild to moderate depression of the cardiovascular and/or respiratory systems [5, 6, 9, 11, 17, 18].

The breathing systems used with laboratory anaesthetic equipment tend to be open-style circuits. Some studies have suggested that occupational exposure to wasted anaesthetic gases may produce untoward effects in researchers and other operating theatre personnel [2] and time-weighted limits for the exposure of such personnel have thus been suggested. According to the National Institute for Occupational Safety and Health, exposure to inhaled anaesthetics should not exceed 2 parts per million [21]. On account of this, effective passive or active scavenging systems should be used in laboratories where gaseous anaesthesia is performed [7].

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