THE INFLUENCE OF BODY TEMPERATURE ON MAC OF HALOTHANE IN THE RABBIT DETERMINED USING CONTROLLED MECHANICAL AND HEAT STIMULATION

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DECLARATION

I declare that this thesis is my own work and composed by my self except for the acknowledged contributions.

A.T.H. Sobair

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PUBLISHED WORK

Part of this work has been published as a paper (Appendix F of this Thesis) entitled "A Mechanical Stimulator for the Determination of the Minimum Alveolar Concentration (MAC) of Halothane in the Rabbit" by Sobair, A.T.H, Cottrell, D.F. and Camburn M.A. In: Veterinary Research Communications 17 (1993) 375-385.

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ABSTRACT

The determination of the minimum alveolar concentration (MAC) of any inhalation anaesthetic requires repeated application of the stimulus. The investigation of the influence of body temperature on MAC in the same animal, requires even more applications of the stimulus. The use of what is commonly known as a "supramaximal stimulus", beyond which there is no increase in MAC with any further increase in stimulus intensity, is generally held to provide for the reproducibility of MAC. However, there are many conflicting reports with regard to the MAC values obtained using "supramaximal stimuli" and with the interpretation of results.

In this study the unreliability of the "supramaximal stimulus", in the form of tail clamping using a haemostat for the determination of MAC of halothane in the rabbit, was established. The use of this technique was demonstrated, both grossly and histologically, to cause severe trauma and lacerations that may have altered the sensitivity of the sensory mechanisms as indicated by the great variability in the values of MAC obtained on subsequent determinations in the same animal. Also, the technique appeared to disregard modern physiological concepts on receptor thresholds, inflammation and hyperalgesia.

Therefore, it was decided to explore more reliable techniques of stimulation. Controlled mechanical and heat stimulators were devised and used for the determination of MAC of halothane in the rabbit as the body temperature was manipulated to test the effect of environmental heating or cooling on MAC.

The mechanical stimulator is a form of pincer driven by compressed air, and is operated from a control panel with a manual trigger. It is capable of delivering a precise stimulus at a preselected pressure to the target site (ear pinna). The stimulus was calculated in terms of Newtons per unit surface area after calibration of the device using gram weights. The heat stimulator was designed to deliver a controlled, preset focal heat stimulus from a heat lamp for a preselected duration The device is

operated through a system of electronic circuits controlling the temperature and time. With both stimulators, a just noxious level of stimulation was applied.

The mechanical and heat stimulators shared some common advantages over the established clamping techniques used for the determination of MAC. Firstly, both of them were shown to be capable of delivering precise stimuli in terms of amount, duration and rate of application. The control of such components of the stimulus is essential for the reproducibility and interpretation of results, particularly in the ever growing field of comparative studies. Secondly, the stimulus applied by both stimulators was recordable. This allows for the calculation and the calibration of the applied stimulus and, together with the electronically measured and recorded movement response, it was also possible to calculate the response time.

At normothermia, the MAC values of halothane determined in the rabbit usingnon-destructive stimuli from either stimulator were found to be consistent and reproducible. Hyperthermia (rectal temperature of 40.6 ^{o}C +/- 0.3 (SD) was demonstrated to cause a significant increase in MAC of halothane (12.3% per 1^{o}C rise in body temperature) when determined using a mechanical stimulus of 1.37 +/- 0.04 (SD) N/mm^2. On the other hand, no significant difference was detected in MAC determined using a focal heat stimulus of 54.35 ^{o}C +/- 0.09 (SD) in hyperthermic rabbits (41.6 ^{o}C +/- 0.4 (SD). Hypothermia of 4 ^{o}C was shown to reduce the MAC of halothane in the rabbit by 50% (12.8% per 1 ^{o}C drop in body temperature) to a focal heat stimulus of 54.3 ^{o}C, and by 35.1% (9 % per 1 ^{o}C decrease in body temperature) to a mechanical stimulus of 1.39 - 1.41 N/mm^2. By extrapolation of results, it was predicted that the "anaesthetising body temperature", at which no halothane would be required to abolish movement response to such stimuli, would be 31.0 ^{o}C and 27.7 ^{o}C for the heat and mechanical stimuli respectively. It is concluded that, normal doses of potent anaesthetics similar to halothane should probably be considerably reduced in hypothermic rabbits, which must be closely monitored for signs of overdose.

In the initial stages of this study some problems were encountered regarding the determination of MAC in hyperthermic rabbits due to the difficulty in the control of animal heating. As a result a section of this thesis was devoted to the establishment of a controlled and reliable heating and cooling protocol, with some emphasis on selected cardiopulmonary parameters and some stimulus-related responses. A body temperature of 42.0 ^{o}C appeared to represent a critical temperature in the rabbit above which the normal physiological responses are interfered with and also above that temperature, induced cooling was ineffective in reducing the body temperature.

Stimulus-related responses, other than movement, included changes in arterial blood pressure, apnoea and hyperpnoea. Such responses, particularly those of the arterial blood pressure, showed both quantitative and qualitative inconsistency. Therefore it was concluded that these responses are not reliable end points for the determination of MAC in the rabbit. On the other hand, the provoked movement of the head and or limbs, remains unchallenged as the most reliable end point for the determination of MAC.

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CHAPTER 1

LITERATURE REVIEW

1.1: Anaesthetic Depth And Potency:

Until the middle of the 19th century, the elimination of pain during surgical operations represented a real challenge to the medical and veterinary professions. The first breakthrough came as a result of the pioneering work of Dr. Morton, a skilful dentist, and Bigelow (1847) who used ether vapour for carrying out a number of different surgical operations without pain sensation on the part of the patient.

Despite that progress, the road to insensibility appeared to have been blocked by the problem of controlling the dosages of these agents used for the production of insensibility. The first approach towards the solution of that problem was triggered by Snow (1858) who drew the attention to the use of pricking stimuli to ascertain patient insensibility with the conclusion that blunting of responses to such stimuli, was related to anaesthetic depth. With the introduction of chloroform and ether, Snow introduced the concept of anaesthetic potency by describing chloroform as being about six times more potent than ether.

Since then, the work of subsequent investigators has lead to the development of a number of ways and indices for the expression of the anaesthetic potencies and dosages of inhalation agents:

1.1.1: The Anaesthetic Tension:

This term was employed by Boothby (1914): "to indicate the partial pressure of ether vapour that, after equilibrium is established, can for an indefinite period, maintain the subject in the stage of ideal surgical anaesthesia." Apparent variation in the anaesthetic tension in different patients, were attributed to changes in volume of respiration, changes in rate of circulation and changes in the chemical environment in which the reversible reactions between ether and lipoid, take place. With the advancement of knowledge and the development of what is commonly known as balanced anaesthesia, it might be difficult to define "ideal anaesthesia". In one of the earliest attempts to define anaesthesia, unconsciousness, analgesia, muscle relaxation and the suppression of motor somatic and autonomic reflexes to noxious stimulation were all considered to be components of anaesthesia (Woodbridge, 1957). More recently, however, it was considered to be more logical to restrict the definition of anaesthesia to the state of unconsciousness that ensures the suppression of pain perception to noxious stimulation. The other components such as analgesia, muscle relaxation and the blockade of autonomic activity, were found to be achievable independently by specific drugs (Prys-Roberts, 1987).

1.1.2: Physiological Responses:

Various degrees of etherisation (anaesthetic depth) were expressed in terms of ether tensions that were associated with specific physiological responses (Haggard, 1924). Accordingly and at specific tensions, the animal can be regarded as being: a) lightly anaesthetised, when the corneal reflex is present; b) moderately anaesthetised when the corneal reflex is absent; and c) deeply anaesthetised. In addition ether tensions associated with various degrees of respiratory depression have also been determined.

Although some ocular reflexes such as eyeball movement and the palpebral reflex are still regarded to be fairly reliable signs of anaesthetic depth in large animals (Hall and Clarke, 1991a), their reliability in laboratory animals have been questioned as these signs can be affected by the type of anaesthetic and the concomitantly used drugs as well as the species of animals. For instance, the palpebral reflex in the rabbit might still be detected until dangerously deep levels of anaesthesia have been reached (Flecknell, 1987a).

The use of these physiological responses were shown not to be fully applicable to other anaesthetic agents, such as cyclopropane, which lack the irritant properties of agents such as ether and chloroform which, by causing laryngospasm, are more protective against the inhalation of extremely high concentrations. Also unlike ether, cyclopropane is not a respiratory stimulant (Waters and Schmidt, 1934). At present, these problems can be avoided by using modern calibrated vaporisers that allow the various inhalation anaesthetics to be delivered in a constant and controlled concentrations (Hall and Clarke, 1983).

1.1.3: Anaesthetic Dose 50 (AD50):

This is one of the anaesthetic indices of anaesthetic potency, defined by Robbins (1946) as "the anaesthetic concentration in mice contained in a bottle, rotated at 14 revolutions per minute, that prevent 50% of the mice keep themselves upright over a period of 15 seconds".

Similarly he defined the anaesthetic concentrations required to cause death in 50% of the mice, in ten minutes, as the Fatal dose 50 (FD50) and the FD50/AD50 was selected as an index of the margin of anaesthetic safety.

1.1.4: Guedel`s Clinical Signs For Ether:

In this investigation a correlation was established between the various levels of etherisation and the associated changes in specific signs such as respiration, muscle tone, eye movement and the degree of pupillary dilatation. Accordingly, inhalation anaesthesia is divided into four stages namely, the first stage (analgesia), second stage (delirium), third stage (surgical) and fourth stage (respiratory paralysis) (Guedel, 1937)

In the light of the current information on anaesthesia, the use of clinical signs as anaesthetic depth indicators have been questioned on a number of grounds. Firstly,

they are not drug specific i.e. they tend to vary both quantitatively and qualitatively with the various anaesthetics (Cullen, Eger, Stevens, Smith, Cromwell, Cullen, Gregory, Bahlman, Dolan, Stoelting and Fourcade, 1972). Secondly, they vary with the species particularly in laboratory animals (Flecknell, 1987a). Thirdly, clinical signs such as movement and those related to sympathetic activity e.g. pupillary dilatation, tachycardia and hypertension may be masked when drugs such as muscle relaxants, opioids, cholinergic and Beta-adrenergic antagonists, vasodilators and antihypertensive agents are concomitantly administered with the anaesthetic agent (Ghoneim and Block, 1992).

1.1.5: Faulconer`s Graphic Patterns:

Faulconer (1952) reported a correlation between various levels of anaesthetic ether in the arterial blood and electroencephalographic (EEG) patterns. Out of the seven EEG patterns identified , the first and second appear during induction. Patterns 3, 4 and 5 are noticed during light, moderate and deep anaesthesia. Patterns 6 and 7 are detected at very deep anaesthetic levels. A wide variability in the EEG patterns between individual patients has been reported. They might probably be linked to variation in individual tolerance for the drug (Faulconer, 1952). Recently, it was revealed that the use of the raw EEG is of limited value in quantifying anaesthetic depth because of its inconsistency in the various anaesthetics, bulky recordings and the difficulty in its interpretation (Jones, 1993). The EEG interpretation can also be

complicated by factors such as the Pa CO_2, body temperature and sensory input to the CNS (Ghoneim and Block, 1992).

1.1.6: Minimum Alveolar Concentration (MAC):

In 1963, a more reliable index of anaesthetic potency, the Minimum Alveolar Concentration (MAC) was described and used by Merkel and Eger (1963) in a comparative investigation of the effect of halothane and halopropane on a number of physiological patterns in dogs.. It was defined as " the minimal anaesthetic concentration in the alveolus required to keep a dog from responding by gross movement to a painful stimulus such as tail clamping or varying electrical currents applied to sensitive mucous membranes."

MAC is considered to be the most widely accepted measure of anaesthetic potency because of its stability, reproducibility and relative ease of measurement (Quasha, Eger, and Tinker, 1980) in both man (Saidman and Eger, 1964) and animals (Eger, Saidman and Bradnstater, 1965a). MAC has also been advocated as a reliable measure for comparing the pharmacodynamics of equipotent concentrations of various inhalation anaesthetics, expressed as multiples or fractions of their MAC values (Merkel and Eger, 1963; Regan and Eger, 1967) to replace the use of clinical signs that differ widely from one drug to the other (Cullen et al, 1972). MAC is measured when the inspired, alveolar, arterial and cerebral anaesthetic concentrations are assumed to be equal i.e. it is in fact a partial pressure and not a concentration as

its name implies (Merkel and Eger, 1963). This gives MAC the additional advantage of representing the partial pressure of anaesthetic (in percent of 1 atmosphere) at the site of action (the brain) that makes it applicable to all inhalation anaesthetics (Eger, 1974).

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The procedure for MAC determination involves three essential components which are: 1) the measurement of the end-tidal anaesthetic concentration; 2) the application of the stimulus and 3) the seeking of a response (Merkel and Eger, 1963). In animals MAC 1.0 is considered to be the average of the concentration that allows movement and that which prevents movement in response to stimulation. In man, as repeated stimulation is not acceptable in the same subject, MAC 1.0 is regarded as the concentration at which a movement response to a single surgical incision was detected in 50% of patients (Quasha et al, 1980).

1.1.6.1: The Measurement Of The Alveolar Concentration:

Because of the difficulty of the direct measurements of the alveolar concentration the end-tidal concentration was measured on the assumption that at equilibrium the brain, arterial, alveolar and end-tidal concentrations were equal (Larson, Eger, and Severinghaus, 1962). That was specifically the case in healthy unanaesthetised patients. In anaesthetised and or sick patients, however, that assumption could be interfered with through a number of anaesthetic and or disease-related factors. Firstly, the presence of diffusion barriers that are associated with some pathological

conditions (Forster, 1957; Forster, 1963; George, Lassen, Mellemgaard and Vinther, 1965). Secondly, the end-tidal gas might be contaminated by gases released from the lining of tracheobronchial tree. The degree of contamination might be particularly greater with gases having high tissue solubilities such as methoxyflurane (Eger and Shargel, 1963). Thirdly ventilation/perfusion mismatch was reported to be one of the most important factors that contribute towards the discrepancy between the end-tidal and arterial partial pressures (Eger and Saidman, 1964; Eger and Severinghus, 1964). Such an effect was found to be more pronounced with highly soluble anaesthetics such as methoxyflurane (Holady, Garfield and Ginsberg, 1965).

The end-tidal (alveolar) to arterial differences were reported to be minimal when: 1) using poorly soluble anaesthetics; 2) normal ventilation and cardiac output and 3) allowing for nearly complete equilibration (Saidman, Eger, Munson, Babad and Muallem, 1967; Eger and Bahlman, 1971).

The time required for equilibration between alveolar and cerebral tensions can be calculated from the equation: $T = \lambda/CBF \times Log_e 20$, where T= time to 95% brain-blood equilibration in minutes; λ = anaesthetic brain-blood partition coefficient; CBF = cerebral blood flow per 100 gram of brain tissue. Using this equation equilibration time is calculated to be 8 minutes for fluoxene (Munson, Saidman and Eger, 1965). An equilibration period of 15 minutes was used for the determination of MAC of the moderately soluble anaesthetics in both man and animals (Saidman and Eger, 1964; Quasha, et al, 1980). An equilibration time of less than 15 minutes was also

suggested because of the relatively higher blood flow to the gray matter (Miller, 1986). Hyperventilation at a constant inspired partial pressure was predicted to reduce the time for cerebral anaesthetic equilibration with anaesthetic agents having the greatest blood/gas partition coefficient. A large reduction of cerebral equilibration time, regardless of solubility was likely to be brought about by normal or greater than normal CBF as is the case during rebreathing or inhalation of carbon dioxide (Munson and Bowers, 1967) or with cerebral vasodilators such as halothane (Smith and Wollman, 1972).

The measurement of the anaesthetic end-tidal concentration requires two major steps: 1) end-tidal sampling, and 2) anaesthetic gas analysis. End-tidal sampling could either be intermittent (Davis, Nunnally and Malinin, 1975) or continuous (Drummond, 1985; Doorley, 1988) by aspiration through a catheter located at the distal end of the endotracheal tube.

1.1.6.1.1: Anaesthetic Gas Analysis:

During anaesthesia gas analysis is usually carried out with the aim of 1) identifying the nature and the concentration of the delivered anaesthetic gases, and 2) evaluating the cardiorespiratory function through the analysis of the respired gases or the detection of contaminating gases or vapours in the expired gases (Sykes, Vikers and Hull, 1991). Methods of gas analysis are either chemical or physical. Because of their

speed the physical methods are the most commonly used in anaesthetic gas analysis. They are either non-specific or specific. Non-specific methods use some characteristics that are shared by a number of gases, but to a varying degree such as density, viscosity, thermal conductivity, refractive index, velocity of sound in the gas and magnetic susceptibility. The specific methods use a specific feature that is unique to a specific gas such as absorption and emission of radiation of a particular wavelength, identification of atomic nuclear properties or the conduction of electricity in response to the application of a specific voltage (Sykes et al, 1991).

In order to understand the limitations of the halothane gas analyser used in this study, it was decided to review the historical development of the range of techniques for anaesthetic gas analysis. A detailed account on this topic is presented in Appendix E.

1.1.6.2: Stimuli For MAC Determination:

The stimuli used for the determination of the minimum alveolar concentration (MAC) include skin incision (mainly in man), tail clamping in animals and electrical stimulation in both man and animals.

1.1.6.2.1: Tail Clamping:

Tail clamping is the most commonly used stimulus for MAC determination in animals. Quite a number of different clamps have been used, not only in the different animal species but within the individuals of the same species. For example, in the rabbit the stimulus for MAC determination involved the use of a 10 inch rubber-shod haemostat (Drummond, 1985) in one investigation and a 2.5 inch John Hopkins arterial clamp in another (Davis et al, 1975). Other clamps used include a large Kelly haemostat in pigs (Tranquilli, Thurmon, Benson and Steffey, 1983) and an alligator clip in mice (Koblin, Deady and Eger, 1982).

In order to circumvent, or at least minimise, the traumatic effects caused by the various clamping devices some investigators used, for instance, a rubber-shod haemostat (Drummond, 1985, Drummond, Todd and Shapiro, 1983) a haemostat with taped jaws (Doorly, Waters, Terrell and Robinson, 1988) or by always stimulating the tail proximal to a test site i.e. avoiding repeating the stimulus at the same site (Stone, Moscicki and Difazio, 1992).

Tail clamping was performed in a number of ways. In one investigation a rubber covered 10 inch haemostat was applied to the cat`s tail and continuously rotated to and fro about its long axis to produce wagging motion of the tail (Drummond et al, 1983). In another investigation a 10 inch haemostat was clamped on the shaved tail which was moved continuously with the haemostat for the duration of the stimulation

(Eger et al, 1965a). In a third investigation the tail clamp was applied and oscillated for 60 seconds (Koblin et al, 1982) or applied and moved continuously for 60 seconds (Masumura, Cole, Schell and Wong, 1991).

Although the site for tail clamping was precisely mentioned by some investigators to be 2 - 4 inches from the base of the dog`s tail (Eger et al, 1965a), other broader terms were also used to describe the site for tail clamping such as the proximal quarter of the cat`s shaved tail (Drummond et al, 1983), the base of the pig`s tail (Eisele, Talken and Eisele, 1985; Lundeen, Manohar and Parks, 1983), the distal third of the rat`s tail (White, Johnston and Eger, 1974) or even by always clamping the tail proximal to an earlier test site (Stone et al, 1992).

The duration for which the tail clamp was applied showed an even greater variability in the various investigations for the determination of MAC. The duration ranged between 2-60 seconds. These durations included 30 seconds (Viegas and Stoelting, 1976; Schieber, Namnoum, Sugden, Shiu, Orr and Cook (1986) and 60 seconds (Tranquilli et al, 1983; Deady, Koblin, Eger, Heavner and Aoust, 1981; Lundeen et al., 1983; White et al, 1974, Drummond et al, 1983, Eisele et al, 1985, and Koblin et al, 1982). Some investigators applied the stimulus for an unspecified duration of up to 30 seconds (Davis et al, 1975).

1.1.6.2.2: Skin Incision:

This is the most commonly used stimulus for the determination of MAC in man (Saidman and Eger, 1964; Saidman et al., 1967). Skin incision was occasionally used in animals for comparative studies involving other types of stimulation e.g. tail clamping and electrical stimulation in order to test for a supramaximal stimulus (Eger et al, 1965a; Lundeen et al, 1983; and Tranquilli et al, 1983).

1.1.6.2.3: Electrical Stimulation:

Electrical stimulation has been less frequently used than tail clamping and skin incision as a stimulus for MAC determination in both man and animals. In man, electrical currents of 30 to 45 volts AC with a 1.2 msec pulse at 50 cycles /sec. for less than 50 seconds, was applied via 20-gauge needles in the forearm (Saidman and Eger, 1964).

In an investigation involving 10 horses the electrical stimulus was applied to the oral mucous membranes using 50 V, 10 msec pulse width at 5 cycles /sec. for 60 seconds (Steffey, Howland, Giri and Eger, 1977). Electrical stimulation of 10, 30 and 50 volts, 50 cycles/sec for 10 msec pulse width was also used in another investigation for comparison with tail clamping, manual movement of the endotracheal tube in its longitudinal axis, skin incision and a paw clamp with the ultimate goal of seeking a "supramaximal stimulus" (Eger, Saidman and Brandstater, 1965a).

1.1.6.2.4: Rotating Chamber:

This is the most commonly used stimulus in mice and newts. In one of the early studies a closed 2.7 litre bottle, 12 cm internal diameter attached to a revolving machine at 14 revolutions per minute, was used as a stimulus for studying the anaesthetic activities of fluorinated hydrocarbons (Robbins, 1946).

More recently rotating chambers were being used for the investigation of anaesthetic potencies of inhalation anaesthetics (Miller, Paton, Smith and Smith., 1972; Clarke, Daniels, Harrison, Jordan, Paton, Smith and Smith, 1978; Koblin, Dong, Deady and Eger, 1980). Although the rotating chamber is still being used for the investigating anaesthetic potencies, it does not appear to provide for the application of a noxious stimulus in the strictest sense as previously reported by Merkel and Eger (1963) for the determination of MAC.

Nevertheless many investigations involving the use of the rotating chamber were tabulated in two reviews on MAC determination and considered to be MAC determination procedures (Cullen, 1986; and Quasha et al , 1980).

1.1.6.2.5: Supramaximal Stimulus:

In 1965 Eger et al (1965a) reported that a consistently higher alveolar concentration was required for the suppression of movement in dogs in response to tail clamping or electrical stimulation than in response to surgical incision which in turn was more

severe than paw clamp. From their findings those investigators introduced the concept of the supramaximal stimulus which was defined as the stimulus beyond which no further response was detected with the application of a more intense stimulus . Therefore tail clamping was considered to be a supramaximal stimulus and hence chosen to be the standard stimulus for MAC determination (Eger et al, 1965a).

However, Lundeen et al (1983), used tail clamping, a full thickness skin incision and a pedal reflex elicited by application of a haemostat at the skin fold between the digits of the hind limb as stimuli for the determination of MAC of isoflurane in each animal of a group of pigs. They concluded that tail clamping was probably equivalent to surgical incision but neither of them was a supramaximal stimulus because even at 2.18% end-tidal isoflurane (1.5 MAC), 70% of the pigs responded by gross purposeful muscular movement to elicitation of the pedal stimulation.

That conclusion was later confirmed by Eger, Johnson, Weiskopf, Holmes, Yasuda, Targ and Rampil (1988) who found that in pigs MAC values for a new fluorinated anaesthetic, I-653 and isoflurane obtained by clamping the tail were more variable and lower than the MAC values of the same anaesthetics obtained by clamping the dew claw. They concluded that the MAC values obtained for I-653 and isofurane is affected by the stimulus applied and that tail clamping is not a supramaximal stimulus in pigs, as a greater stimulus is provided by clamping the dew claw (Eger et al , 1988).

In a separate investigation, halothane potency was determined in pigs. It was found that in several pigs a higher end-tidal halothane concentration was required to abolish the response to tail clamping than to abolish the response to skin incision and therefore it appeared that the tail clamping technique provides a supramaximal stimulus in pigs (Tranquilli et al 1983). Further confirmation of that appeared in an investigation carried out by Lerman, Oyston, Gallagher, Miyasaka, Volgyesi, Eng and Burros (1990) who studied the minimum alveolar concentration (MAC) and haemodynamic effects of halothane, isoflurane and sevoflurane in new-born swine . In their investigation they determined the MAC values for the three anaesthetics in 36 new-born swine. They applied the stimulus by clamping a haemostat to the coronary ligament of the hoof for 30-45 seconds and each animal was observed for a movement response defined as withdrawal of one or more extremities. They found that new-born swine consistently withdrew an extremity in response to hoof clamping even though they did not respond to tail clamping at the same anaesthetic concentration which suggested that tail clamping was a less intense stimulus than was hoof clamping and therefore supports the use of hoof clamping for the determination of MAC in new-born swine (Lerman et al, 1990).

1.1.6.3: The seeking of a response:

When MAC was introduced for the first time as a measure of anaesthetic potency (Merkel and Eger, 1963), the response was defined as a gross muscular movement. A

more precise definition of the positive response was latter given by Eger et al (1965a) as a gross purposeful muscular movement, usually of the head (jerking or twisting) or extremities (running or clawing).

This definition excludes responses such as chewing, coughing and swallowing, which represent signs of increased central nervous system activity, as well as changes in ventilation (Eger et al, 1965a).

Since 1965 the movement response has been increasingly used for the determination of MAC in both man and animal. However in mice and newts the most commonly used end point or response was the rolling reflex (Miller et al, 1972) or the loss of the righting reflex (Robbins, 1946 and Clarke et al, 1978). The rolling reflex is comparable to the righting reflex and measured the ability of the mouse to maintain its normal posture in a chamber revolving at 4 revolutions per minute. Mice which rolled over completely during any one revolution were assigned a score of zero for that revolution; other mice which reacted sufficiently to avoid this were regarded as giving a positive response and given a score of one. Three sets of five revolution sequences were executed at three minute interval and a final score out of a possible 15 was allotted to each animal. The total score for each group of mice tested was then expressed as the percentage of positive responses. This procedure provides an internal check that equilibrium has been achieved (8 minutes for nitrous oxide and 25 minutes for highly water soluble fluorinated compounds (Miller et al, 1972).

On the other hand the loss of the righting reflex was tested in mice, specially bred to study their resistance to nitrous oxide, using a 20 litre hyperbaric chamber rotated at 4 revolution per minute. The chamber was flushed with a 100% oxygen for 10 minutes before 1.29 atmosphere nitrous oxide was added. After 30 minute equilibrium, animals rolling over twice during 5 complete turns of the rotator failed the test and were considered anaesthetised (Koblin et al, 1980).

1.1.6.3.1: Other Stimulus Related Responses:

One of the limitations of the measurement of MAC is that it ignores other important responses to the stimulus applied such as changes in ventilation, arterial blood pressure and pulse rate as well as signs of increased central nervous system activity such as coughing, chewing or swallowing (Eger et al, 1965a).

In modern anaesthetic practice and because of the widespread use of neuromuscular blocking drugs , the ability of an anaesthetic agent to prevent heart rate and systemic blood pressure responses to noxious stimuli would appear to be a more clinically relevant index of anaesthetic potency than the ability to prevent purposeful movement, as end point for MAC determination (Gibbs, Larach, Skeehan and Schuler, 1989).

Noxious surgical stimulation is accompanied by an increased activity of the hypothalamus associated with increased activity of the pituitary and sympathetic nervous system. The pituitary activity results in hormonal and metabolic changes and

the increased sympathetic activity is manifested by the general signs of light anaesthesia such as dilatation of the pupils, sweating, tachycardia and hypertension which are indirectly used to assess the depth of anaesthesia (Derbyshire and Smith, 1984).

In 1981 Roizen, Horrigan and Frazer, studied the adrenergic and cardiovascular responses to skin incision at different anaesthetic levels of halothane, enflurane, morphine and spinal anaesthesia in man with the objective of assessing the possible protective role of anaesthesia against the detrimental adrenergic and cardiovascular responses. The dose of anaesthesia that blocked the adrenergic response (plasma norepinephrine) in 50% of individuals who had skin incision (MAC- BAR) was determined for the various anaesthetics. Cardiovascular responses to incision as measured by rate- pressure product was found to decrease with increasing doses of halothane and morphine but not enflurane or even disappeared completely with adequate level of spinal analgesia (Roizen et al, 1981).

The abolition of heart rate response to a noxious stimulus was investigated in rats as one of three points of anaesthetic potencies of isofurane , halothane and diethyl ether. It was suggested that the heart rate response to noxious stimuli (1-kg weight on the middle of the tail, pressure surface of 0.25 cm^2) in contrast to the righting reflex is depressed by inhalation anaesthetics through a mechanism similar to that underlying the depression of purposeful movement response to the same noxious stimuli which might suggest the use of the heart rate response to noxious stimuli as an index of

anaesthetic potency (Kissin, Morgan and Smith, 1983). For similar arguments, this suggestion was also made by Kissin and Green (1984).

However some other investigators concluded that " the mean arterial blood pressure (MAP) responses to noxious stimuli can not be used as a linear index of anaesthetic depth in the rat and that monitoring of both MAP and heart rate is necessary to correctly interpret data from animal experiments involving the application of noxious stimuli " (Gibbs et al, 1989).

As to the nature of the blood pressure responses to noxious stimuli, it was found to be dependent mainly on the end-tidal anaesthetic concentration and the site of stimulation. For instance at a certain end-tidal concentration of halothane in the rat, the presser (increase) effects changed into depressor (decrease) responses (Gibbs et al, 1989). Similar findings were reported in the rabbit where halothane inspired concentrations in the range of 2.25% and 2.5% were found to convert the presser response of arterial blood pressure into depressor effects (Fukunaga, Tanigughi and Kikuta, 1990). Haemodynamic responses in the rat such as mean arterial blood pressure and heart rate were found to be significantly reduced by increasing the anaesthetic concentration from 0.6 x MAC to 0.75 x MAC of either the halothane or the isoflurane (Gibbs et al, 1989).

1.2: Pain Mechanisms And Nociceptors:

With the introduction of the concept of the "supramaximal stimulus" for the determination of MAC, it is generally held that the variability in MAC resulting from variation in stimuli is removed (Eger et al., 1965a). However it might be difficult to understand the exact meaning and predict the reliability of this concept in the light of concepts in sensory physiology. Therefore, it was decided to review briefly the literature on some aspects of pain mechanisms and nociceptors.

1.2.1: Pain Mechanisms:

There are three theories to explain the nature of pain (Melzack and Wall, 1965) :

1) Specificity theory: according to this theory, specific cutaneous receptors are involved in the perception of each sensation resulting from cutaneous stimulation. For instance cold and warm sensations are perceived via specific classes of A-delta and C-fibres respectively (Raja, Meyer and Campbell, 1988; Bischoff, 1979).

2) Pattern theory: this theory suggested that it is the pattern of input from the skin that determines the nature of a particular sensation i.e. no specific somatosensory receptors are involved (Raja et al, 1988).

3) Gate control theory of pain: this theory proposes that pain phenomena are determined by interactions among three spinal cord systems, namely: the substantia gelatinosa which acts as a gate control system, dorsal column fibres and the first central transmission (T) cells in the dorsal horn. According to this theory three features of the afferent input are significant for pain: a) the ongoing activity which precedes the stimulus, b) the stimulus evoked activity and c) the relative balance of activity in large and small fibres (Melzack and Wall, 1965).

According to Woolf (1991), there are two kinds of pain: 1) physiological pain resulting from low intensity innocuous stimuli that caused no tissue damage or inflammation and is considered to be protective in nature. 2) Pathological pain which is caused by high intensity noxious stimuli that are accompanied by tissue injury or inflammation and is characterised by a reduction in pain threshold (allodynia), increase in response to noxious stimuli (hyperalgesia), a prolongation in the response to a transient stimulus (persistent pain) and the spread of pain to undamaged tissues. With the occurrence of damage to tissues there will be no clear distinction between the two types of pain and in such case the resulting pain is called clinical pain (Woolf, 1989).

1.2.2: Classification Of Cutaneous Nociceptors:

It is the work of Adrian (1931) which led to the discovery of the presence of small amplitude action potentials in mammalian nerves when the stimulus is enough to cause some deformation of the skin surface. These findings, in addition to investigations

carried out by Zotterman (1933) and Zotterman (1939) provided the backbone for the understanding of the presence of specific receptors and neuronal pathways which are involved in pain mechanisms.

The studies that followed, in both man (Torebjork and Ochoa, 1980; and Vallbo, 1981) and other animals (Meyer and Campbell, 1981; and Lamotte, 1978) have put the flesh on the bones and identified a family of specific cutaneous receptor, called nociceptors, that respond preferentially to high intensity stimuli.

Nociceptors could be classified according to: 1) The kind of response to the various modalities of intense stimulation; 2) The features of their response; 3) The conduction velocity of their axons; and 4) the type of their fibres.

There might be some difficulty and confusion with the classification of nociceptors, due to the diversity of the terms used to describe the various groups (Dubner and Bennett, 1983). For example, three terms have been used to describe a group of receptors that only respond to intense mechanical stimulation. Those terms are: high threshold mechanoreceptors, moderate pressure mechanoreceptors and low sensitivity mechanoreceptors (Dubner and Bennett, 1983). On the other hand the whole class of these receptors has been referred to by some investigators as mechanical nociceptors (Iggo and Ogawa, 1971; Burgess and Perl, 1973). Adding further to the confusion in the classification of nociceptors, the same group of mechanical nociceptors have been described as mechanothermal fibres in some

investigations because of their heat sensitivity property (Campbell, Meyer and Lamotte, 1979) or alternatively called thermal nociceptor or heat nociceptor where they lack the heat sensitivity property (Iggo and Ogawa, 1971).

With a minor exception, the conduction of all nociceptive afferents is in the A-delta or C-fibre range which suggested that they have thinly myelinated or unmyelinated fibres (Dubner and Bennett, 1983).

The following classes of nociceptors have been identified:

1.2.2.1: Polymodal Nociceptors:

As the name implied, this is a group of nociceptors that respond to three modalities of stimulation which are : mechanical, thermal and chemical stimulation.

According to Bessou and Perl (1969), this group of nociceptors has the following features: a) The thermal threshold varies between 42 and 56 degree C, with no response reported for thermal changes between 20 and 40 degree C. b) They become more sensitive as a result of exposure of the skin to increased temperatures, indicated by alterations in their response in successive heating trials. For instance, activity equivalent to that obtained at over 50 degree C in the first test was observed at about 46 degree C. c) The duration of enhanced sensitivity is usually as long as the duration of the investigation (upto 2 hours). Therefore a meaningful estimation of threshold required the use of skin not previously subject to noxious stimuli. d) Prolonged

heating using temperatures in excess of 60 degree C or transient contact with very hot objects (+ 75 degree C) were found to cause inactivation of polymodal nociceptors or a reversal of increased response and may have reflected damage to the nerve terminals. e) Mechanical excitation did not result in sensitisation to subsequent mechanical stimuli. f) The discharge of a polymodal unit to dilute acid was low in frequency which was reported to be strongly enhanced by preheating the receptor terminal prior to the application of the acid.

1.2.2.2: Mechanical Nociceptors:

These respond to noxious mechanical and heat stimuli. They are either innervated by myelinated type A-fibres (A-mechanoheat receptors = AMHs) (Fitzgerald and Lynn, 1977; Campbell et al, 1979) or unmyelinated type C-fibres (C-mechanoheat receptors = CMHs) (Campbell and Meyer, 1983; Dubner and Bennet, 1983). The pain threshold of CMHs in animals was found to be similar to that in humans (about 43 degree C) (Lamotte and Campbell, 1978).

1.2.2.2.1: Types Of AMHs:

Two types of AMHs have been identified. Those are type I and type II AMHs, with thresholds greater than 49 and near 43 degree C respectively. The receptor utilisation time (time between stimulus onset and initiation of action potential activity) was found to be long (>600 ms) for type I AMHs and short (200 ms) for type II AMHs

(Raja et al, 1988) making the latter to be possibly related to the signalling of the first pain sensations in "dual pain" (Price and Dubner, 1977).

1.2.2.3: High Threshold Mechanoreceptors (HTMs):

These were also called mechanical nociceptors (Dubner and Bennett, 1983). They are characterised as follows:

a) They respond only to intense mechanical stimuli and although rarely responsive to initial heat stimuli in the range of 45 to 55 degree C, some will respond to thermal stimulation following repeated exposure to heat. b)The myelinated units of this group have the property of heat sensitisation, after repetitive heat stimulation, which is not accompanied by altered responses to mechanical stimulation (Fitzgerald and Lynn, 1977; Campbell et al 1979). The main features of sensitisation include a decrease in threshold temperature, an augmented response to suprathreshold stimuli reflected as a decreased latency or greater number of impulses or both and the development of a low frequency spontaneous discharge (Bessou and Perl, 1969).

c) The myelinated units usually have conduction velocities in the 5 to 35 m/sec. (A-delta range), while those of some units were found to be in the A-beta range i.e. 40 to 55 m/sec.(Dubner and Bennett, 1983). d) Unmyelinated afferents of the high threshold mechanoreceptors which do not respond to thermal stimulation have been

identified in the cat and monkey, but in the latter they comprises only 10-15% of the C fibre population (Price and Dubner, 1977).

In addition to the above mentioned primary classes of nociceptors, small group of myelinated and unmyelinated units that respond to noxious mechanical and cold stimuli, but not heat have been reported (Georgopoulos, 1976). Also other groups that respond only to intense cold (Lamotte and Thalhammer, 1982) or intense thermal stimulation (Beck, Handwarker and Zimmerman, 1974) have been identified.

1.3: Environmental Temperature And Anaesthesia:

When discussing the non-drug factors that influence the onset of general anaesthesia following the use of intravenous drugs, Dundee (1988) stressed the importance, in clinical practice, of environmental temperature which is often forgotten. Few and contradictory reports and observations, supported with little experimental work, are available on aspects of the possible influence of environmental temperature on the pharmacodynamics and pharmacokinetics of anaesthetic drugs and their clinical implications. During hot weather, the time to the onset of action of thiopentone, pentobarbitone (Dundee, 1957) and hexobarbitone (Ruddell, 1953) was reduced. This was probably related to change in forearm blood flow associated with the increased cardiac output and vasodilatation at the high environmental temperature (Dundee, 1957). Krahenmann (1969) had the opportunity of working at veterinary surgical clinics in both temperate (Zurich, Switzerland) and tropical (Khartoum,

Sudan) climates. He noticed that higher dosages of thiopentone sodium, pentobarbitone sodium and thiambutene-HCL were required in temperate climates. He suggested that this was probably due to the influence of environmental temperature.

Heat stress is also reported to prolong mean standing time (recovery time) and to delay the return of pain sensation in heifers that received xylazine at a dose of 0.1 mg/kg (Fayed, Abdalla, Anderson, Spencer and Johnson, 1989). Some contradictory reports are also available on the influence of room temperature on the sleeping time of barbiturates. The sleeping times of pentobarbitone, allyl-barbituric acid and thiopentone are longer at a low rather than at a high room temperature, while that of phenobarbitone is actually shortened by low room temperature (Raventos, 1938). The prolongation of the sleeping time of pentobarbitone associated with low room temperature, was shown to be a dose-related effect in such a way that it occurred only at high doses (Shaw and Shanky, 1948).

The effects of environmental temperature on the toxicity of some anaesthetics and other drugs, has received relatively more attention from investigators. In rats, pentobarbitone was reported to be five to eight times more toxic, at 36 ^{o}C than at 26 ^{o}C (Keplinger, Lanier and Deichmann, 1959). Similarly the toxicity of aspirin, Cyclizine and trimethobenzamide, was reported to increase in heat-stressed rats (Shields, Marra, Goodwin and Vernikos-Danellis, 1975). On the other hand cold environment was found to increase the toxicity of pentobarbitone and barbital in rats

and dogs. The animals, however, died more quickly in an hot environment. Also, the toxicity of thiopentone and pentobarbitone was shown to decrease with a lowering of room temperature (Richards, 1941; Richards and Taylor, 1956).

The mechanisms of the effects of environmental temperature on the toxicity of drugs including anaesthetic agents are not obvious. These mechanisms might be related to alterations in normal body functions brought about by changes in environmental temperature. These alterations might involve changes in pharmacokinetics such as absorption, diffusion or metabolism (Keplinger, et al., 1959). More recently, it was suggested that anaesthesia may be affected by changes in temperature in three ways, namely: 1) transportation of the anaesthetic to or from the site of action might be affected by changes in blood or other solubilities and changes in blood flow; 2) changes of concentration at the site of action; and 3) a direct effect on the site of action or elsewhere in the organism as indicated by hypothermia which appears to have an anaesthetic like action in warm-blooded animals (Halsey, 1989).

Changes in both the transport and the concentration of anaesthetic at the site of action are attributed to changes in solubility which are related to changes in temperature. These changes might be considered in both closed and open systems. If a closed system, containing water in which an organism is immersed, is equilibrated, for instance, with 1% halothane at 1 atmosphere, the organism will be subjected to a partial pressure of 7.6 mm Hg. Since the temperature coefficient for halothane in water is 4.01%, then a 5 °C reduction in temperature will increase the solubility by

20% and as a result there will be a drop in the partial pressure of halothane in water. Since the system is a closed one, the resultant gradient will diminish, resulting in a limited availability of halothane in the aqueous phase. However, the halothane concentration at the site of action (lipid phase) would increase as a result of the high affinity of anaesthetics for lipids and the small volume of the lipid phase leading to an increased depth of anaesthesia (White and Halsey, 1974).

In an open system in which the gas phase is connected to an anaesthetic machine, the anaesthetic concentration in the gas phase will be maintained while the solubility in the lipid phase will be increased by 22% as the temperature falls by 5 °C. Partial pressure gradients will be set up which, through a repeated re-equilibration process, will increase the halothane concentration in the brain thereby deepening anaesthesia (White and Halsey, 1974).

1.3.1: Body Temperature And MAC:

Both hyperthermia and hypothermia have been reported to influence the MAC value in the anaesthetised subject. However, very few reports are available on the influence of hyperthermia, probably because in clinical anaesthesia, it is hypothermia which is more frequently encountered especially with the use of modern air conditioning facilities. Patient hyperthermia can either be induced intentionally for e.g. cancer therapy or research purposes or occur spontaneously during the course of anaesthesia. Heat alone or in combination with chemotherapy or radiotherapy is increasingly being

used for the treatment of malignant tumours in both man and animals (Page, Thrall, Dewhirst and Meyer, 1987; Grier, Brewer and Theilen, 1980; Robins, 1984; vander Zee, van Rhoon, Faithfull and van den Berg, 1990).

Factors that contribute towards the development of hyperthermia during anaesthesia include increased metabolic activity associated with light levels of anaesthesia, obesity, heavy insulation by surgical draping, breathing a fully humidified gas mixture (Haskins, 1987), faulty warming devices, infection, hyperthyroidism as well as malignant hyperthermia (Feldman, Harrop-Griffiths and Hirsch, 1989a). Also heat retention frequently occurs at environmental temperatures exceeding 75 degree F. (wet bulb) especially when a to and fro circuit is used (Clark et al., 1954).

During anaesthesia, hypothermia can be deliberately induced to achieve one or more of its beneficial effects such as cerebral protection against hypoxic conditions (Carlsson, Hagerdal and Siesjo, 1976), the improvement of the chances of survival of hypoxic animals and its widespread beneficial use in the treatment of hypothermic circulatory arrest (Steen and Michenfelder, 1979; Artru and Michenfelder, 1981). When no warming facilities are used during anaesthesia, varying degrees of hypothermia can also develop spontaneously due to factors that include operating room temperatures of below 21 degree C. (Morris and Wilkey, 1970); prolonged laparotomy procedures (Stephen, 1961; Goldberge and Roe, 1966); the use of non-rebreathing anaesthetic circuits (Clark et al., 1954); infusion of cold fluids or blood and the administration of cold, dry anaesthetic gases (Hall, 1978).

1.3.1.1: Influence Of Hypothermia On MAC:

The MAC decreasing effect of hypothermia was reported to be related to the hypothermia-related increase in the lipid solubility of the anaesthetic agent at the site of action (the brain) (Eger, Saidman and Brandstater, 1965b).

In dogs cooled to a rectal temperature of 28 °C, "cold narcosis" develops. Animals remain in a state of relaxation without any need of giving additional anaesthetic doses of ethyl or vinyl ether (Bigelow, Lindsay and Greenwood, 1950). Similarly, unconsciousness and loss of motor activity are observed as the body temperature of immature monkeys is reduced to 20 °C (Callaghan, McQueen, Scott and Bigelow, 1954). These observations motivated subsequent workers to carry out a more quantitative investigations on the effect of hypothermia on MAC.

A direct linear relationship was reported to exist between the body temperature and MAC values in dogs. A 50% and 25% reduction of the MAC values of halothane and cyclopropane respectively, were demonstrated in dogs as their body temperatures were lowered by 10 °C (Eger, Saidman and Brandstater, 1965b). The MAC values of halothane, methoxyflurane, cyclopropane, ether and fluroxene were reported to decrease by 52, 53, 20, 42 and 37% respectively, in dogs exposed to hypothermia of 28 °C (Regan and Eger, 1967). Also, rats subjected to two levels of hypothermia at 32 and 27 °C, showed a reduction in MAC values of halothane and isoflurane by

4.82% and 5.28% respectively per 1 °C reduction in body temperature (Vitez, White and Eger, 1974).

1.3.1.2: Influence Of Hyperthermia On MAC:

The MAC increasing effect of hyperthermia was attributed to the hyperthermia-related decrease in the lipid solubility of the agent involved (Steffey and Eger, 1974). Increasing the oesophageal temperatures of dogs from 37.3 to 40.7 °C was shown to increase MAC of halothane by 8% per 1 °C rise in temperature. At temperatures exceeding 42 °C MAC decreased, with death occurring at 45.9 °C (Steffey and Eger, 1974). These findings were in agreement with the previously reported increase in the anaesthetising partial pressures of diethyl ether, chloroform, halothane and methoxyflurane with the increase of body temperature of the gold fish (Cherkin and Catchpool, 1964).

CHAPTER 2

GENERAL INTRODUCTION AND AREAS OF INVESTIGATION

2.1: General Introduction:

The MAC determination procedure requires repeated measurement of end-tidal anaesthetic concentration and repeated application of the stimulus. Even more measurements and stimulation are required in the same animal when investigating the influence of body temperature on MAC. This highlights the importance of using reliable techniques for gas analysis and stimulation, particularly when comparing the results obtained in various investigations. The use of a "supramaximal stimulus", beyond which there is no increase in MAC with any further increase in stimulus intensity, is generally held to remove any variability in MAC resulting from variation in stimuli (Eger et al., 1965a). However, there are conflicting reports in the literature regarding the concept of the "supramaximal stimulus". For instance, the tail clamping technique, which is most commonly used in animals, was considered to be a "supramaximal stimulus" by some investigators (Tranquilli et al., 1983; Eger et al., 1965a), but not by others (Lundeen et al., 1983; Eger et al., 1988; Lerman et al, 1990). Unquantifiable stimulation, such as tail clamping, have been carried out using various clamping devices even in the same animal (Drummond, 1985; Davis et al., 1975) and the stimulus have been applied in different ways and for various durations

(Eger et al., 1965a; Drummond, 1985; Koblin et al., 1982; Masumura et al, 1991). Such variations in the components of stimulation is reported to affect the degree of firing of somatosensory nociceptors (Iggo and Kornhuber, 1977; Iggo, 1985); which may contribute towards the variability of results in the determination of MAC. For instance, the MAC value of halothane in the rabbit was reported to be 0.82 +/- 0.25 (SD)% (Davis et al., 1975) in one investigation and 1.39 +/- 0.23 (SD)% in another one (Drummond, 1985).

As for the measurement of the end-tidal anaesthetic concentration, it has been revealed within the last few years that some gas analysers, particularly those using infra red absorption mechanisms, can significantly contribute towards the variability of MAC (Foley, Wood, Peel, Jones, and Lawler, 1990; Moens, Gootjes, and Lagerweij, 1991). This reflects the importance of testing the accuracy of the gas monitor in all studies. It also suggests that some of the previously reported MAC values are questionable as a result of significant monitor errors. For example, a positive offset of 0.65% halothane (nearly 1.0 MAC), was obtained with Datex Capnomac monitor in one alcoholic human subject (Foley et al., 1990).

Previous investigations concerning the influence of both hyperthermia and hypothermia on MAC, could be questioned as a consequence of one or more of the sources of variability associated with the stimulus application or end-tidal measurement as indicated before. In addition, none of these investigations was carried

out in the rabbit. It was therefore decided to investigate some effects of hyperthermia and hypothermia on MAC of halothane in the rabbit.

2.2: Areas Of The Investigation:

In the light of the information available in the literature and the problems indicated above, the areas covered by this investigation include:

1. The testing of the accuracy of Siemens Servo Gas Monitor 120 for the measurement of the end-tidal concentration of halothane in the rabbit (Chapter 3).
2. The investigation of the reliability of the tail clamping technique for the determination of MAC (Chapter 4).
3. The development of techniques to enable the use of controlled mechanical (Chapter 4) and heat stimulation (Chapter 6) for the determination of MAC.
4. The establishment of a reliable heating and cooling protocol for the determination of MAC at normothermia, hyperthermia and hypothermia, with a test of reliability of using stimulus-related cardiopulmonary responses as an end point for the determination of MAC of halothane in the rabbit (Chapter 5).
5. The investigation of the influence of a defined hyperthermia on MAC of halothane in the rabbit, determined with a controlled mechanical and heat stimulators (chapter 6).
6. The investigation of the influence of a defined hypothermia on MAC of halothane in the rabbit, determined with either of the stimulators (Chapter 7).

CHAPTER 3

MATERIALS AND GENERAL METHODS

In this section the materials and the general methods involved in this investigation are discussed. Any other special methods or modifications to these techniques will be dealt with in the relevant sections. Healthy New Zealand White rabbits were supplied by Hylyne Rabbits limited (Statham Lymm, Cheshire, England) and Interfauna U.K. Limited (Abbots Ripton Road, Wyton, Huntingdon, England. Animals were housed in an approved accommodation in the University of Edinburgh. they were fed on fresh hay greens and checked daily for any health problems. They were individually housed in clean aluminium cages with provisions for feed and water. Electric lighting was available 8 hours/day during the working day and for about 3 hours/day at the weekends. All animal procedures were carried out with the approval of the Home Office as indicated in the project licence PPL No. 60/01091 and the personal licence PIL 60/03972. The general experimental set-up is shown in the schematic diagram in Figure 3.1. and is photographically illustrated in Figure 3.2.

3.1: Anaesthetic Induction And Maintenance:

Although both nitrous oxide and premedication are known to have the advantage of hastening and smoothening anaesthetic induction, they were not used in these experiments for the determination of the minimum alveolar concentration (MAC) because of their well documented effects on anaesthetic requirements (Saidman and Eger, 1964; Munson, Saidman and Eger, 1965). During the induction procedure restraint was provided by wrapping the animal in a towel. Halothane anaesthesia was induced in the non fasting, New Zealand White rabbits, using a small rubber mask and Jackson's Ree modified Ayre's T-piece (non- rebreathing anaesthetic circuit). Halothane was delivered in 100% oxygen at 2-3L/min (twice the animal`s minute volume) (Flecknell, 1987b) from a Drager vaporiser (Dragerwerk Lubeck Germany), with inspired concentrations of up to 4 volume % (Hall and Clarke, 1991b) for induction and later adjusted for maintenance levels.

Figure 3.1: A schematic diagram of the general set-up. **a**, the heat stimulus applied to the ear pinna using a heat stimulator shown in Figure 6.1 (Chapter 6); **b**, flow transducer which was used together with an amplifier on the 8 channel recorder (**h**) for the measurement of the tidal volume; **c,** force transducer for the detection of head movement response; **d**, gas monitor (Siemens Servo Gas Monitor 120) for the measurement of the halothane concentration; **e**, the mechanical stimulus applied to the ear pinna using a mechanical stimulator shown in Figure 4.1 (Chapter 4); **f**, Stathem blood pressure transducer for the measurement of the arterial blood pressure via an arterial catheter placed into the femoral artery; **g**, thermistor scale to display the rectal temperature measured using a rectal probe. Parameters are recorded on the 8 channel recorder (**h**) and displayed on an oscilloscope (i).

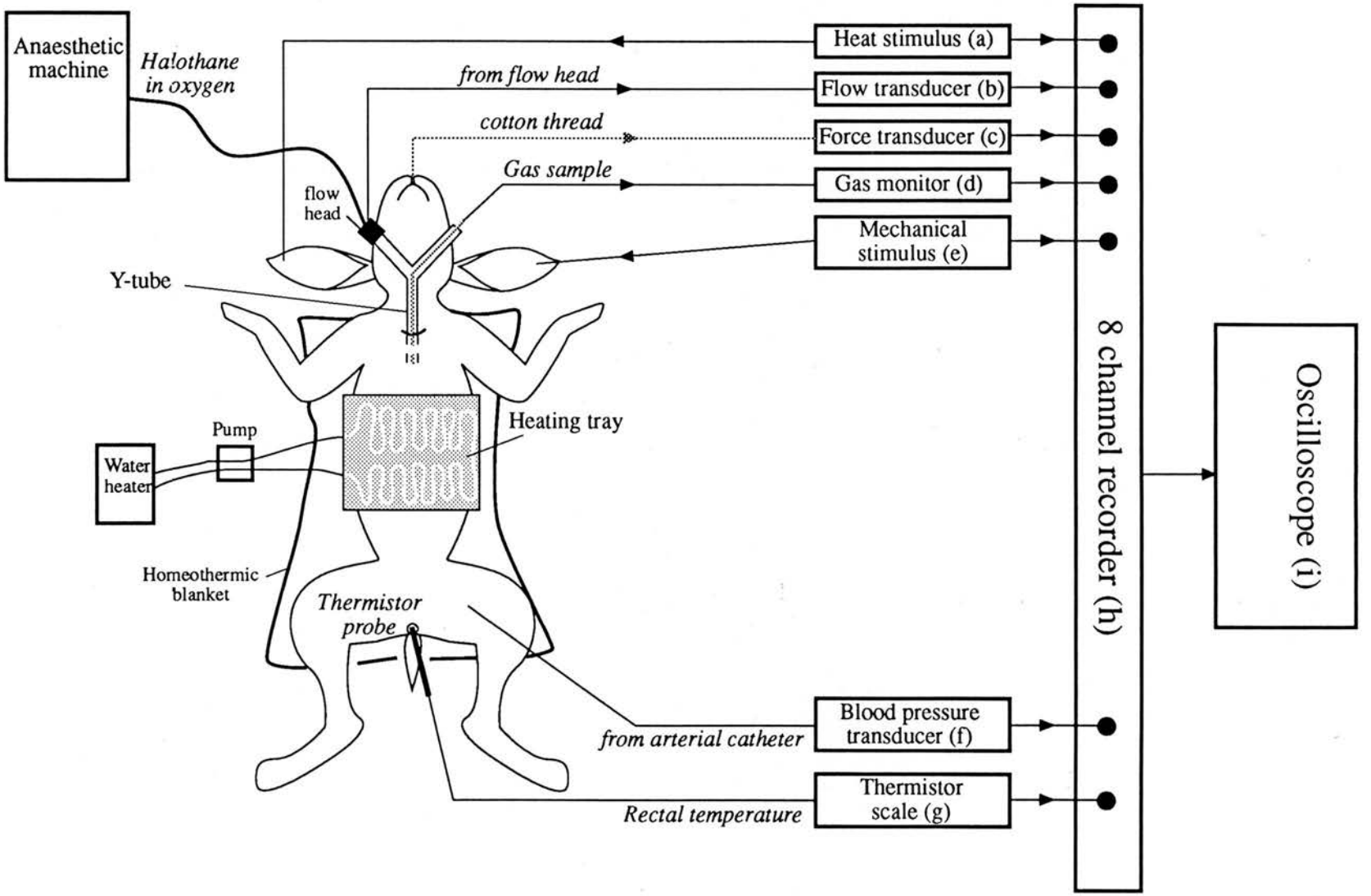
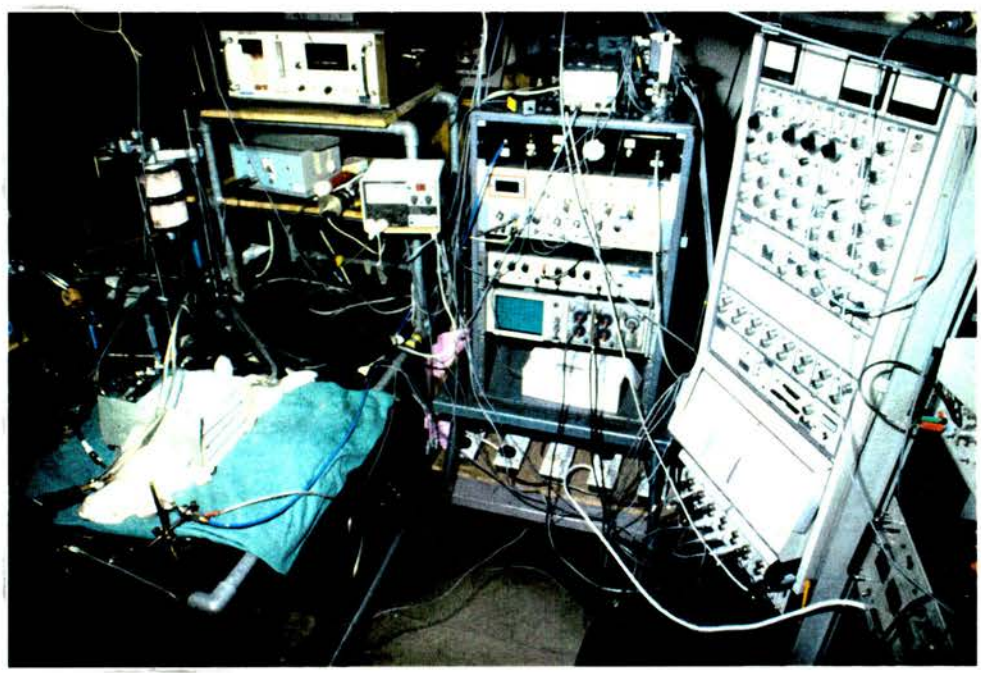

Anaesthetic machine
Halothane in oxygen
from flow head
cotton thread
Gas sample
flow head
Y-tube
Heat stimulus (a)
Flow transducer (b)
Force transducer (c)
Gas monitor (d)
Mechanical stimulus (e)
8 channel recorder (h)
Oscilloscope (i)
Pump
Water heater
Heating tray
Homeothermic blanket
Thermistor probe
from arterial catheter
Blood pressure transducer (f)
Rectal temperature
Thermistor scale (g)

Figure 3.2: A photographic illustration of the general set-up showing the instrumentation of the animal for measuring the various parameters indicated in the text and the schematic diagram of the general set-up (Figure 3.1).



3.2: Tracheotomy:

Tracheotomy was performed using a standard technique. A mid line incision was made over the trachea about 2 cms distal to the larynx. The trachea was isolated by division of the overlying muscles and then separated from the deep tissues by blunt dissection, taking care to minimise soft tissue damage and avoiding trauma to the vagus and major blood vessels. A bevelled plastic tube (3-4 mm ID) was inserted into the trachea via an incision between two tracheal rings. The tracheotomy tube was securely anchored in place by cotton ligatures encircling the trachea. The soft tissues were apposed and the skin wound was closed with cotton sutures. To ensure the security of the tracheotomy tube, it was anchored to the skin with an additional suture. Although it was considered important to mimise trauma, because of its possible effects on pain thresholds, it was essential to ensure adequate stabilization of the tracheal tube as head movement was to be one of the end points used for the determination of MAC in these experiments.

Immediately after intubation, the tracheal tube was then connected to a small plastic Y-tube which provided for connection with the breathing circuit through one of its limbs and for end tidal sampling through the other. Additional fixation of the tracheal tube was obtained by suturing the skin incision to provide for "a securing pocket" specially against side ways movement of the tube.

3.3: Catheterisation Of The Femoral Artery:

The arterial catheter (size 3 FG-pink Luer mount) was prepared by bevelling its free end, connecting it to a three-way tap and then filling them with heparinised saline (50 U/ml). The area of the inner side of the thigh was clipped. Using the scalpel, a skin

incision was made along the site of the pulsating femoral artery. The facia was then bluntly and carefully dissected to expose the artery, which is usually in close proximation with the vein and nerve, from which it was carefully separated. The artery was then ligated distally using a cotton suture. A Dieffenbach clip (R 21022 Rocket of London Limited) was applied to the far proximal part of the isolated artery. Another cotton ligature was loosely placed proximally to the clip. A small cut was made in the arterial wall distal to the Dieffenbach clip through which the bevelled end of the catheter was inserted into the artery to the level of the clip which was then gently released and the catheter was advanced further into the artery to a reasonable distance and secured in place by tightening the preplaced ligature. Correct placement of the catheter was indicated by drawing blood and then flushing the catheter using a syringe filled with heparinised saline. The skin incision was then sutured using a cotton thread. At the distal end of the incision the two ends of the thread were used to make another ligature around the catheter with the aim of further securing it in place.

3.4: Blood Pressure Measurement, Recording And Display:

Blood pressure was directly and continuously measured by connecting the arterial catheter to a Statham transducer (Bell and Howell LTD- England). The output of the transducer was amplified using a blood pressure amplifier(3552) mounted on an 8-channel recorder (Lectromed). The amplifier output was connected, on one hand, to a pen driver amplifier (3380) to be recorded on a heat sensitive paper using a heated stylus, and on the other hand connected to a blood pressure conditioning unit and a meter unit (3471-S3479-4) to display the numerical value of the blood pressure. The amplifier output was also displayed on an oscilloscope. The arterial line was intermittently flushed with heparinised saline. Calibration of the blood pressure

transducer-amplifier system was carried using a mercury manometer and an internal calibration signal according to the manufacturer`s instructions.

3.5: Measurement Of The Respiratory Tidal Volume :

The respiratory tidal volume was continuously measured using a Fleisch pneumotachograph flow head (Flow transducer Number 1, Gould Godart BV) which was placed between the breathing limb of the Y-tube and the breathing circuit connector. The flow head was connected to a transducer the output of which was then processed through a system, mounted on an 8-channel recorder, comprising a pressure amplifier (3552), a limit switch (3655) and an integrator (3630). Calibration of the transducer-amplifier system for the measurement of the tidal volume was carried out using a 10 or 20-ml syringe. The syringe was attached to a three-way tap and an air-tight connection was established between the latter and the pneumotachograph flow head. Air was then injected from the syringe via the pneumotachograph flow head and the output recorded on a heat sensitive paper was examined for zero stability, gain and linearity or otherwise adjusted using the various controls as indicated in the manufacturers manual. The flow and the tidal volume were continuously recorded on a heat sensitive paper using a heated stylus.

3.6: Measurement And Display Of The Respiratory Rate :

The output of the flow pressure amplifier(3552) was connected to a rate conditioning unit and then recorded on a heat sensitive paper using a heated stylus and displayed on a meter unit (3471-S3479-5). Alternatively the respiratory rate was calculated in breath per minute (BPM) from the tidal volume tracing run at a faster speed (25 divisions per second) for few seconds.

3.7: Measurement Of Body Temperature:

The body temperature was measured using a thermister probe inserted into the rectum. The thermister output was continuously displayed on a scale. The thermister output was amplified using a D.C. amplifier (high gain 3551) mounted on an 8-channel recorder and then continuously recorded on a heat sensitive paper using a heated stylus. Calibration of the thermistor was carried out by the simultaneous measurement of the temperatures of water baths using the thermistor and a mercury thermometer as a reference.

The oesophagus is regarded by many as being more representative of core body temperature (Azar, 1981). However, the repiratory gases were reported to have some cooling effects on the oesophageal temperatrure (Whitby and Dunkin, 1969; Whitby and Dunkin, 1971). Other sites for measuring the body temperature included the nasopharynx, tympanic membrane, pulmonary artery and the urinary bladder (Harrow and Rosberg, 1988). All these sites are expected to involve more stimulating procedures and therefore are less likely to be compatible with the MAC determination procedure. Therefore, the rectum was chosen to be the site for measuring the body temperature in this investigation.

3.8: Induction of Hyperthermia:

Hyperthermia (defined as the elevation of normal body temperature by 2-2.5 ^{o}C) was induced using circulating hot water. The heating system (Figure 3.3) was made of a water heater, a pump and a home-made heating copper tray with a coiled copper tubing on the outer surface to provide for the even distribution of heat throughout the

tray which delivers radiant heat to the animal. The water heater was provided with a control knob which allows for the selection of a wide range of constant water temperatures between 0-90 ° C.

In addition to the pumping of the heated water through the plastic tubing via the coiled tubing of the heating tray, the pump was also useful in controlling the rate of heating by changing the speed of the circulating water through a specific speed control knob.

In order to avoid the skin burns which might result from the direct contact between the animal skin and the heating tray, the latter was placed over the animal and the sites of the heating tray which were expected to come in contact with the animal skin, were covered with a protective paper towel, thus heating was by dry radiant heat.

At both normothermia and during heating, and inorder to improve the heating efficiency by reducing the heat loss, the animal was placed on a homeothermic blanket with thermistor feed back (homeothermic blanket control CFP 8140) preset at 39 ° C. Also a radiant light bulb was directed towards the animal.

3.9: Determination Of MAC:

As indicated in Chapter 1, the definition of MAC indicates that there are three major components involved in the determination of MAC. These are : 1) the measurement of the end- tidal concentration of the anaesthetic 2) the application of a stimulus and 3) the seeking of a response defined as the purposeful movement of the head and or the limbs.

Figure 3.3: A photographic illustration of the heating system, comprising a water heater (**W**), a water pump (**P**) and a heating tray (**H**).


M
d

3.9.1: Measurement Of The End -tidal Anaesthetic Concentration:

The end-tidal anaesthetic concentration is considered to be the minimum concentration of halothane at the end of the expiratory phase measured at the level of the distal end of the tracheal tube. The end-tidal concentration of halothane was measured using a Siemens Servo Gas Monitor 120 designed for the measurement of the concentrations of halothane, enflurane and isoflurane under anaesthetic conditions. After the machine was set-up according to the instructions in the manufacturer`s manual, it was calibrated using a certified gas concentration (1.52% isoflurane in 35.5% oxygen) provided by the manufacturer (Siemens-Elema). Calibration with any one of the three gases is a calibration for the other two (Siemens-Elema Instruction manual)

Gas sampling was continuous at 300 ml/min. through a small catheter (size 5 FG) inserted through the free limb of Y-tube and into the tracheal tube via an air-tight rubber cab. The sampling catheter was then connected to the sampling limb of the gas monitor which was then connected to the gas monitor through 0.8 μm micropore filters (Siemens-Elema AB) that served to remove condensation.

The concentration of halothane was continuously displayed on a dial and a digital value of the anaesthetic concentration was alternatively obtainable. The electrical output from the gas monitor was taken from the machine through a 25-way "D" connector fitted with a potential divider (1K + 2K7) in order to reduce the large manufacturers` voltage output to a smaller recordable one, which was then connected to SKI 1n1 on the 8-channel recorder for continuous recording of the anaesthetic concentration on a heat sensitive paper after carrying out the necessary wiring and calibration.

3.9.1.1: Gas Monitor Accuracy Testing:

Both indigenous and exogenous contaminants of expired gases were recently reported to influence the performance of anaesthetic infrared monitors. A false positive reading of 6% halothane was reported to be caused by cleaning the anaesthetic circuit components with 70% isopropyl alcohol (Doyle, 1988). Using the Datex Capnomac Monitor, a reading of 0.65% halothane (nearly one MAC) was observed in a human subject following the consumption of 4.4 litres of beer (Foley, et al, 1990). A positive offset of 5% halothane was reported with another infrared anaesthetic agent monitor (AAM-222, Puritan Bennett Corporation). It was discovered to be the result of the contamination of nitrous oxide with hydrocarbon contaminants such as methane, ethane, propane, isobutane, pentane and hexane (Johnson, 1987).

In veterinary practice it was observed, probably for the first time, that the performance of the Datex Normac infrared gas analyser was affected by an unknown contaminant of the normally expired gases (Taylor, 1990). That contaminant was later believed to be methane gas on the basis of the highly significant linear relationship between the difference in the halothane concentration simultaneously measured using Capnomac Datex (infrared absorption of 3.3 um) and Anaesthetic gas monitor (AGM) 1304 and the methane concentration as measured by infrared multigas analyser (MGA) 1302 (Moens, et al, 1991). These findings lead these investigators to conclude that gas analysers using high spectrum infrared absorption such as AGM 1304 are not affected by methane and therefore preferred for measuring

the concentration of inhalation agents in species where methane is excreted with the expired gases.

The amount of methane gas in the expired air is influenced by factors such as species (McKay and Eastwood, 1984), the time and type of feeding (Hungate, 1968) as well as some pathological conditions of the gastrointestinal tract (Haines, Metz, Dlawari, Blendis, and Wiggins, 1977).

Therefore it was decided to carry out the following experiments to test the accuracy of the Siemens Servo Gas Moniter 120 which was used through out this investigation.

3.9.1.1.1: The Effect of Normally Expired Gases of The Rabbit:

Four nonfasting New Zealand White rabbits (2 males and 2 females; mean age of 3.0 +/-0.6 (SD) month; mean weight of 3.1 +/- 0.8 (SD) kg.) were used in this experiment. Anaesthesia was induced and maintained using diazepam (Valium) and fentanyl/fluanisone (Hypnorm, Janssen) at an intramuscular doses of 2 mg/kg and 0.3 ml/kg. respectively (Flecknell, 1987c). The animals were intubated and instrumented as explained above and they were breathing oxygen from a T- piece. Anaesthetic concentration was continuously measured and recorded.

In two rabbits (No.3.III and 3.IV in Table 3.1) the anaesthetic concentration was continuously measured at normothermia and after the induction of hyperthermia (2 - 2.5 ^{o}C above the normothermic value). In one of the rabbits (No.3.II in Table 3.1), and for comparison, the anaesthetic concentration was also measured using another gas monitor (Siemens Circust Multigas monitor) which is an infrared analyser.

The results of these experiments are shown in Table 3.1. Throughout these experiments, the Siemens Servo gas monitor was reading between Zero and + 0.1 at all three modes of anaesthetic gases, namely halothane, isoflurane and enflurane and in both normothermic and hyperthermic experiments. Those readings are within the manufactorers` quoted error rates (Siemens Instruction Manual).

As for the rabbit where the anaesthetic concentration was measured using the Siemens Circust Multigas monitor, the machine was positively reading 0.4%, 0.1% and 0.2% on the halothane, enflurane and isoflurane measuring modes respectively. These results were expected as abnormally high readings of halothane were noticed with another infrared analyser (Capnomac infrared analyser) (Moens et al, 1991). As the highest reading was obtained at the halothane mode, the results might also suggest that the suspected material in the expired gases of the rabbit is absorbed at a band closer to that of halothane than those of the other two gases.

From these experiments it was concluded that the normally expired gases of the rabbit appeared to affect the readings of the Siemens Circust Multigas analyser, while not affecting the readings of the Siemens Servo Gas monitor. The reason for that might be due to the difference in the gas analysis mechanisms used in the two monitors, namely infrared absorption in the former and changes in the frequency of a "doped" crystal in the latter (Siemens Operating Manual for Servo Gas Monitor 120).

Also it appeared that although both gas monitors could be used for clinical monitoring, it was the Siemens servo gas monitor that was more appropriate for research purposes.

Table 3.1: The readings for Siemens Servo Gas Monitor 120 (Servo) and Siemens Circust Multigas monitor (Circust) (animal No. 2) in 4 rabbits anaesthetised with hypnorm and diazepam.

Animal No.	Monitor	halothane Conc.%	Isoflurane Conc.%	Enflurane Conc.%	Duration of experiment/hrs
3.I	Servo	0.0	0.0	0.0	2.5
3.II	Servo	0.0	0.0	0.0	3
3.II	Circust	0.4	0.2	0.1	3
3.III	Servo	0.1	0.1	0.1	6.5
3.IV	Servo	0.0	0.0	0.0	4.5

3.9.1.1.2: Effect Of Methane On The Reading Of The Servo Gas Monitor 120:

Since methane was suspected to be one of the factors that might affect a gas monitor readings (Moens et al, 1991), it was decided to test whether the Siemens sevo gas monitor is affected by such gas.

The experiment involved the filling of a ballon with methane gas using a syringe and a three way tap. Then the sampling tube of the gas monitor was connected to the ballon through an air tight connection. The effect of methane was tested on the three anaesthetic gases measuring modes. The results indicated that the monitor was not affected by methane. At no stage did the machine show any other value apart from zero.

3.9.1.1.3: The Effect Of Changes In The Temperature Of The Sampled Gases:

Some of the experiments in this project involved induction of hyperthermia and hypothermia in the experimental animals which is likely to affect the temperature of the expired gases. Therefore it was decided to test the effect of the temperature of the sampled gases on the reading of the machine.

In this experiment various concentrations of halothane in oxygen (1.5%, 1.2% and 0.7%) were delivered from the anaesthetic machine through an extended tube. Gases in the extended tube were heated or cooled to more or less the same range of temperatures used in the hyperthermia or the hypothermia experiments. The machine was connected to sample from the warmed gases. The temperature inside the tubing was measured using a mercury thermometer. The gas monitor reading was recorded

periodically and then at intervals as the gases were gradually being warmed up or cooled down.

The results are shown in Table 3.2. It is concluded that the gas monitor in question appeared not to be affected by the range of the sampled gas temperatures used in these experiments and, by implication, by those temperatures used in the hyperthermic and hypothermic experiments.

3.9.2: Application Of The Stimulus:

Generally speaking three kinds of stimuli were used in this project for the determination of MAC. Those were: tail clamping using artery forceps, an air operated mechanical stimulator in a form of a pincher and a controlled heat stimulator. The description and the use of these stimulators is presented in the relevant sections (Chapters 4, 6 and 7). The use of the tail clamping technique using a haemostat was restricted to a pilot study which proved that the technique was ethically unacceptable and scientifically unreliable (Chapter 4).

3.9.3: Detection And Recording Of Response:

The response was defined as the provoked movement of the head and or limbs. The response was observed and also recorded using a force transducer (1 kg strain gauge transducer) connected by a cotton thread to the upper incisor teeth (Figure 3.4).

Head movement was selected for recording because it occurred more often in response to ear stimulation than the limbs. In fact it occurred in all animals when ear stimulation was performed at "a response concentration". Also the flaccid nature of

the limbs as well as the relative difficulty of anchoring them to the transducer, make them less suitable candidates for the recording of the movement response.

The transducer output signal was amplified and continuously recorded on a heat sensitive paper and an oscilloscope after carrying out the required wiring and calibration at the patch panel of the channel recorder. Movement artefacts, due to respiration were of lower amplitude and frequency than evoked responses which are observable and of high frequency and amplitude and coincided with the stimulus application.

Table 3.2: the effect of changes in temperature of the sampled gases on the readings of Siemens Servo gas monitor 120. The monitor was tested at 1.5%, 1.2% and 0.7% halothane.

Gas temperature ^{o}C	Monitor reading (halothane con.%)	Vaporiser temperature ^{o}C
20	1.5	18
warming		
25	1.5	18
30	1.5	19
	Adjusted to 1.2%	
35	1.2	20
37	1.2	20
39	1.2	20
41	1.2	20
43	1.2	20
45	1.2	20.5
cooling		
43	1.2	20.5
41	1.2	20.5
39	1.2	20.5
37	1.2	20.5
35	1.2	20.5
30	1.2	20.5
warming	Adjusted to 0.7%	
35	0.7	20.5
37	0.7	20.5
39	0.7	21
41	0.8	21
43	0.8	21
44	0.8	21
cooling		
42	0.8	21
41	0.8	21
39	0.8	21
37	0.8	21
35	0.8	21

Figure 3.4: A photographic illustration of the method for the detection of the head movement response using a force transducer connected by a cotton thread to the upper incisor teeth of the rabbit.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CHAPTER 4

THE DETERMINATION OF MAC OF HALOTHANE USING CONTROLLED MECHANICAL STIMULATION

4.1: Introduction:

The determination of MAC involves three major steps which are : 1) the measurement of the end-tidal anaesthetic concentration 2) the application of the stimulus and 3) the seeking of a response (Merkel and Eger, 1963).

Reproducibility, which is claimed to be one of the main attributes of MAC, requires the standardisation of techniques for the determination of these three components. In order to remove the variability in MAC, that is related to any variability in stimuli, the concept of a "supramaximal stimulus" was established (Eger et al., 1965a). This was defined as the stimulus beyond which there is no increase in MAC value with any further increase in stimulus intensity. However, a wide variety of stimuli have been used which quite often appear to be extreme, resulting in actually or potentially damaging injury to the skin and the underlying tissues. These stimuli have included tail clamping and a rotating chamber, that tests for the righting reflex in animals, skin incision in man and electrical stimulation in both man and animals (Cullen, 1986; Muir, Wagner and Hinchcliff, 1992).

Tail clamping is the most widely used mechanical stimulus for the determination of

MAC in experimental animals. It is performed in different ways and for various durations using a wide variety of clamps, even in the same species of animals. For instance, in rabbits it was carried out using a 25 cm (10-inch) rubber- shod haemostat (Drummond, 1985) or 6.25 cm (2.5-inch) John Hopkins arterial clamp (Davis et al., 1975). The actual forces applied in these investigations were neither measured nor recorded.

The variability of the stimuli used may in part explain the differences in the values of MAC obtained (Eger et al., 1965a; Eger et al., 1988; Sobair, Cottrell and Camburn, 1993). For instance, the MAC of halothane in the rabbit was reported to have mean values of 0.82 +/- 0.25 (SD)% (Davis et al., 1975), 1.39 +/- 0.23 (SD)% (Drummond, 1985) and 0.63 +/- 0.06 (SEM) % (Mackenzie, 1977). This demonstrates the crucial need for the development of reliable and controlled techniques that provide for the application of a consistent stimulus.

No investigators have accurately quantified the mechanical stimuli used for MAC determination. In a crude attempt, a noxious stimulus resulting from 1 kg. weight applied to the middle of the tail (pressure surface of 0.25 cm^2) was used to investigate the anaesthetic potency of halothane, isoflurane and diethyl ether in the rat (Kissin, Morgan and Smith, 1983).

The purpose of this section is to investigate the reliability of the tail clamping technique using a haemostat in the rabbit and the attempt to define a consistent mechanical stimulus applied by using an air-operated pincer. This takes into

consideration modern concepts in sensory physiology and to establish the MAC at the nociceptive threshold. This approach should minimise the effect of sensory sensitisatization by inflammatory mediators or desensitization of nociceptors due to excessive trauma and, by accurately recording the forces used, should allow meaningful comparison between investigations. Also, it was decided to attempt to determine MAC of halothane in hyperthermic rabbits using the controlled mechanical stimulation.

4.2 Materials and methods:

Unless stated otherwise, all procedures in this section were performed as described in Chapter 3 (Materials and General Methods). Halothane anaesthesia was induced without premedication in 9 non-fasting New Zealand White rabbits (5 males, 4 females; mean age of 4.5 +/- 1.1 (SD) month and a mean weight of 3.6 +/- 0.3 (SD) kg.).

Animal instrumentation was carried out as previously described to provide for the measurement and recording of end-tidal halothane concentration, head movement response, arterial blood pressure, tidal volume and rectal temperature.

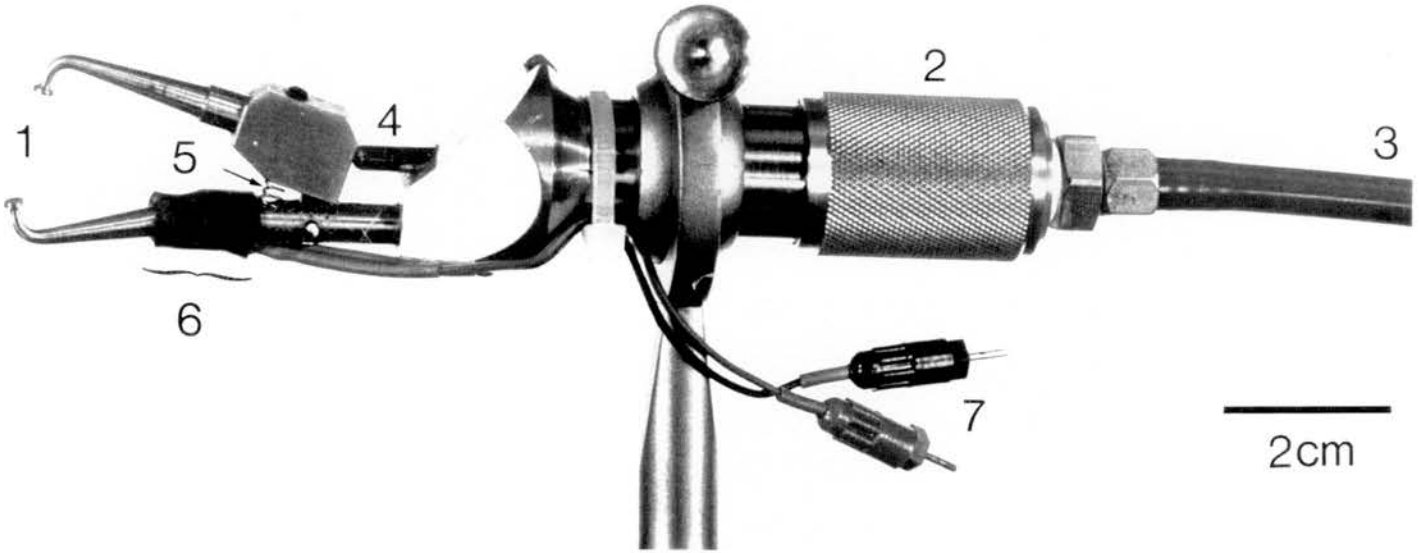
4.2.1 Description of the mechanical stimulator:

The mechanical stimulator consisted of a "pincer" driven by compressed air and was operated from a control panel with a manual trigger (Figure 4.1). The compressed air was delivered through a pressure regulator (range 0 - 415 Kpa; 0-60 psi) to an electronic valve (12 V DC) and a flow valve to control the rate of change of force of the "pinch" applied.

Before applying the stimulus, the gap between the two pinching tips (1) (Figure 4.1) can be adjusted to suit the thickness of the stimulus site, by turning the knurled ring (2). The compressed air was fed to the stimulator through the line (3), forcing the cylinder (4) to move forward so that the pinching tips (1) closed. The stimulus intensity was controlled by a preselected driving pressure on a pressure gauge. At the end of the stimulus, the air pressure was released by releasing the manual trigger and the tips (1) were forced open by a return spring (5).

A foil strain gauge transducer (120 Ohm; Showa measuring instrument Co.; Tokyo) was mounted on the lower arm of the pincer, protected in a water proof seal (6), so as to provide for the measurement of the force applied to the target site. The signal in the line (7) was displayed on a chart recorder and oscilloscope. This allowed for the calculation and calibration of the stimulus and, together with the simultaneously recorded movement response, it also enable the calculation of the response time. The mechanical stimulator was triggered to apply a precise stimulus at a preselected pressure, rate of change of force and duration of application. Calibration of the force applied was carried out by applying gram weights to the lower arm of the pincer and reading the voltage deflection from the recording paper. The force applied was calculated after measuring the surface area of the circular pinching disc (3.8 mm^2) and converted to Newtons per unit surface area (4.90 N represents the force from a mass of 0.5 kg.). Throughout this section the stimulus was kept steady, a mean force of 1.35 +/- 0.05 (SD) N/mm^2 being applied for 30 seconds by the stimulator at a preselected pressure of 275 Kpa (40 psi) and a rate of force application of 1.6 N/S. The latter was calculated from photographs taken from a storage oscilloscope.

Figure 4.1: The air-operated mechanical stimulator (pincher) used for the application of a controlled and recordable mechanical stimulus for the determination of MAC. **1**, two pinching tips made of two circular discs, each with a surface area of 3.88 mm^2; **2,** knurled ring for adjusting the gap between the pinching tips to suit the thickness of the stimulus site; **3**, line through which air is fed to the pincher for the application of a stimulus at a driving pressure preselected on the control panel; **4**, a cylinder which when pushed forward, causes the two pinching tips to close; **5**, return spring which, at the end of the stimulus, opens the two pinching tips; **6**, a water-proof protective seal containing the strain gauge foil transducer; **7**, a line for pincher output.


1
2
3
4
5
6
7
2cm

Attempts were made to determine MAC of halothane in the same animals after hyperthermia was induced (as described in Chapter 5) using a water heater setting of upto 90 °C and a pump setting of 9. However, these attempts were frustrated by the occurrence of spontaneous movements and the continuous rise in the rectal body temperature even after cessation of heating and as a result, animals either died at around 44 °C or were killed by injecting a lethal dose of pentobarbitone.

4.2.2 Experimental protocol:

In normothermic animals (rectal temperature of 39.5 +/- 0.3 °C), anaesthesia was induced and then maintained for at least 15 minutes at an end-tidal concentration that was believed to approximate to MAC. The stimulus force provided by a driving pressure of 275 Kpa (40 psi) was chosen on the basis of known nociceptor thresholds (Iggo, 1985; Livingstone, Waterman, Nolan, Morris, Ley, and Headley, 1992) and also by taking into consideration the results of a pilot study. This stimulus force did not cause an inflammatory response and could just be tolerated when applied to the human thumb web. The stimulus was applied to the ear pinna for a duration of 30 seconds and it was interrupted whenever a positive response was indicated by movement of the head. For any one end-tidal concentration, the same stimulus was repeated three times, separated by 1.5 - 3 minutes, before considering the response as negative.

The end-tidal halothane concentration was then adjusted by 0.1% (increased after a positive response or decreased after a negative one) and maintained for at least 15 minutes at the newly adjusted end-tidal concentration before the same stimulus was reapplied. The MAC was considered to be the average of the lowest end-tidal concentration at which a negative response was detected and the highest end-tidal concentration at which a positive response was found.

4.2.3 Data analysis:

Data were presented as Means +/- Standard deviations for all relevant measurements.

4.3 Results:

4.3.1 Pilot study:

The main objective of the pilot study was to test for the reliability of using the tail clamping technique, using a padded haemostat, for the determination of MAC. Two rabbits were used for this purpose, with the artery forceps applied to the ear in one of them and to the tail of the other. This technique demonstrated that there was altered tissue sensitivity. During the initial application of the haemostat to the ear, a MAC value of 1.65% was obtained (an average of a response concentration of 1.6% and a no-response concentration of 1.7%). During a second attempt to determine MAC of halothane using the same ear, a positive response was still being detected at an end-tidal halothane concentration of 1.9%. This implied an increased tissue sensitivity.

Alternatively, the damage to the underlying nerves might be so severe as to make them incapable of conducting impulses (Wolff and Wolf, 1958; Sunderland, 1978) as was apparently the case in the second rabbit, when the haemostat was applied to the tail. During this trial, MAC of halothane was initially calculated at 1.5% (an average of a response concentration of 1.4% and a no-response concentration of 1.6%). When the haemostat was applied to the previous site, there was no response at an end-tidal halothane concentration of 0.9%. The implication of this might be that the

sensory conduction pathways were probably functionally severed. When the resultant pathology of tails taken from animals which were fully anaesthetised, was analysed, it was found that repeated application of the artery forceps had caused severe trauma and lacerations (Figure 4.2). This damage was also demonstrated in a histological section of the tail at the site of stimulation (Figure 4.3). It was considered that the extent of these lacerations and contusions, even in the absence of actual nerve damage, would alter the sensitivity of the sensory nerve terminals either directly or through the release of inflammatory mediators (Willoughby, 1987), which might have accounted for the variability in the MAC values obtained when the ear was similarly traumatised.

As a result of these findings, of physical damage and the possibly associated altered sensitivity, it was decided to look more carefully at the techniques involved in the determination of MAC. In particular, it was decided to devise an air-operated mechanical stimulator that would accurately deliver a controlled and measured stimulus, taking into consideration the information reported on mechanonociceptors thresholds (Iggo, 1985; Waterman, Livingstone, Ley, and Brandt, 1992). In the only previous quantitative study involving mechanical noxious stimuli, normal conscious sheep were shown to have a pedal withdrawal to pressure from a blunt-pin (of unreported surface area) of 4.35 N on the skin overlying the lower end of the radius (Waterman et al., 1992).

As part of the pilot study, the use of the air-operated mechanical stimulator was tried on a third anaesthetised rabbit. Driving pressures greater than 207 Kpa (30 psi)

applied to the tail were found to cause an unacceptable level of tissue damage, that increased with the driving pressure used. A driving pressure of 207 Kpa (30 psi) applied to the tail produced a similar reactive force to the mean value of the stimulus from 275 Kpa (40 psi) applied by the stimulator to the ear (1.35 +/- 0.07 (SD) N/mm^2). This finding demonstrates the contribution of the site of application to the actual force measured, i.e. the stimulus pressure against the bone of the tail produced a greater reactive force than the same pressure across the cartilage of the ear.

The importance of measuring the actual force applied for each stimulus application is further demonstrated with reference to Table 4.1. It is seen that even when the driving pressure and the rate of application are constant and the pincer diameter remained the same, the actual force applied varies with the nature of the tissue being compressed, even in a relatively uniform tissue like a rabbit`s pinna. It was concluded that the absence of accurate measurements in other studies may have led to differences in the stimulus applied, which may partially account for the different values of MAC reported.

4.3.2 Main investigation:

The mechanical stimulator was used throughout the rest of this investigation. The mechanical stimuli, calculated in terms of $Newtons/mm^2$, and the movement responses at various halothane end-tidal concentrations for the individual rabbits are shown in Table 4.1. The mean stimuli and the concentrations at which either a response or no response was obtained are shown in Table 4.2. The end-tidal halothane concentrations that allowed a movement response ranged between 0.80% and 0.90% with a mean value of 0.85 +/- 0.05 (SD)% (n = 6). The concentrations of halothane that prevented a movement response ranged between 0.90% and 1.20% with a mean value of 0.98 +/- 11 (SD)% (n = 24).

Table 4.1 : Head movement response to stimulus generated from a pre-set driving pressure of 275 Kpa (40 psi) and applied to the ear for a duration of 30 seconds and at a rate of force application of 1.6 N/second. At any one end-tidal concentration the stimulus was applied for three times before considering the response as negative.

Animal No.	End-tidal halothane conc.%	Applied stimulus N/mm^2	Head movement response	Animal No.	End-tidal halothane conc.%	Applied stimulus N/mm^2	Head movement response
4.I	0.9	1.42	-	4.IV	1.2	1.39	-
	0.9	1.42	-		1.2	1.39	-
	0.9	1.42	-		1.2	1.34	-
	0.8	1.42	+		0.9	1.24	+
	Mean	1.42			Mean	1.34	
	SD	0			SD	0.06	
4.II	0.9	1.38	-	4.V	0.9	1.29	-
	0.9	1.38	-		0.9	1.38	-
	0.9	1.46	+		0.9	1.20	-
	1.0	1.29	-		0.8	1.46	-
	1.0	1.29	-		0.8	1.38	-
	1.0	1.38	-		0.8	1.29	+
	Mean	1.36			Mean	1.33	
	SD	0.056			SD	0.08	
4.III	0.9	1.29	-	4.VI	1.0	1.43	-
	0.9	1.29	-		1.0	1.29	-
	0.9	1.20	-		1.0	1.29	-
	0.8	1.29	-		0.9	1.43	-
	0.8	1.29	+		0.9	1.35	+
	Mean	1.27			Mean	1.36	
	SD	0.04			SD	0.06	

Figure 4.2: A photographic illustration of a rabbit`s tail showing the severe trauma and lacerations inflicted by the application of a padded haemostat for the determination of MAC. This stimulus was so severe that its use was discontinued. Its use was restricted to animals in the pilot study which were deeply anaesthetised and did not recover consciousness

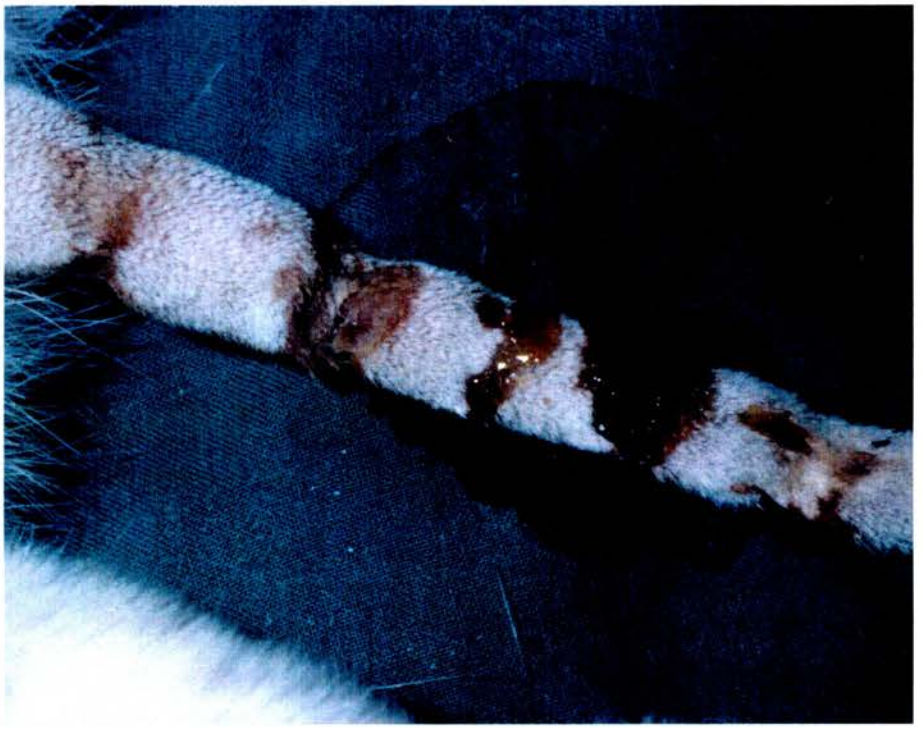
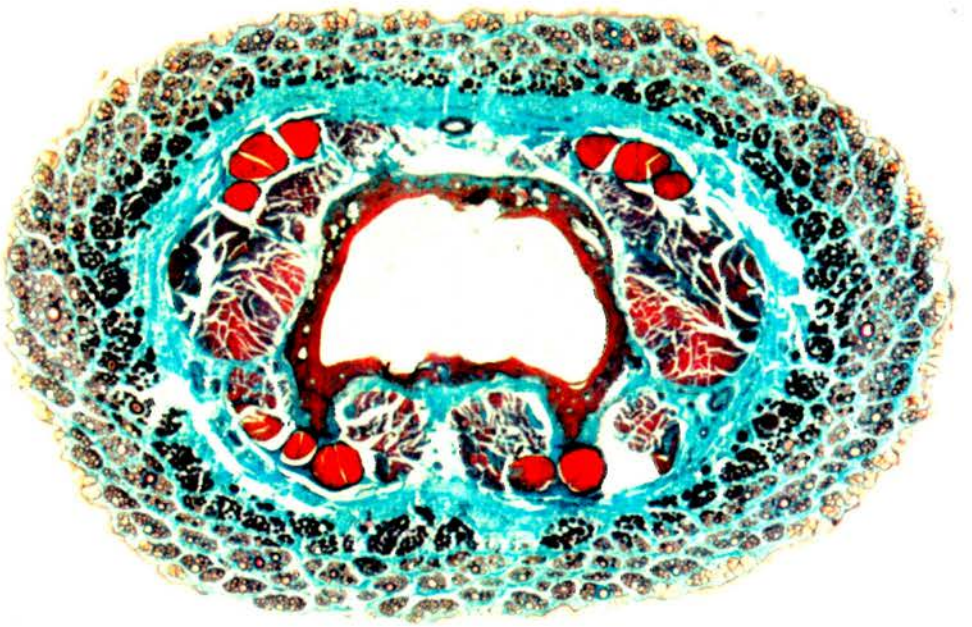
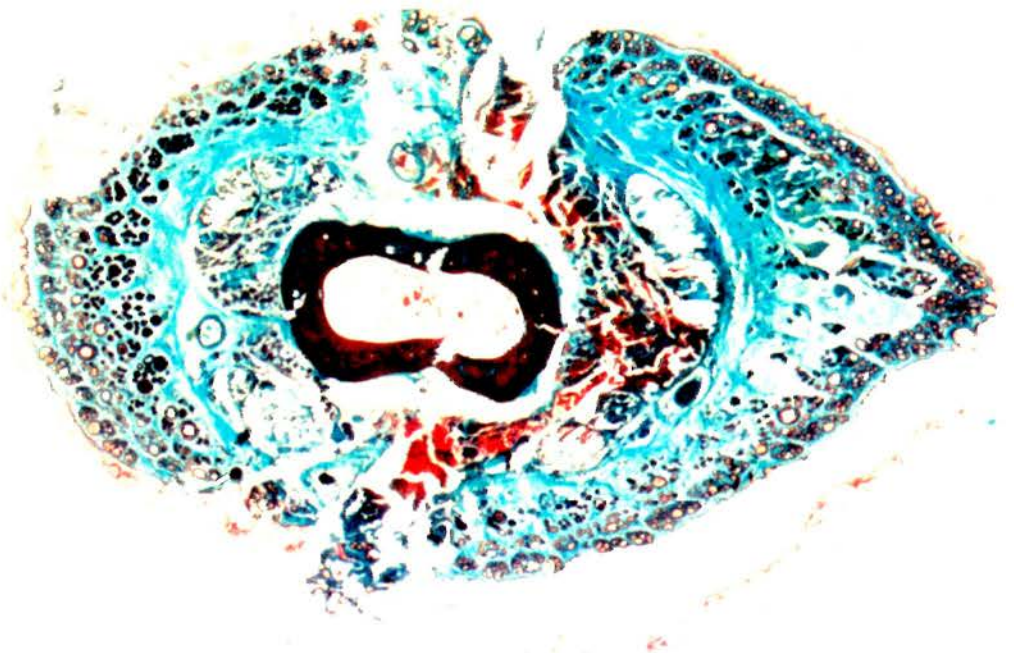


Figure 4.3: Histological illustration of the result of the application of a padded haemostat to the rabbit`s tail. (**A**) a transverse histological section of the tail obtained from a non-stimulated area, showing a normal histological picture; (**B**) a transverse section prepared from a stimulated site, revealing gross tissue disruption including fracture of the caudal vertebra, areas of haemorrhage and muscular contusion (please refer to Figure legend 4.2).

A



B



The MAC values (Table 4.2) calculated as the average of the halothane concentrations that allowed either a response or no response, ranged between 0.85% and 1.05% with a mean value of 0.92 +/- 0.07 (SD)% (n = 6).

The mean value of the stimulus for the 30 applications in Table 4.1 was a force of 1.35 +/- 0.07 (SD) N/mm^2 and the mean value of the stimulus in the 6 animals was 1.35 +/- 0.05 (SD) N/mm^2 (Table 4.2). An example of the simultaneously recorded three components of MAC, namely the end-tidal halothane concentration, the stimulus and the movement response to the stimulus is shown in Figure 4.4.

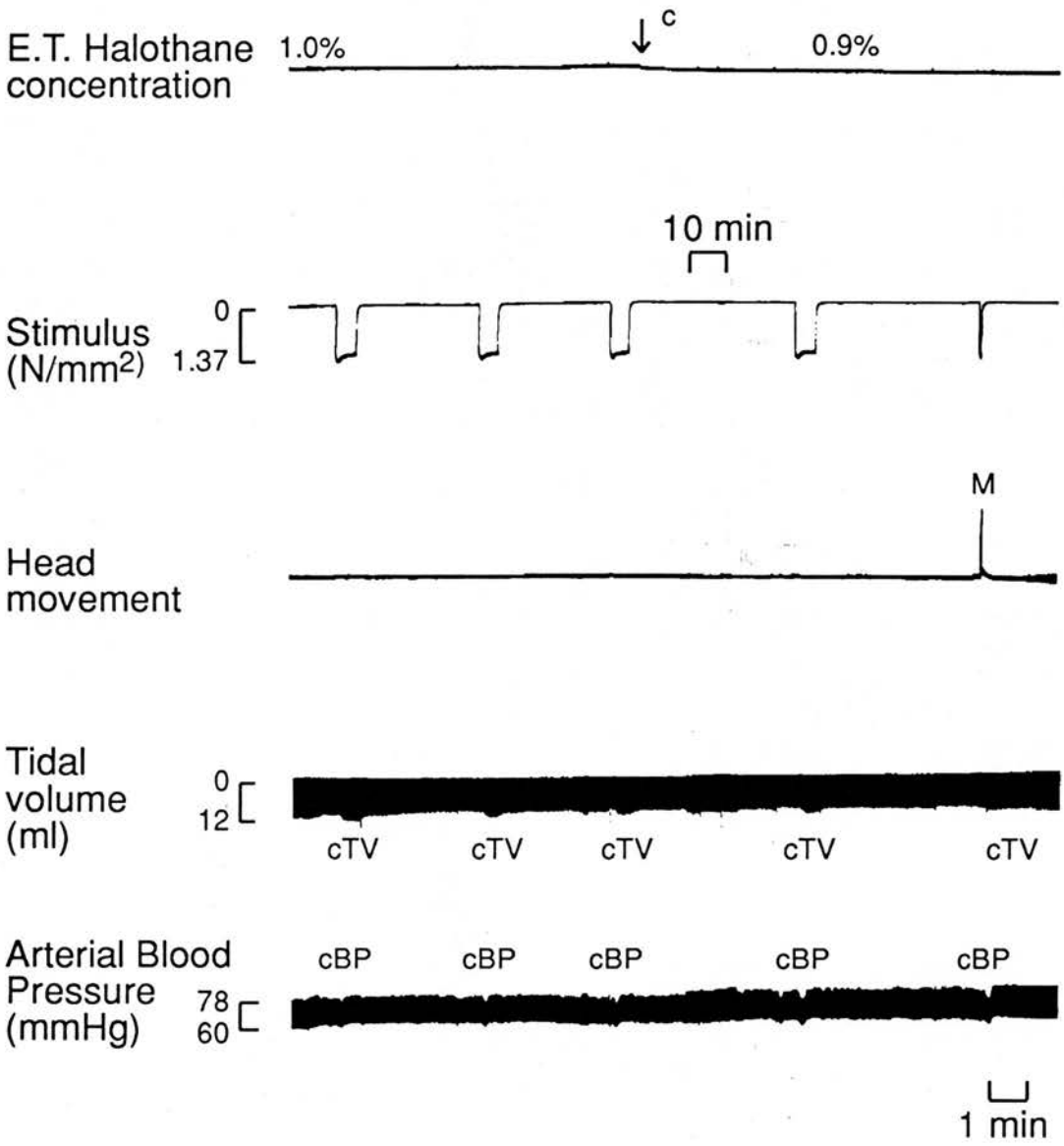
The response time, defined as the time from the application of the stimulus until the animal responded, showed considerable variability and it ranged between 1.2 and 12 seconds with a mean value of 7.4 +/- 4.5 (SD) seconds (n = 6). The normothermic rectal body temperature (Table 4.2) ranged between 39.0 °C and 40 °C with a mean value of 39.5 +/- 0.3 (SD) °C (n = 6).

Other effects of the stimulus that were frequently seen included a sudden drop in arterial blood pressure, an increase in the tidal volume and a transitory apnoea (Figure 4.4).

4.4: Discussion:

Of the three components of MAC, it is the stimulus applied that appears to have been least quantified by investigators. This was probably because it was generally held that MAC is not affected by the stimulus, provided that the stimulus is "supramaximal" in nature (Eger et al., 1965a). The stimulus required to determine MAC must be substantial but it must not be of sufficient intensity either to desensitise or sensitise the tissues.

Figure 4.4: An example of the measurement and recording of the three components of MAC determination namely, the end-tidal concentration, the mechanical stimulus (downward movement) and head movement response. At an end-tidal halothane concentration of 1.0% (no-response concentration), a consistent mechanical stimulus from a driving pressure of 275 Kpa (40 psi) was applied for three times and no response was detected. Then, the end-tidal concentration was reduced at (**c**) to 0.9% (response concentration) at which the animal was maintained for at least 15 minutes before the same stimulus was reapplied and the head movement response occurred at (**M**) within 1.2 seconds (response time) from the start of the second stimulus application. The stimulus was interrupted soon after the occurrence of the movement response. Note the drop in blood pressure (cBP) and the increase in tidal volume (cTV) that coincided with the stimulus application.


E.T. Halothane concentration
1.0%
C
0.9%
10 min
Stimulus (N/mm2)
0
1.37
M
Head movement
Tidal volume (ml)
0
12
cTV
cTV
cTV
cTV
cTV
Arterial Blood Pressure (mmHg)
78
60
cBP
cBP
cBP
cBP
cBP
1 min

Theoretically, the concept of a "supramaximal stimulus" might appear quite appealing since, by definition, it should provide for the reproducibility of MAC. However, there are a number of sources of concern when using a "supramaximal stimulus" for the determination of MAC. Firstly, in terms of sensory physiology it is conceptually difficult to interpret what "supramaximal", a term often used by anaesthetists, means - does it mean exciting a discrete population of nociceptors maximally, or all somatosensory nociceptors maximally?. Secondly, there are conflicting reports regarding the reliability of the "supramaximal stimuli" that are used. For instance, tail clamping is considered to be a supramaximal stimulus by some investigators (Eger et al., 1965a; Tranquilli et al., 1983) but not by others (Lundeen et al., 1983; Eger et al., 1988). Similarly, skin incision has been shown not to be a supramaximal stimulus in the dog (Eger et al., 1965a) and pig (Lundeen et al., 1983; Tranquilli et al., 1983), but it is considered to be a standard stimulus for MAC determination in man (Saidman et al., 1967)).

Thirdly, the unreliability of the use of the clamping technique using a haemostat for the determination of MAC in the rabbit was demonstrated in the pilot study of this investigation. This technique was shown to cause severe trauma and lacerations (Figures 4.2 and 4.3) that produced an altered sensitivity to stimulation when applied to either the ear or tail. In the tail, the technique may even have prevented sensory impulse conduction (Woolf, 1983; Woolf, 1991; Handwerker and Reeh, 1991; Dubner, 1991).

Table 4.2: Mean stimulus generated by a driving pressure of 275 Kpa (40psi) applied to the ear and MAC values calculated as the average of the response and no-response concentration.

Animal	Stimulus (Force) N/mm^2	Response Conc.%	No response. Conc.%	MAC Conc.%	Rectal temp. degree C	Response times (sec.)
4.I	1.42	0.8	0.9	0.85	39.5	12
4.II	1.36	0.9	1.0	0.95	40.0	4.8
4.III	1.27	0.8	0.9	0.85	39.2	9.6
4.IV	1.34	0.9	1.2	1.05	39.5	4.8
4.V	1.33	0.8	0.9	0.85	39.0	12
4.VI	1.36	0.9	1.0	0.95	39.7	1.2
Mean	1.35	0.85	0.98	0.92	39.5	7.4
SD	0.05	0.05	0.11	0.07	0.3	4.5

The stimulus-related sensitisation or desensitisation might eventually lead to a significant overestimation or under estimation respectively of the MAC value, with all the consequences and clinical implications regarding the dosage and the pharmacodynamics of the particular inhalation anaesthetic. In a previous report, MAC of halothane and methoxyflurane was determined in the rat using two stimulus intensities namely, a bull-dog clamp and a surgical haemostat applied to the tail. The difference between the values of MAC obtained for each of the two agents at the two stimulus intensities was suggested to be attributable to the deeper tail compression and greater trauma caused by the application of the surgical haemostat that are likely to result in greater recruitment of deep sensory fibres than would occur with the bull-dog clamp (Waizer and Orkin, 1973). Elsewhere, chronic inflammatory disease of the hoof significantly reduces the flexor reflex threshold in conscious sheep from control values of 4.35 N to 2.49 N (Waterman et al., 1992). Therefore it was decided not to continue using such a severe stimulus even in non-recovery work and it was considered to be ethically unacceptable and scientifically unreliable for the determination of MAC in recovery procedures. Furthermore, when the stimulus is of such intensity as to cause trauma that produces inflammation, sensitisation or even desensitisation, this makes scientific interpretation of MAC data impossible (Zavisca, Stanley, Cronau, and Iacono, 1993).

Other investigators have obviously been aware of this and they have implicitly acknowledge the problems related to the use of the clamping technique for determining MAC. This is evidenced from their attempts to circumvent or at least

minimise the trauma caused by the various clamping devices, for instance by using a rubber-shod haemostat (Drummond, 1985) or a haemostat with taped jaws (Doorley et al., 1988) or by always stimulating the tail proximal to an earlier test site i.e. avoiding repeating the stimulus at the same site (Stone et al., 1992; Kuroda, Strebel, Rafferty and Bullock, 1993). However, and on the basis of the pilot study in this investigation and the modern concepts of sensory physiology, it was concluded that these precautions and measures may not have succeeded in solving the problem of tissue damage provoking altered somatosensory sensitivity.

The MAC values (as a volume percent) obtained in this investigation, employing controlled mechanical stimulation that take into consideration knowledge of nociceptor threshold, ranged between 0.85% and 1.05%, with a mean value of 0.92 +/- 0.07 (SD)%. This is similar to the value obtained by Davis et al. (1975) of 0.82 +/- 0.25 (SD)%. It is significantly lower than the 1.39 +/- 0.23 (SD)% obtained by Drummond (1985). However, the comparison of the MAC values in this investigation with those obtained in those investigations is confounded because of a number of factors, mainly related to the stimulus used for MAC determination. Firstly, the stimulus used in this investigation was different from theirs, which was a rubber-shod haemostat (Drummond, 1985) and a Johns Hopkins arterial clamp (Davis et al., 1975). Secondly, in those investigations the stimulus was applied for an unspecified duration of upto 30 seconds (Davis et al., 1975) or for 60 seconds during which time the haemostat was continuously moved (Drummond, 1985). Neither of these investigators reported the rate of application of the clamps and they may not have

been in a position to do so because of the difficulty in controlling the manual application of the clamps. Both the rate of application and the duration of the stimulus have been found to affect the degree of firing of somatosensory nociceptors (Iggo and Kornhuber, 1977; Iggo, 1985) and are therefore intimately involved in eliciting any movement response. In this investigation a specific force (1.35 +/- 0.05 (SD) N/mm^2) applied at a rate of application of 1.6 N/second and for a constant duration of 30 seconds was used for the determination of MAC. Thirdly, in this investigation the stimulus was applied to the ear pinna rather than the tail (Davis et al., 1975; Drummond, 1985). The ear, unlike the small tapering tail, offers a large, flat surface area of more or less uniform thickness. These features provide for the application of consistent stimuli with less chance of repeating the stimulus at the same test site. Elsewhere, MAC obtained by clamping the tail of the pig was reported to show more variability than the MAC determined by clamping the dew claw of the same animal (Eger et al., 1988). These factors could account both for the differences between our results and those of the previous investigators and also for the greater variability in the MAC values obtained by Davis et al. (1975) and Drummond (1985).

In one rabbit, MAC was estimated to have an unexpectedly higher value of 1.05% (The underlined value in Table 4.2). This was probably accounted for by the larger anaesthetic increments (0.3%) used to determine the response on that occasion. The true MAC value for that rabbit could have had any value between 0.90 and 1.20%. This demonstrates that for a meaningful comparison of MAC data obtained from different investigations, it is important to state the size of increments or decrements

used for determining MAC, particularly that of potent anaesthetic agents. Also, it is equally important to test for the accuracy of the gas monitor used for the measurement of the end-tidal concentration, particularly in the light of significant reading errors reported with some infrared analysers (Foley et al., 1990), (see Chapter 3).

In one other rabbit (data not included in this investigation) a stimulus of 1.69 +/- 0.08 (SD) N/mm^2 (a mean of 9 applications) generated from a driving pressure of 50 psi was incidentally used for the determination of MAC in exactly the same way as described in this section. The MAC value of halothane was found to be 1.15%. This finding indicates the correlation between the stimulus and the various driving pressures. It also demonstrated the importance of quantifying the stimulus particularly if the MAC values from various investigations are to be meaningfully compared (see chapter 8).

One of the advantages of the mechanical stimulator used in this investigation was that, by simultaneously recording the stimulus and the movement response, the response time could be calculated. Positive movement responses occurred within 1.2-12 seconds after the start of the stimulus (Table 4.2), with a mean value of 7.4 +/- 4.5 (SD) seconds, indicating wide variability. If the stimulus was applied for no more than 10 seconds, as used by Merkel and Eger (1963), two of the positive responses would have been reported as negative and a different value obtained for MAC. For similar reasons, Eger et al. (1965a) suggested that the stimulus should be applied for

at least 30-40 seconds, usually for one minute. However, our results suggest that the stimulus need not be applied for longer than 30 seconds, which will minimise the tissue damage. These arguments demonstrate that a meaningful comparison of MAC data within the same investigation or between different investigations, requires the use of a consistent duration of stimulus application.

In addition to movement, other stimulus-related responses included a sudden drop in blood pressure (Figure 4.4) as seen in the "playing dead" reaction (Folkow and Neil, 1971). In one of the rabbits, for comparison, the stimulus from the same preselected pressure of 275 Kpa (40 psi) was applied to the nostril. At this site the stimulus was consistently associated with an increase in blood pressure as part of the positive response, as was previously reported to be a reaction to stress in human subjects (Wolf, Pfeiffer, Ripley, Winter, and Wolff, 1948). This indicates the importance of the selection of the site at which the stimulus is applied when investigating autonomic reflexes and requires further study.

When, after the initial stages of this experimental protocol, attempts were made in this group of experimental rabbits to raise progressively the body temperature and record any apparent or real changes in the MAC value of halothane, a number of problems arose. During the continuous heating phase frequent spontaneous movements were recorded. These movements included spontaneous paddling of the fore limbs that was occasionally accompanied by slight head movements. All other signs, however, indicate that these rabbits were anaesthetised. It was also found that during the

period of evaluation of MAC at the raised body temperature, the rectal temperature continued to rise over the period of measurement even after all active heating had been discontinued. These rabbits either died at a rectal temperature of around 44 °C or the experiment was terminated as the rabbits were killed by injecting a lethal dose of pentobarbitone while still under the influence of anaesthesia.

As no spontaneous movements occurred in normothermic animals, it therefore seems that the heating protocol employed in this section is the culprit behind such movements which may imply an increase in MAC of halothane with hyperthermia, previously reported in dogs (Steffey and Eger, 1974). However, spontaneous movements were reported to interfere with the MAC determination of isoflurane and I-653 in normothermic pigs (Eger et al., 1988).

These factors mean that if a study of the influence of body temperature on the MAC value of halothane is to be carried out, it is important that a more reliable heating and cooling protocol be established.

CHAPTER 5

HEATING AND COOLING PROTOCOL FOR RABBITS

5.1: Introduction:

In a previous report, investigating the influence of hyperthermia on MAC of halothane in dogs, whole body hyperthermia was induced using heating lamps (Steffey and Eger, 1974). In that report, MAC was repeatedly determined as the body temperature was continuously raised by 1 °C. every 45 or 60 minutes. During the heating protocol as described in the previous chapter, attempts to determine MAC of halothane in the rabbit were frustrated by the occurrence of spontaneous movements. Also there was a continuous rise of body temperature even after heating was discontinued, eventually leading to the death of the animal.

The purpose of this section is to establish a controlled and a reliable heating protocol that could be used in subsequent sections of this study for the determination of MAC in the rabbit. In the light of the widespread use of neuromuscular blockers, some investigators have suggested the use of selected cardiovascular responses to noxious stimuli, such as the systemic blood pressure responses (Gibbs et al., 1989) and heart rate (Roizen et al., 1981; Kissin and Green, 1984), as alternative end points to the provoked movement response for the determination of MAC and as an index of anaesthetic potency. Therefore, it is also one of the aims of this section to investigate the reliability of some of the stimulus-related cardiopulmonary responses as a possible end points for the determination of MAC of halothane in the rabbit.

5.2: Materials And Methods:

5.2.1: Experimental Protocol:

Unless stated otherwise, all the procedures employed in this section were performed as indicated in the section on Materials and General Methods (Chapter 3). A total of 11 New Zealand White rabbits were used (mean age of 4.5 +/- 0.7 (SD) month and a mean body weight of 3.9 +/- 0.5 (SD) kg). In each animal anaesthesia was induced and then maintained, throughout the experiment, on an end-tidal concentration of halothane of 1.8% i.e. approximately twice MAC 1.0 of halothane, as determined by Sobair et al. (1993). Animal instrumentation allowed the measurement and recording of end-tidal halothane concentration, rectal body temperature, mechanical stimulus (Sobair et al., 1993), head movement, arterial blood pressure, heart rate, tidal volume and respiratory rate.

Hyperthermia (2-2.5 ^{o}C above the initial body temperature) was induced using the heating protocol described in the previous Chapter with the following modifications: the pump output was reduced by using a speed setting of 7 instead of 9; the water heater was set at 70 ^{o}C instead of 90 ^{o}C and those parts of the heating tray in direct contact with the animal skin were covered with paper towels, with the aim of insulating them from the effects of conducted heat, due to direct contact. Animals were maintained at a hyperthermic plateau for up to 40 minutes after switching off the

heating. Cooling was then started by circulating iced water, using the same apparatus, to bring down the rectal temperature to approximately the pre-heating value.

Due to the variability in the initial rectal temperature and the rate of heating in the individual animals, a system of a fixed point markers was employed for data collection and analysis (Faithfull, Reinhold, van den Berg, van Rhoon, van der Zee, and Wike-hooley, 1984). Accordingly, the above mentioned cardiopulmonary parameters were measured at the following markers: the initial temperature or normothermia (Marker 0); the start of heating (Marker 1); 60 minutes after the start of heating - 60ASH (Marker 2); the end of heating (Marker 3); 30 minutes after switching off the heating = P30 (Marker 4); the start of cooling (Marker 5); 60 minutes after the start of cooling (Marker 6); 90 minutes after the start of cooling = 90 ASC (Marker 7); the end of cooling (Marker 8) and 30 minutes after the end of cooling =CP30 (Marker 9). Heating rates were calculated as "first or initial heating rate (FHR)" and "second heating rate (SHR)". The former was defined as the rate of heating in ^{o}C/ hour during the first 1 ^{o}C rise in rectal body temperature, and the latter as the rate of heating during the second ^{o}C rise in body temperature. Similarly cooling rates were calculated as "first" and "second" cooling rates.

In 3 animals, induced active cooling failed to bring down the temperature and they eventually died. The remaining 8 animals were divided into two groups of 4. In one group (stimulated group), some selected stimulus-related responses (changes in systolic and diastolic blood pressures in mmHg, changes in tidal volume, the

occurrence of paddling movements and limb and head movement) were observed, recorded and quantified in each animal for each stimulus application at 3 markers namely, normothermia, hyperthermia and after cooling to approximately the normothermic body temperature. The stimulus applied was a consistent mechanical stimulus generated from a preselected gauge pressure of 275 Kpa (40 psi). It was applied to the ear pinna and calculated in the same way as explained in Chapter 4. At each of the three selected markers in each animal, the stimulus was applied three times (Appendix B).

The frequency of spontaneously occurring changes including : (paddling (slight twitching) of limbs, head and or limb movement, changes in systolic and diastolic blood pressures and changes in tidal volume) was calculated in each animal for both the stimulated and non-stimulated groups for a fixed period of 15 minutes at specific markers. Those markers were : normothermia, hyperthermia (hyperthermic plateau after switching off the heating), 15 minutes before switching off the heating and after cooling to approximately the normothermic temperature (see Table 5.5 and Table 5.6).

5.2.2: Analysis Of Data:

Data were presented as means +/- the standard deviations (SD). All the calculations were carried out on a Microsoft spread sheet programme. Statistical analysis was performed using analysis of variance, available on a Genstat software, to test for

significant differences between the heating rates, cooling rates and between the heating and cooling rates (Table 5. 1). The lowest level of significance was considered to be "$P < 0.05$". For the comparison of the mean values of the measured cardiopulmonary parameters at the different markers (Table 5. 3), paired t-tests and Bonferonni correction factor for inequality were used. The correction factor is applied by dividing the value of alpha (0.05) by the total number of comparisons (9) with the aim of preventing type 1 errors (Fisher, 1986). Therefore, instead of $P = 0.05$ or less being significant, $P = 0.05/9$ i.e. $= 0.0055$ or less is significant. During the heating phase, Markers from 1-3 (Table 5. 3) were compared with Marker 0. After switching off the heating and during the cooling phase Markers 4-9 were compared with Marker 3.

5.3: Results:

The influence of heating and cooling on the measured cardiopulmonary parameters and the provoked as well as the spontaneously occurring changes in the rabbit, showed a considerable variation between the various markers. The heating and cooling rates in 8 rabbits are shown in Table 5. 1. The heating rates in the three animals in which induced active cooling failed to bring down the body temperate and eventually died are presented in Table 5. 2.

The heating and cooling rates in 8 rabbits are presented in Table 5. 1. The mean value of the second heating rate (1.36 +/- 0.14 (SD) ^{o}C/hr) was significantly higher ($P <$

0.01) than that of the first heating rate (0.78 +/- 0.13 (SD) °C/hr). Similarly, the mean value of the second cooling rate (1.53 +/- 0.46 (SD) °C/hr) was significantly higher ($P < 0.05$) than that of the first cooling rate (1.12 +/- 0.42 (SD) °C/hr). Also the mean value of the average cooling rate (1.32 +/- 0.38 (SD) °C/hr) was significantly higher ($P < 0.05$) than that of the average heating rate (1.07 +/- 0.13 (SD) °C/hr).

5.3.1: Cardiopulmonary Parameters:

The values of the cardiopulmonary changes, measured at the different markers are shown as mean +/- SD in Table 5. 3. A summary of these results in the individual animals is presented in Appendix A. These cardiovascular and respiratory parameters are graphically illustrated in Figures 5.1 and 5.2 respectively. During the heating phase (marker 1 - 3), the heart rate/min. was the first of the cardiopulmonary parameters to show a significant increase ($P < 0.0055$) from a mean value of 272.3 +/- 22.7 (SD) at normothermia (marker 0) (39.2 +/- 0.2 (SD) °C) to a mean value of 284.1 +/- 22.8 (SD) at marker 2 (60 minutes after the start of heating). By the time heating was switched off (marker 3), the mean value of the rectal temperature was 41.4 +/- 0.2 (SD) °C. At this marker, the respiratory rate /min. was significantly increased ($P < 0.001$) from a mean value of 79.0 +/- 10.2 (SD) at normothermia to a mean value of 163.1 +/- 28.1 (SD). In contrast, the tidal volume was significantly reduced ($P < 0.001$) from a normothermic mean value of 25.0 +/- 3.8 (SD) ml, to 9.4 +/- 1.8 (SD). At marker 4 (30 minutes after switching off the heating), the mean

value of the diastolic B.P. (47.8 +/- 6.4 (SD) mmHg) was significantly lower ($P < 0.0055$) than its mean value at normothermia (51.4 +/- 5.0 (SD) mmHg).

During the cooling phase (marker 5 - 8), the systolic blood pressure was the first of all measured parameters to show a significant decrease ($P < 0.0055$) in its mean value, from 87.4 +/- 14.1 (SD) at marker 3 (switching off the heating) to 78.9 +/- 10.9 (SD) mmHg at marker 6 (60 minutes after the start of cooling). At marker 7 (90 minutes after the start of heating), the systolic blood pressure still continued to be the only parameter to show a significant reduction ($P < 0.0055$) compared to its mean value at marker 3. By the time cooling was switched off (marker 8), the respiratory rate per minute was significantly reduced ($P < 0.0055$) from a mean value of 163.1 +/- 28.1 +/- (SD) at marker 3 to 99.7 +/- 15.8 (SD). In contrast, the tidal volume was significantly increased ($P < 0.0055$) from a mean value of 9.4 +/- 1.8 at marker 3 to 16.7 +/- 4.4 ml at marker 8. At marker 9 (30 minutes after switching off the cooling), the tidal volume continued to show a significant increase ($P < 0.0055$) while the respiratory rate per minute became highly significantly reduced ($P < 0.001$) compared to their mean values at marker 3.

5.3.2: Stimulus-Related Responses:

Using a consistent mechanical stimulus generated from a preselected gauge pressure of 275 Kpa (40 psi), the stimulus-related responses, calculated as means +/- SD (s), showed a considerable variation between animals, even at the same marker (Table 5. 4). Such variation is very obvious from the values of SD (s) and it is photographically illustrated at normothermia (Figure 5.3), hyperthermia (Figure 5.4) and after cooling (Figure 5.5). The mean values of stimulus-related changes in both the systolic blood pressure (12.3 +/- 8.7 (SD) mmHg) and the diastolic blood pressure (7 +/- 5.5 (SD) mmHg) are significantly

Table 5. 1: The heating and cooling rates in 8 rabbits anaesthetised with halothane.

Animal No.	First heating rate ^{o}C./Hr	Second heating rate ^{o}C./Hr	Average heating rate ^{o}C./Hr	First cooling rate ^{o}C./Hr	Second cooling rate ^{o}C./H	Average cooling rate ^{o}C/Hr
	0.81	1.36	1.09	1.13	1.76	1.45
5.II	0.79	1.46	1.13	0.58	0.52	0.55
5.III	1.07	1.5	1.29	0.92	1.2	1.06
5.IV	0.75	1.33	1.04	1.25	1.94	1.595
5.V	0.66	1.13	0.90	1.43	1.67	1.55
5.VI	0.63	1.18	0.91	0.54	1.82	1.18
5.VII	0.75	1.5	1.13	1.71	1.71	1.71
8.VIII	0.78	1.43	1.11	1.4	1.58	1.49
Mean	0.78	1.36 **	1.07	1.12	1.53 *	1.32 *
SD	0.13	0.14	0.13	0.42	0.46	0.38

* $P < 0.05$; ** $P < 0.01$

Table 5.2: Heating rates in three animals where active cooling failed to bring down the body temperature after heating.

Animal No.	First heating rate ^{o}C./Hr	Second heating rate ^{o}C./Hr	First heating rate after heating off ^{o}C./Hr	Rectal temperature ^{o}C at the start of cooling	First heating rate after cooling ^{o}C./Hr
5.IX	0.92	1.88	1.94	42.8	2.31
5.X	0.59	1.18	1.25	42.8	1.88
5.XI	0.62	1.18	2.5	42.5	3.75
mean	0.71	1.41	1.90	42.7	2.65
SD	0.18	0.40	0.63	0.2	0.98

Table 5. 3: Changes in cardiopulmonary parameters during heating followed by cooling of anaesthetised rabbits.

Marker♣	Statistic	Rectal temp. °C	Systolic B.P. mmHg	Diastolic B. P. mmHg	Heart rate/min.	Tidal Vol. ml	Resp. rate/min.
0	Mean	39.2	78.5	51.4	272.3	25.0	79.0
	SD	0.2	9.3	5.0	22.7	3.8	10.2
1	Mean	38.9	76.8	48.5	272.0	25.0	79.0
	SD	0.4	9.1	4.6	21.3	2.9	8.1
2	Mean	39.5	80.5	50.8	284.1 *	19.4	95.0
	SD	0.4	12.1	6.1	22.8	4.3	14.4
3	Mean	41.4	87.4	50.9	308.0	9.4 **	163.1 **
	SD	0.2	14.1	6.2	40.4	1.8	28.1
4	Mean	41.5	83.8	47.8 *	321.0	9.2	159.5
	SD	0.2	12.3	6.4	30.8	2.6	35.1

Table 5. 3: Continued.

Marker♣	Statistic	Rectal temp. ^{o}C	Systolic B.P. mmHg	Diastolic B. P. mmHg	Heart rate/min.	Tidal Vol. ml	Resp. rate/min.
5	Mean	41.4	81.1	48.3	314.1	10.9	151.6
	SD	0.1	13.6	6.4	28.0	4.8	37.3
6	Mean	40.3	78.9 *	45.4	287.6	13.0	127.0
	SD	0.5	10.9	6.3	22.3	5.2	21.3
7	Mean	39.9	75.5 *	42.7	288.8	13.2	119.0
	SD	0.7	10.4	5.0	26.3	2.6	19.6
8	Mean	39.1	74.9	45.1	268.6	16.7 *	99.7 *
	SD	0.4	13.7	4.7	8.8	4.4	15.8
9	Mean	39.0	69.8	41.3	273.7	21.7 *	86.7 **
	SD	0.4	14.1	5.3	8.0	5.2	7.8

♣ Key for Markers: Marker 0 = Normothermia i.e. before the start of heating Marker 1 = Start of heating Marker 2 = 60 minutes after start of heating Marker 3 = Switching off the heating Marker 4 = 30 minutes after switching off the heating Marker 5 = Start of cooling Marker 6 = 60 minutes after the start of cooling Marker 7 = 90 minutes after the start of cooling Marker 8 = Switching off the cooling. Marker 9 = 30 minutes after switching off the cooling.
* $P < 0.0055$; ** $P < 0.001$

Table 5. 4: Stimulus related responses of the arterial blood pressure and the tidal volume of anaesthetised rabbits at normothermia, hyperthermia and after cooling.

Animal No.	Marker	Statistic	Rectal temp oC	Stimulus N/mm2	Change in. systolic B.P	Change in Diastolic B.P.	Change in tidal vol
5.V	Normothermia		39.0	1.4	0	0	0
5.VI	Normothermia		39.3	1.38	2.0	1.3	0.3
5.VII	Normothermia		39.0	1.34	5.3	2.7	0.0
5.VIII	Normothermia		39.0	1.47	4.0	2.0	5.3
		Mean	39.1	1.40	2.8	1.5	1.4
		SD	0.1	0.05	2.3	1.1	2.6
5.V	Hyperthermia		41.3	1.28	2.7	1.3	0
5.VI	Hyperthermia		41.5	1.40	2.0	2.7	0.7
5.VII	Hyperthermia		41.8	1.30	10.0	4.7	0.3
5.VIII	Hyperthermia		41.5	1.32	0.0	0.0	3.3
		Mean	41.5	1.33	3.7	2.2	1.1
		SD	0.2	0.05	4.4	2.0	1.5
5.V	After cooling		39.0	1.38	0	0	0
5.VI	After cooling		39.0	1.40	19.3	11.3	2.3
5.VII	After cooling		39.3	1.28	17.3	11.3	0.0
5.VIII	After cooling		38.3	1.32	12.7	5.3	0.7
		Mean	38.9	1.34	12.3	7.0	0.8
		SD	0.4	0.05	8.7	5.5	1.1

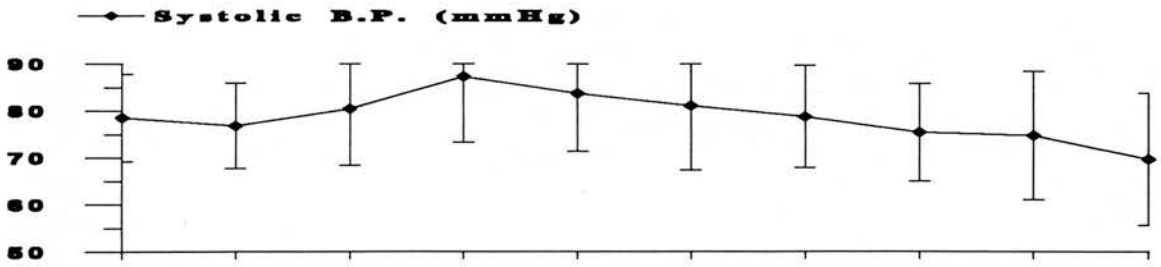
Table 5. 5: The frequency of spontaneously occurring Changes in non-stimulated anaesthetised rabbits at four selected markers. In animal No. 5.I the tidal volume was not measured as indicated by "?" mark.

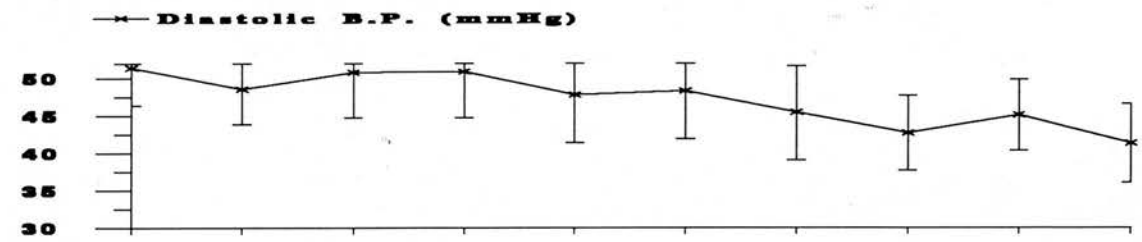
Animal No.	Marker	Rectal temp. ^{o}C	paddling of limbs	head and or limb movement	Change in Systolic B.P.	Change in Diastolic B.P.	Change in tidal volume
5.I	Normothermia	39.5	0	0	6	6	?
5.II	Normothermia	39.5	0	0	1	1	1
5.III	Normothermia	39	0	0	3	3	3
5.IV	Normothermia	39	0	0	2	2	0
	Mean	39.3	0.0	0.0	3.0	3.0	1.3
	SD	0.3	0.0	0.0	2.2	2.2	1.5
5.I	Hyperthermia	41.5	5	0	6	6	?
5.II	Hyperthermia	41.3	2	0	5	5	5
5.III	Hyperthermia	41.5	0	0	0	0	?
5.IV	Hyperthermia	41.5	0	0	0	0	0
	Mean	41.5	1.8	0.0	2.8	2.8	2.5
	SD	0.1	2.4	0.0	3.2	3.2	3.5
5.I	15 mins. before heating off	41.3	2	0	5	5	?
5.II	15 mins. before heating off	40.6	3	0	3	3	3
5.III	15 mins. before heating off	41	4	0	2	2	2
5.IV	15 mins. before heating off	41.3	1	0	2	2	2
	Mean	41.1	2.5	0.0	3.0	3.0	2.3
	SD	0.3	1.3	0.0	1.4	1.4	0.6
5.I	After cooling	39.5	0	0	10	10	?
5.II	After cooling	40	0	0	3	3	3
5.III	After cooling	39	0	0	0	0	0
5.IV	After cooling	38.8	0	0	1	1	1
	Mean	39.3	0.0	0.0	3.5	3.5	1.3
	SD	0.5	0.0	0.0	4.5	4.5	1.5

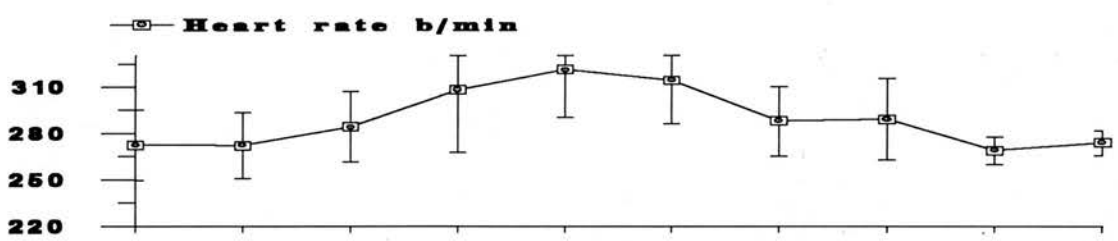
Table 5. 6: The frequency of spontaneously occurring Changes in stimulated anaesthetised rabbits at four selected markers.

Animal No.	Marker	Rectal temp. ^{o}C	paddling of limbs	head and or limb movement	Change in Systolic B.P.	Change in Diastolic B.P.	Change in tidal volume
5.V	Normothermia	39	0	0	3	3	1
5.VI	Normothermia	39.3	0	0	1	1	1
5.VII	Normothermia	39.3	0	0	0	0	0
5.VIII	Normothermia	39	0	0	1	1	0
	Mean	39.2	0	0	1.25	1.25	0.5
	SD	0.2	0	0	1.3	1.3	0.6
5.V	Hyperthermia	41	0	0	3	3	2
5.VI	Hyperthermia	41.5	2	0	2	2	2
5.VII	Hyperthermia	41.5	0	0	2	2	2
5.VIII	Hyperthermia	41.5	1	0	0	0	0
	Mean	41.4	0.8	0	1.8	1.8	1.5
	SD	0.3	1.0	0	1.3	1.3	1.0
5.V	15 mins before heating off	41	1	0	5	5	5
5.VI	15 mins before heating off	41	1	0	2	2	2
5.VII	15 mins before heating off	41.3	0	0	0	0	0
5.VIII	15 mins before heating off	41.5	4	0	4	4	4
	Mean	41.2	1.5	0	2.75	2.75	2.75
	SD	0.2	1.7	0	2.2	2.2	2.2
5.V	After cooling	39	0	0	2	2	0
5.VI	After cooling	39.2	0	0	6	6	6
5.VII	After cooling	39.3	0	0	2	2	1
5.VIII	After cooling	38.3	1	0	2	2	2
	Mean	39.0	0.3	0	3.0	3.0	2.3
	SD	0.5	0.5	0	2.0	2.0	2.6

Figure 5.1: Graphical illustration of the influence of heating followed by cooling on selected cardiovascular parameters in the rabbit measured at a defined markers (see text). During the heating phase (Marker 1 - 3), there was a significant increase ($P < 0.0055$) in the heart rate as compared to its normothermic value. At marker 4 (30 minutes after switching off the heating at a rectal temperature of 41.4 +/- 0.2 (SD) ^{o}C), there was a significant reduction ($P < 0.0055$ in the diastolic blood pressure) as compared to its normothermic value. During the cooling phase (marker 5 - 8), there was a significant drop ($P < 0.0055$) in the systolic blood pressure as compared to its value when heating was switched off (marker 3). The vertical error bars represent standard deviations (SD)s.


Systolic B.P. (mmHg)
90
80
70
60
50


Diastolic B.P. (mmHg)
50
45
40
35
30


Heart rate b/min
310
280
250
220

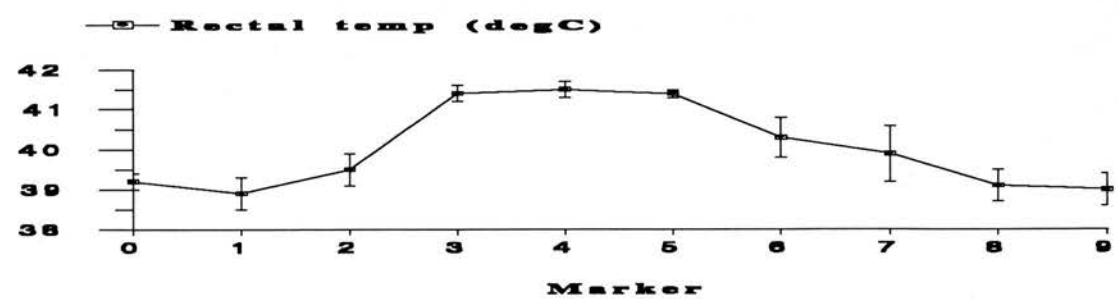
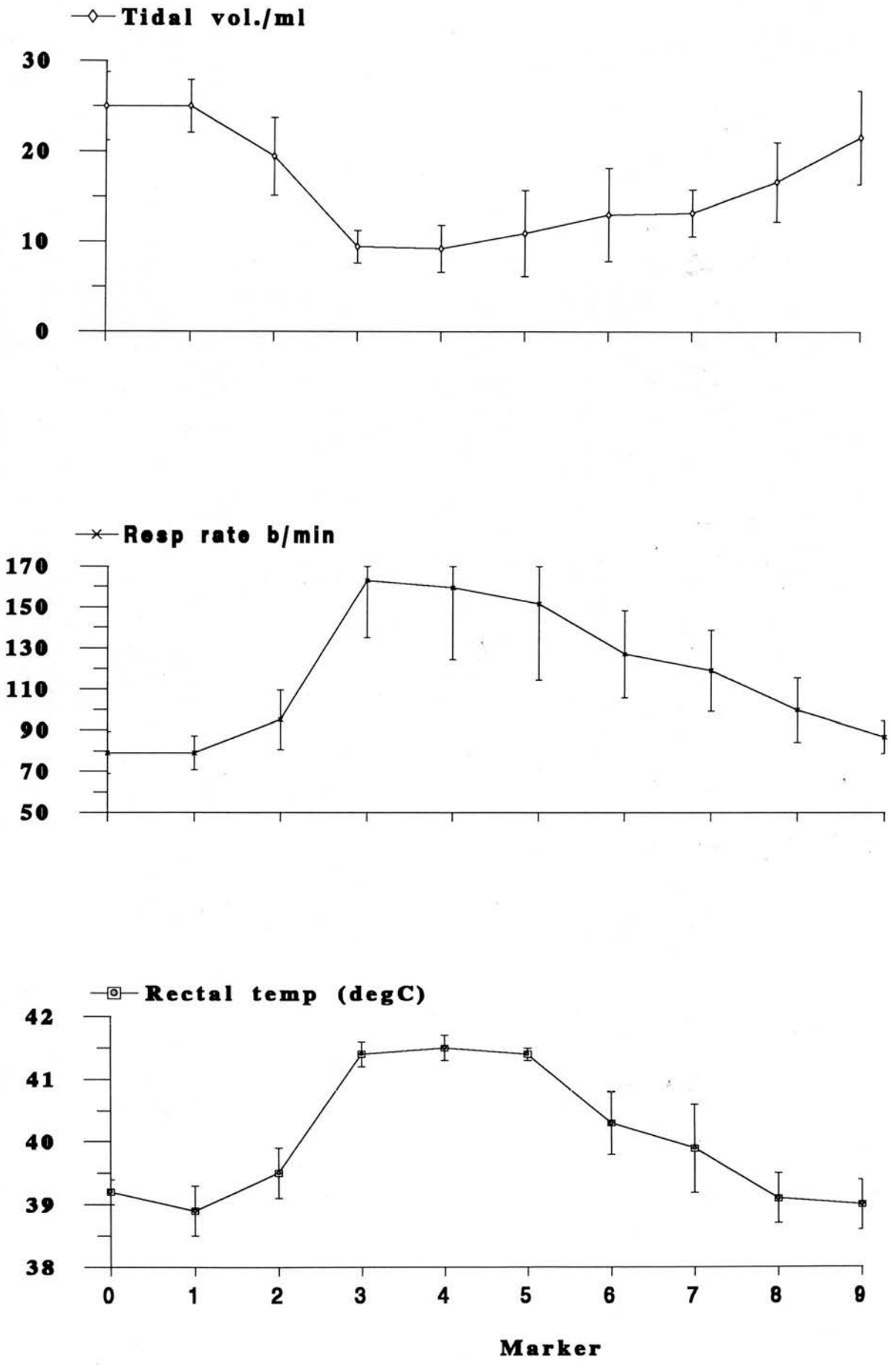

Rectal temp (degC)
42
41
40
39
38
0
1
2
3
4
5
6
7
8
9
Marker

Figure 5.2: Graphical illustration of the influence of heating followed by cooling on selected respiratory parameters. During the heating phase, a significant increase ($P < 0.001$) in the respiratory rate was seen at marker 3 as compared to its normothermic value. This was accompanied by a significant decrease ($P < 0.001$) in the tidal volume. At the end of cooling (marker 8), the respiratory rate was significantly reduced ($P < 0.0055$) while the tidal volume was significantly increased ($P < 0.0055$) as compared to their values at the peak of heating (marker 3). The vertical error bars represent standard deviations (SD)s.


Tidal vol./ml
30
20
10
0
Resp rate b/min
170
150
130
110
90
70
50
Rectal temp (degC)
42
41
40
39
38
0
1
2
3
4
5
6
7
8
9
Marker

greater after cooling than their mean values at both normothermia and hyperthermia. On the other hand, the mean changes in the tidal volume were very small at all three markers, but showed considerable variation. With all but very few stimulus applications, the blood pressure changes were of depressor nature i.e. reductions (Appendix B). Changes in the tidal volume are also mostly reductions.

With all stimulus applications in all 4 animals, there were neither paddling movements nor limb and or head movements. On the other hand, apnoea was very inconsistent in its occurrence. It occurred only with 25% of all applications (36) (Appendix B).

5.3.3: Spontaneously Occurring Changes:

The frequency of the spontaneously occurring changes in the stimulated and the non-stimulated groups are shown in Table 5.5 and Table 5.6 respectively. At all four markers in all animals in both groups, no head and or limb movement was detected. Paddling of limbs showed a wide variability in frequency of occurrence within and between the markers. It was observed at the markers during hyperthermia (Figure 5.6) and 15 minutes before heating was switched off in the non-stimulated group (Table 5. 5) as well as after cooling where it occurred only once in one single animal of the stimulated group (animal No. 8 in Table 5. 6). The frequency of changes in arterial blood pressure and the tidal volume also showed considerable variability in both groups (Table 5. 5 and 5.6).

Figure 5.3: Stimulus-related responses in a normothermic rabbit (rectal temperature of 39.0 °C), maintained on an end-tidal halothane concentration of 1.8%. The application of a consistent mechanical stimulus generated from a driving pressure of 275 Kpa (40 psi), was associated with an obvious drop in arterial blood pressure (cBP) at the third stimulus application but there was neither head movement nor changes in tidal volume. Note the break-down in the continuity of the blood pressure tracing as a result of the blockage of the arterial line, probably by a clot, as indicated by the obvious improvement when the line was flushed at (F).

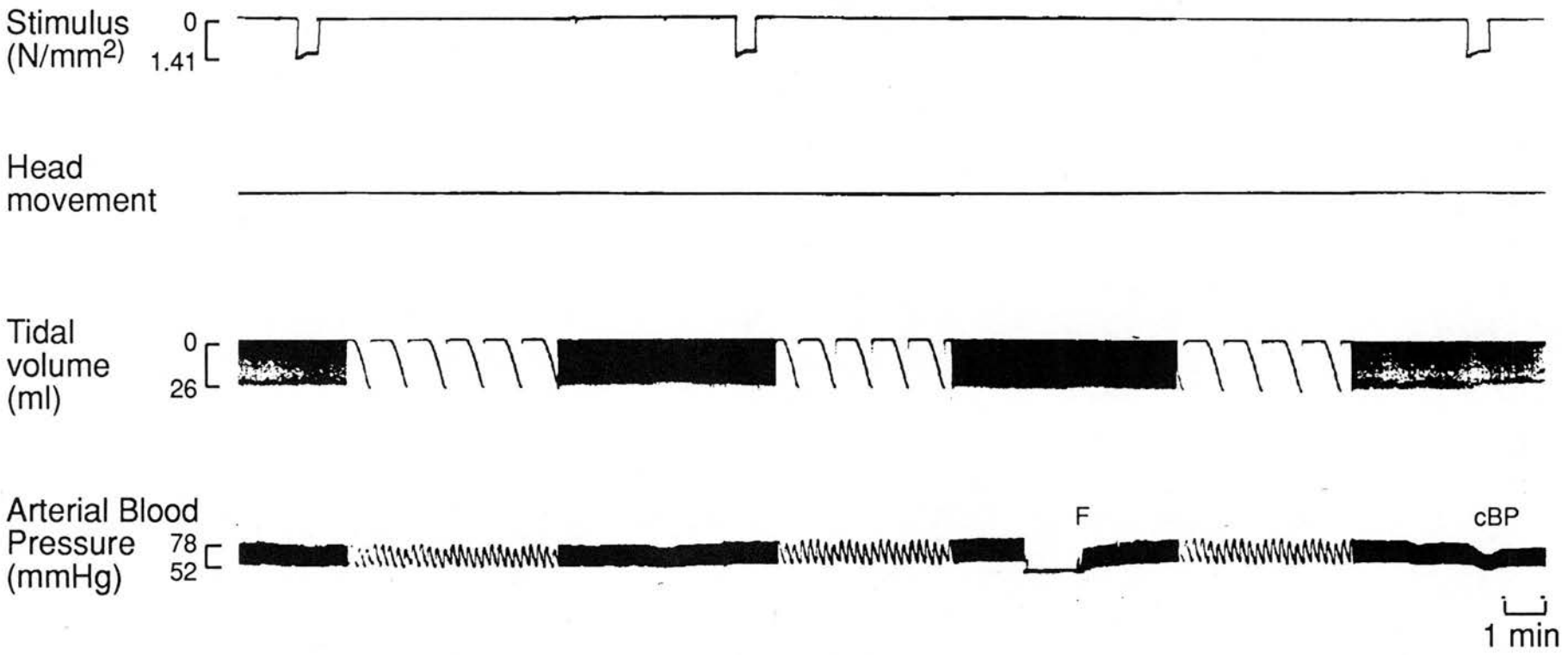
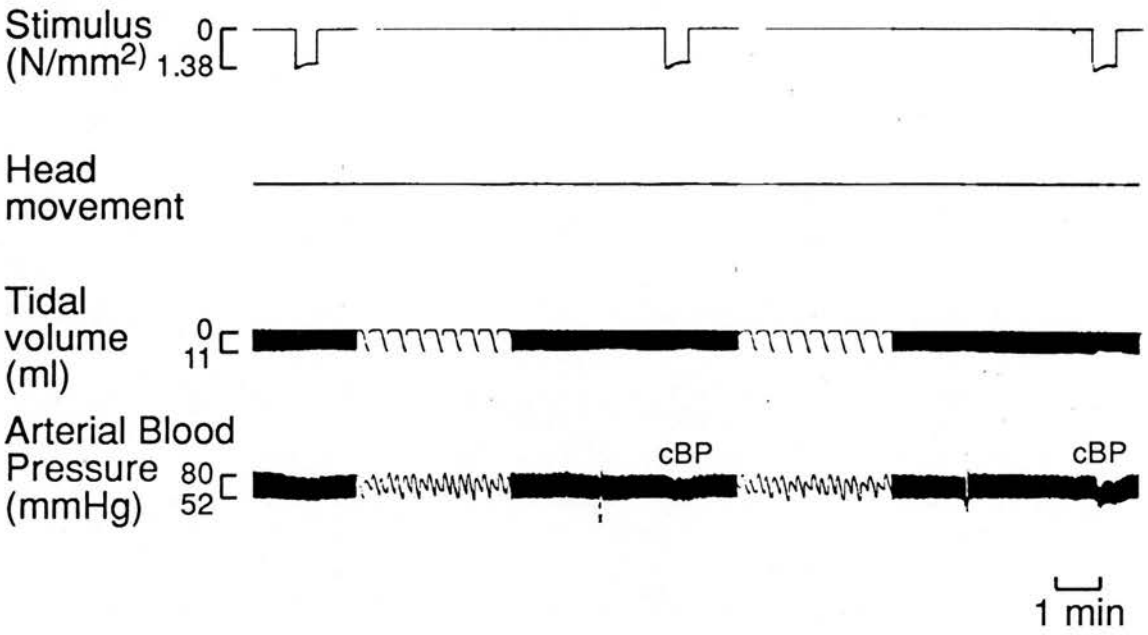

Stimulus
(N/mm2)
0
1.41
Head
movement
Tidal
volume
(ml)
0
26
Arterial Blood
Pressure
(mmHg)
78
52
F
cBP
1 min

Figure 5.4: Stimulus-related responses in a hyperthermic rabbit (rectal temperature of 41.8 °C), maintained on an end-tidal halothane concentration of 1.8%. The application of a consistent mechanical stimuli generated from a driving pressure of 275 Kpa (40 psi), was associated with a drop in arterial blood pressure (cBP) at the second and the third stimulus applications but there was neither head movement nor changes in tidal volume.

Figure 5.5: Stimulus-related responses in a rabbit that was cooled to approximately the preheating rectal temperature (39.3 °C) and maintained on an end-tidal halothane concentration of 1.8%. The application of a consistent mechanical stimuli generated from a driving pressure of 275 Kpa (40 psi), was associated with a drop in arterial blood pressure (cBP) at the second and the third stimulus applications but there was neither head movement nor changes in tidal volume. Note the "saw teeth" appearance of the arterial blood pressure tracing which is a feature frequently encountered in rabbits during the cooling phase.


Stimulus
(N/mm2)
0
1.38
Head
movement
Tidal
volume
(ml)
0
11
Arterial Blood
Pressure
(mmHg)
80
52
cBP
cBP
1 min

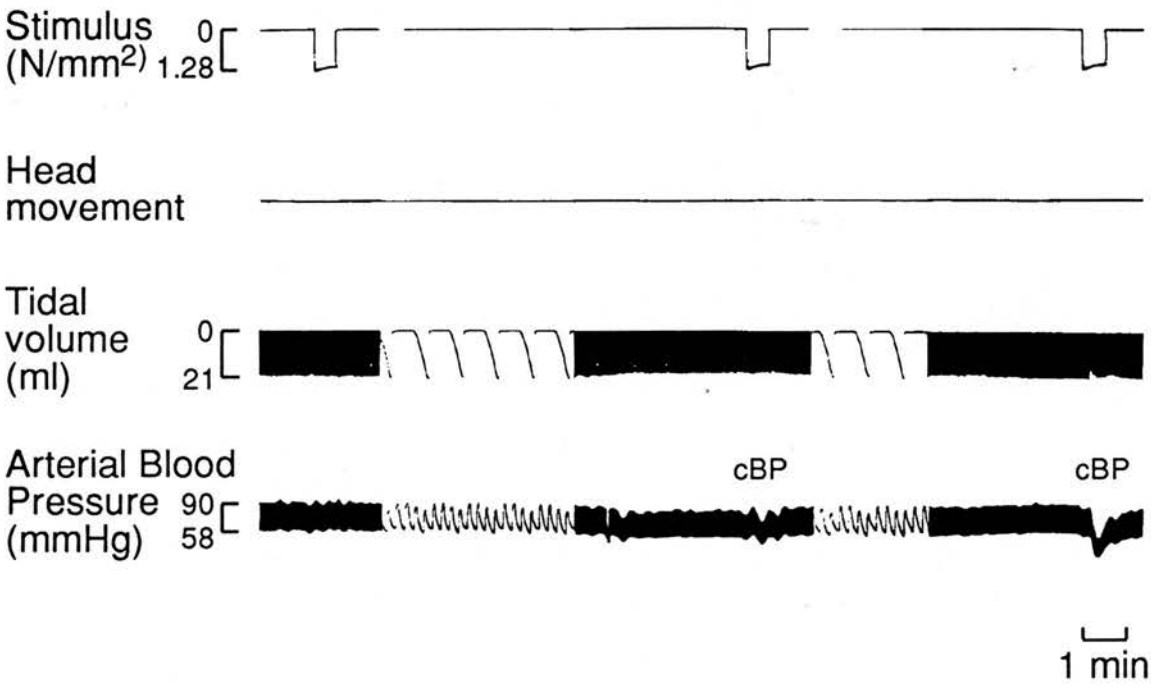
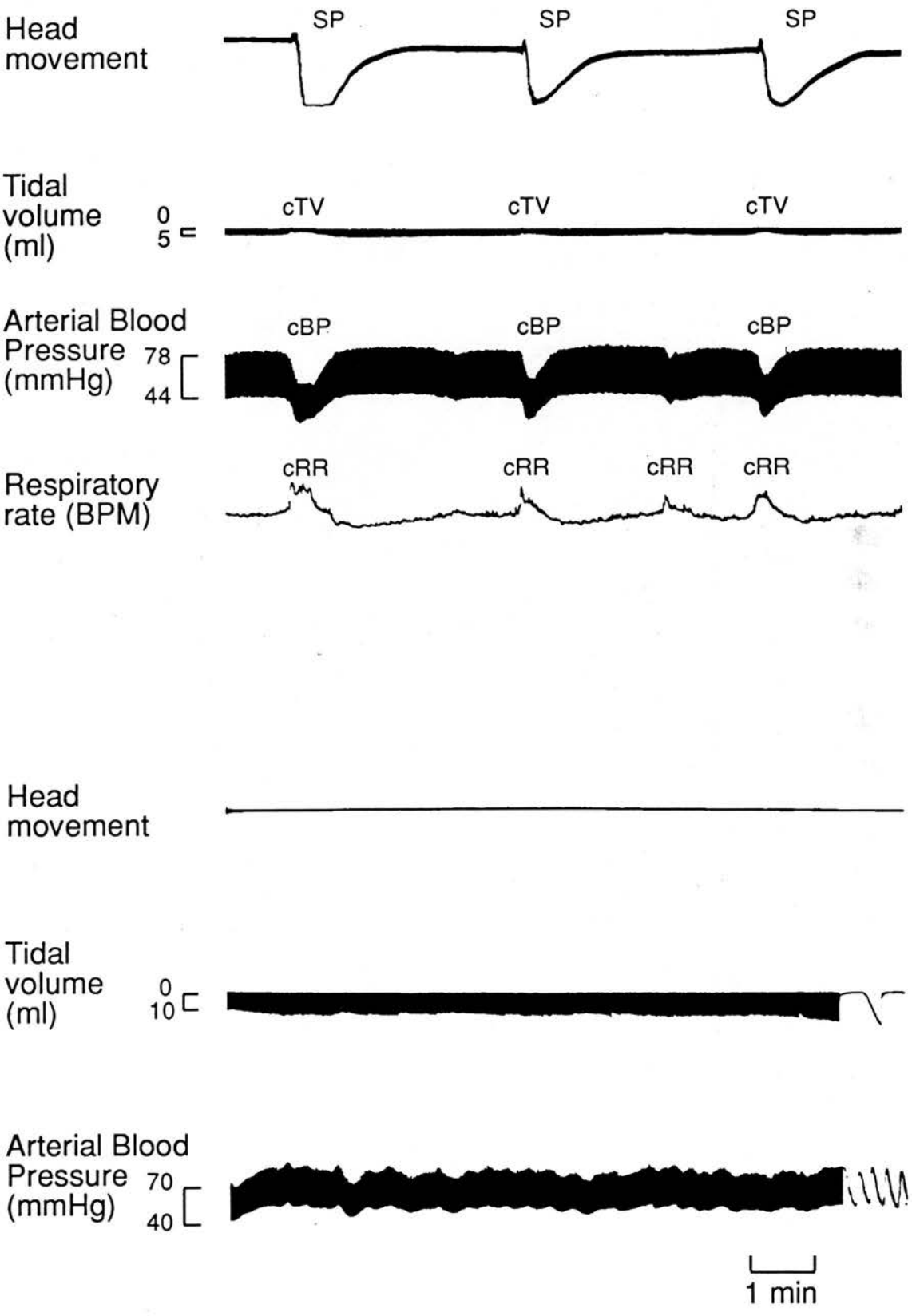

Stimulus
(N/mm2)
0
1.28
Head
movement
Tidal
volume
(ml)
0
21
Arterial Blood
Pressure
(mmHg)
90
58
cBP
cBP
1 min

Figure 5.6: Spontaneously occurring changes in a rabbit maintained on an end-tidal halothane concentration of 1.8%. At hyperthermia (41.5 °C) (upper figure), spontaneous paddling (SP) of fore limbs were associated with a slow and regular head movement that was accompanied by a drop in both arterial blood pressure (cBP) and tidal volume (cTV) as well as an increase in the respiratory rate (cRR). After cooling the same animal to a normothermic rectal temperature of 39.5 °C (lower figure), no head movement was detected and the most obvious feature was the "saw teeth" appearance of the blood pressure tracing which was frequently encountered in rabbits during the cooling phase.


Head movement
SP
SP
SP
Tidal volume (ml)
0
5
cTV
cTV
cTV
Arterial Blood Pressure (mmHg)
78
44
cBP
cBP
cBP
Respiratory rate (BPM)
cRR
cRR
cRR
cRR
Head movement
Tidal volume (ml)
0
10
Arterial Blood Pressure (mmHg)
70
40
1 min

5.4: Discussion:

Depending on the reason for doing so, whole body hyperthermia can be induced using a variety of heating techniques. For instance, for cancer therapy such techniques included patient insulation by molten wax (Pettigrew, Galt, Ludgate, Horn and Smith, 1974), hot water circulation through a water blanket or suit (Gerad, Echo, Whitacre, Ashman, Hinrrich, Foy, Ostrow, Wiernik and Aisner, 1984) and extracorporeal heating (Herman, Zukoski, Andaerson, Hutter, Blitt, Malone, Larson, Dean and Roth, 1982). For the determination of MAC of halothane in hyperthermic dogs, hyperthermia was induced using insulation and heating lamps (Steffey and Eger, 1974).

The hyperthermia techniques employed for cancer therapy, may have one or more features that render them unsuitable for inducing hyperthermia for the determination of MAC. For instance, the total insulation of the patient by molten wax, can make it difficult for the monitoring of movement and could interfere with the easy accessibility to some parts that are essential for the determination of MAC such as the ears, limbs and head. Also the extracorporeal heating or similar techniques used in the rabbit (Hilling, 1987), employ an invasive procedures that might affect the stimulus application or sensitivity to it during the determination of MAC.

With the heating protocol established in this section (Table 5.1), the significant ($P < 0.01$) difference between the "first" and "second" heating rates could probably accounted for by the relatively more active and efficient homeostatic mechanisms of heat loss, particularly through evaporation, that opposes the heat gain during the initial phase with the ultimate result of slower "first" heating rate. On the other hand the relative "exhaustion" of the mechanisms of heat loss during the subsequent heating period, might have allowed the heat gain to go unopposed leading to a higher "second" heating rate. When the animal`s heat loss mechanisms are totally uncompensated, the main determinants will be the environmentally related mechanisms of heat loss.

The "average" heating rate obtained in this investigation (1.07 +/- 0.13 (SD) °C / hr) (Table 5.1), was very much lower than those employed in cancer therapy that were reported to be as high as 13.4 °C / hr (Parks, Minaberry, Smith and Neely, 1979). However, it compares well with an estimated heating rate of 1 °C per 45 - 60 minutes, used for the determination of MAC in hyperthermic dogs (Steffey and Eger, 1974).

In the three animals where active cooling failed to bring down the body temperature (Table 5. 2), the exact reasons for this were unclear. However, these three animals shared three distinctive features, namely significantly higher rates of passive heating i.e. after heating was switched off; significantly higher rates of passive heating during the ineffective active cooling and in all three animals, cooling was started when the rectal temperature was above 42 °C. (Table 5.2). Therefore, it appeared that a rectal temperature of 42.0 °C represents a critical temperature above which normal physiological responses are interfered with. This statement confirmed similar previously reported findings in rabbits (Nichelmann, Martin, Scholz and Lyhs, 1975) and dogs (Steffey and Eger, 1974).

During the heating phase (Table 5. 3), the significant increase in both the heart and respiratory rate was not unexpected, because of their role in the heat dissipating mechanisms. No significant change was detected in either the systolic or the diastolic blood pressure during the heating phase up to marker 3. The reason for that might be the simultaneous occurrence of some of the physiologically antagonistic cardiovascular responses. For instance, a tachycardia associated reduction in the ventricular filling time and the fall in the peripheral resistance that accompany the use of volatile anaesthetics or hyperthermia, can antagonise the expected increase in the stroke volume and blood pressure (Feldman, Harrop-Griffiths and Hirsch, 1989b).

As it is shown in Table 5. 3 and illustrated in Figure 5.2, the increase in the respiratory rate during the heating phase, is accompanied by a decrease in the tidal volume. When calculated as change per 1 °C rise in body temperature, the respiratory rate and tidal volume were found to change maximally (marker 3) by 36.6 breath per minute (increase) and 6.8 ml (decrease) respectively. Similarly, the maximum increase in the heart rate was calculated to be 21.2 beats per minute per 1 °C at marker 4. This value was significantly higher than that calculated in man, of 8.1 beats / min. / °C (Barlogie, Corry, Yip, Lippman, Johnson, Khalil, Tenczynski, Reilly, Lawson, Dosik, Rigor, Hankenson, and Freireich., 1979), 12.8 beats / min./°C (Faithfull et al., 1984) and 14.4 beats / min. / °C (Larkin, Edwards, Smith, and Clarke, 1977). These variations might have been brought about by variations in the heating techniques, anaesthetics used, level of anaesthesia and species related differences. During the cooling phase (Table 5. 3), the maximum changes, expressed as change per 1 °C drop in body temperature, in the heart and respiratory rates and the tidal volume were calculated to be 21.8 ml (decrease), 31.8 ml (decrease) and 5.1 ml (increase) respectively.

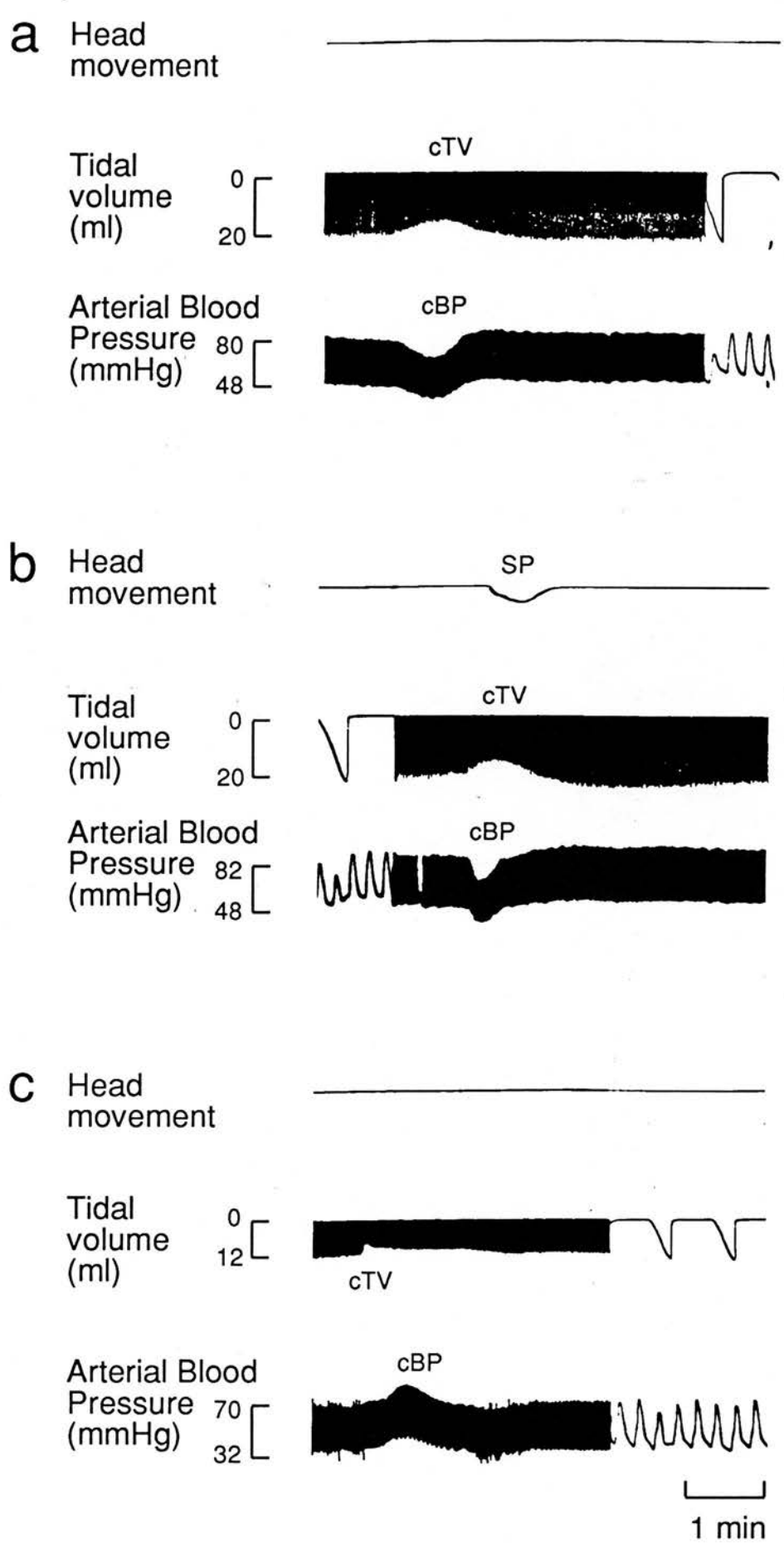
For the investigation of the stimulus-related responses, it was necessary to use consistent stimuli and maintain the animals at the same level of anaesthesia (end-tidal concentration of 1.8%) throughout the experiment. In the rat, it was reported that the nature of arterial blood pressure responses to noxious stimuli (i.e. whether a pressor or a depressor), was found to be dependent on the level of anaesthetic concentration (Fukunaga, Tanigughi and Kikuta, 1990).

The lack of occurrence of provoked movement response to the applied mechanical stimuli during all phases (Appendix B) was not unexpected, since the level of anaesthetic used was approximately twice MAC 1.0, as determined in the previous Chapter and reported by Sobair et al. (1993). Other stimulus-related responses (Table 5. 4), included changes in systolic and diastolic blood pressure and the tidal volume. In the light of the widespread use of the neuromuscular blockers, some investigators have suggested the use of some stimulus-related cardiovascular responses, such as changes in blood pressure (Gibbs et al., 1984; Roizen et al., 1981), heart rate (Kissin and Green, 1984), as end points for the determination of MAC and anaesthetic potency. However, the findings in this section (Table 5. 4) which was also confirmed in chapters 6 and 7, suggested that , in the rabbit, the stimulus-related responses particularly the changes in blood pressure and heart rate were not reliable substitutes for the provoked movement response as an end point for the determination of MAC, for a number of reasons. Firstly, using consistent stimuli at the same anaesthetic level, the arterial blood pressure was found to show both quantitative (the extent of the pressor or depressor effects) and qualitative (pressor or depressor responses) inconsistency. Such inconsistency was very obvious within and between the markers (Table 5. 4 and Figures 5.3, 5.4, 5.5) and it was further demonstrated when determining MAC in chapters 6 and 7. Secondly, these Changes quite often can occur spontaneously (Figure 5.7), in a manner that was neither quantitatively or qualitatively distinguishable from the stimulus -related responses (Table 5. 5 and 5.6).

Thirdly, the measurement of these responses required a relative degree of sophistication and instrumentation and consequently are more liable to the problems

related to such complexity such as the occasional or permanent measurement failure due to factors such as blockade of the arterial line by a blood clot (Figure 5.3) , displacement of the arterial catheter or failure of one or more of the measuring devices. In contrast, the provoked movement response can be reliably observed and or recorded with far less sophistication in instrumentation. Fourthly, the use of the arterial blood pressure responses can be highly subjective. This can easily be demonstrated by considering the fact that, the same blood pressure response can be detectable at one range (sensitivity) of blood pressure amplifier and not at the other. In addition, the blood pressure responses were reported to be dependent on the level of anaesthetic concentration in the rat (Gibbs et al., 1989) and the rabbit (Fukunaga et al., 1990). Therefore, it is concluded that the provoked movement response to noxious stimuli remains unchallenged as the most reliable end point for the determination of MAC in the rabbit.

Figure 5.7: The quantitative and qualitative variability of spontaneously occurring cardiopulmonary changes in a rabbit maintained at an end-tidal halothane concentration of 1.8%. (a) 10 minutes after heating was started (39.0 °C), there was a spontaneous decrease in both arterial blood pressure (cBP) and the tidal volume (cTV) and no head movement was detected. (b) 25 minutes after heating was started (39.3 °C), in addition to the spontaneous drop in arterial blood pressure and the tidal volume there was slight slow head movement (SP) that accompanied spontaneous paddling of the fore limbs. (c) During the cooling phase (40 °C), there was an increase in arterial blood pressure, a decrease in the tidal volume and no head movement was detected. In all three figures, note the individual spikes in the arterial blood pressure and tidal volume tracings, obtained by running the recording paper at a faster speed (25 divisions per second), which were used for calculating the respiratory and heart rates.


a
Head movement
Tidal volume (ml)
0
20
cTV
Arterial Blood Pressure (mmHg)
80
48
cBP
b
Head movement
SP
Tidal volume (ml)
0
20
cTV
Arterial Blood Pressure (mmHg)
82
48
cBP
c
Head movement
Tidal volume (ml)
0
12
cTV
Arterial Blood Pressure (mmHg)
70
32
cBP
1 min

CHAPTER 6

THE INFLUENCE OF HYPERTHERMIA ON MAC OF HALOTHANE DETERMINED USING CONTROLLED HEAT AND MECHANICAL STIMULATION

6.1: Introduction:

Few and contradictory reports and observations, supported with little experimental work, are available on the influence of hyperthermia on the pharmacodynamics and pharmacokinetics of anaesthetic drugs. Those reports and observations have concentrated on the effects of a hot environment or induced hyperthermia on onset of action of anaesthetic drugs (Dundee, 1957; Ruddell, 1953), duration of analgesia and recovery (Fayed et al, 1989; Setnikar and Temelcou, 1961) and dosage (Krahenmann, 1969).

There are a few reports available on the influence of hyperthermia on MAC. In those investigations MAC determination was either carried out using an electrical stimulus (Cherkin and Catchpool, 1964) in the gold fish or a tail clamping technique in the dog. The latter was recently proved to be scientifically unreliable and ethically unacceptable (Sobair et al, 1993; also Chapter 4). In the light of the modern concepts of pain and sensory physiology, the influence of a defined level of hyperthermia on MAC of halothane will be investigated using a heat stimulator that was designed and used in this section and a mechanical stimulator which was described in Chapter 4.

6.2: Materials And Methods:

6.2.1: Description Of The Heat Stimulator:

The device is designed to deliver a controlled preset focal radiant heat stimulus from a heat lamp. The system is capable of delivering a heat stimulus in the range of the ambient temperature to 99 °C for a preset time in a range of 1-999 seconds. As shown in Figure 6.1, the system is made of: 1) a heat source (projector lamp) and 2) a control box that contains a) a heat control circuit, b) a timing circuit, c) a hold temperature function and d) a front control panel.

1) The heat source is mounted on a holder with provisions for moving the lamp forward, backward and side ways, thereby making it easy to change the targeted site.
2) a) The heat control circuit works on the basis of a feedback mechanism coming from a type K thermocouple that is placed at the focal point of the heat source. The temperature at the focal point on the target area, is measured by the thermocouple in terms of voltage that is then compared with a preset reference voltage and as a result a difference voltage is generated. This difference voltage is used to drive an amplifier network which in turn controls a high current transistor through which the lamp is connected , thereby holding the temperature at the focal point on the target site, at a preset value.

2) b) The timing circuit is made of a digital counter and its associated circuitry. The intended duration of the stimulus is entered into the counter by the manual setting of numbered switches to the required time and then pressing a reset control button to load the preset time into the counter display window. The counter is activated by pressing the start button with the result of turning on the heat source and proceeding to count the preset time at the end of which the circuit electronically disconnects the lamp, switching it off and automatically resetting itself so that the process can be repeated once the start button is pressed again to apply the stimulus for the same or a newly adjusted duration.

2) c) The hold temperature function is an additional electronic circuit in the system that provides for maintaining the temperature, at the focal point on the target area, at a preset temperature during the intervals between the stimuli. This circuit operates by keeping the lamp on an intensity just enough to maintain the temperature at the focal point at a preset value. This is done in the same way as for the heat control circuit i.e. by comparing the temperature in voltages to a preset reference voltage. When the timing circuit is activated the value of the reference voltage is switched back to that set for the heat control circuit and remains so until the end of the preset time when it returns to that of the hold temperature function.

2) d) The front control panel is provided with the following controls:

I) Power ON/OFF control.

II) SET LOW control: In the ON position the hold temperature function is operational to maintain, as explained before, the temperature at the focal point at a preset value using the SET LOW control during the intervals between the stimuli. In the OFF position the hold temperature function is out of function and therefore the temperature at the focal point reflects the skin surface temperature when no heat is being applied.

III) SET HIGH control : This is used to preset the stimulation temperature.

IV) SET TIME control: The time (seconds) is set using manually adjusted numbered switches.

V) RESET control : This is used to feed the preset time into the time display window of the digital counter.

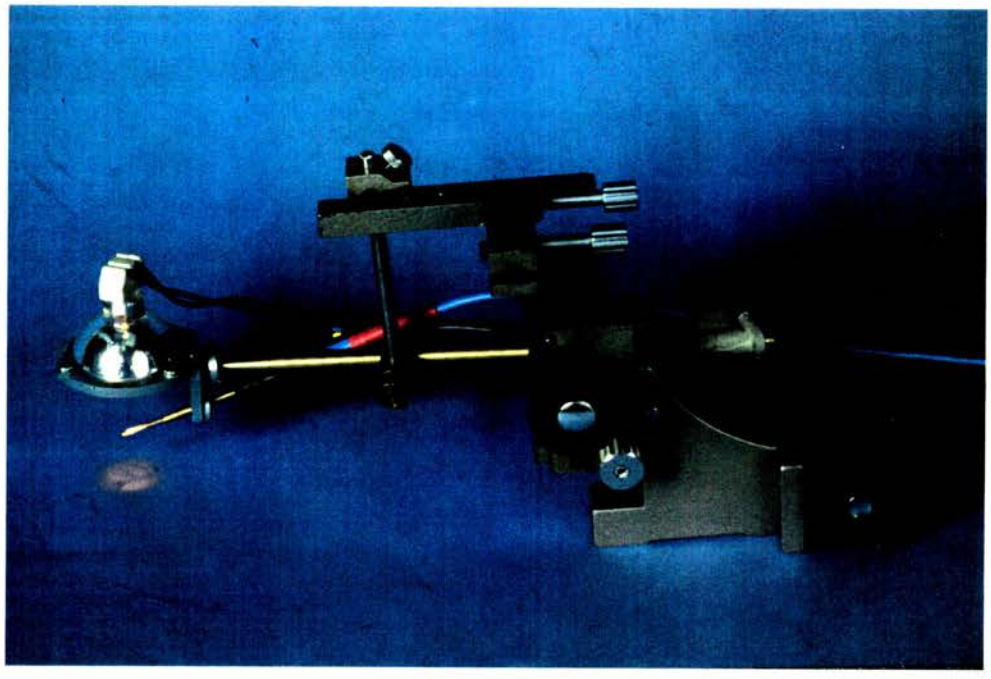
VI) START control : When pressed the system starts applying the preset heat stimulus for the preset time.

VII) TRIGGER controls : In INT position the system is triggered internally. There also provision for external triggering.

VIII) CONTROL/FOCUS control : In the CONTROL position the device is normally operational as described above. In the FOCUS position the system is set to provide for focusing the tip of the thermocouple in the centre of a dim light generated from the projector lamp, so that the measured temperature reflects the temperature at the focal point on the target area selected for stimulation (surface temperature).

In addition to these controls, there are also provisions for connecting the projector lamp, receiving the input from the thermocouple and taking the temperature and time outputs for recording on a chart recorder and or displayed on an oscilloscope.

Figure 6.1: Photographic illustration of the focal heat stimulator. The device comprises a focal heat source (projector lamp) and an electronic control system that is made of 1) a heat control circuit 2) a timing circuit 3) a hold temperature function and 4) a front control panel.



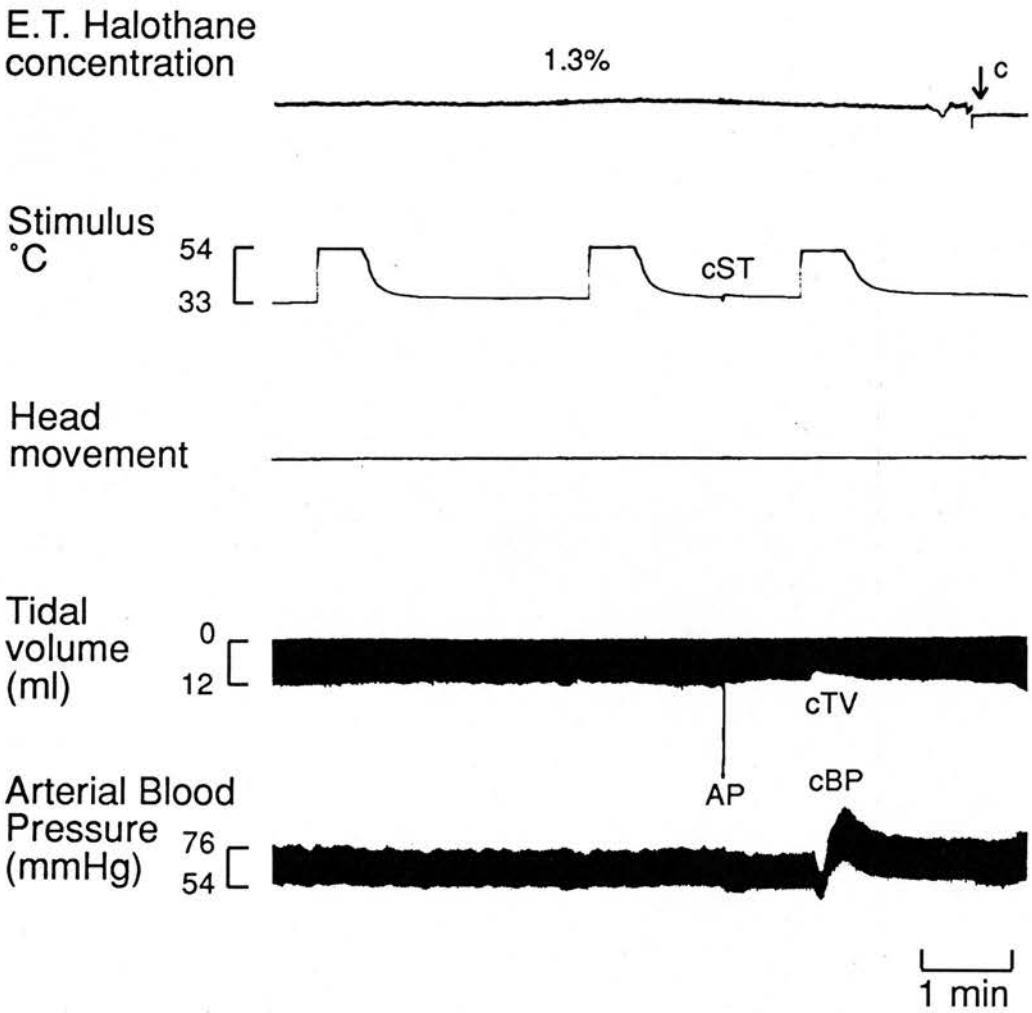
6.2.2: Experimental Protocol:

All the procedures in this section, unless stated otherwise, are carried out as explained in Chapter 3, Materials and General Methods.

A total of 11 animals were used in this section (7 males and 4 females, average age of 4.5 +/- 0.5 (SD) month and average weight of 3.7 +/- 0.2 (SD) kg. In all animals anaesthesia was induced and maintained on halothane in oxygen. Animals were instrumented and the following parameters were measured and recorded: arterial blood pressure, tidal volume, rectal temperature, end-tidal halothane concentration, heat stimulus output, head movement response.

With the heat stimulator focused and set-up, as described above, to deliver a constant stimulus of 55 °C. for 30 seconds to the inner side of the ear pinna, MAC of halothane was determined in 7 animals at normothermia (starting rectal body temperature before heating) and then at hyperthermia (2-2.5 °C. above the normothermic value) in 4 out of the seven animals. In the first three animals (animals No. I-III in Table 6. 1) MAC was not determined at hyperthermia because the MAC determination procedure at normothermia in these animals was too lengthy since it was started with a relatively higher end-tidal concentration in order to predict the MAC value in the remaining animals. The same stimulus was applied three times at three different sites of the same ear pinna before considering the response as negative at any one end-tidal halothane concentration (Figure 6.2).

Figure 6.2: MAC determination at normothermia (rectal temperature of 39.5 °C). At an end-tidal halothane concentration of 1.3% (no response concentration), a consistent focal heat stimulus was applied for 30 seconds to the ear pinna for three times, separated by at least 4 minute intervals, before considering the head movement response as negative. The halothane concentration was adjusted at (c). Note the changes in arterial blood pressure (cBP), Tidal volume (cTV) and the surface temperature (cST). The latter was as a result of slightly moving the thermocouple to another stimulation site.


E.T. Halothane concentration
1.3%
c
Stimulus °C
54
33
cST
Head movement
Tidal volume (ml)
0
12
cTV
AP
cBP
Arterial Blood Pressure (mmHg)
76
54
1 min

Hyperthermia was induced and the heating rates were defined and calculated, as described in Chapter 5, as "first or initial heating rate" and "second heating rate".

In a separate group of 4 rabbits, and for comparison, MAC was determined at normothermia and hyperthermia using the mechanical stimulator and protocol described in Chapter 4. The mechanical stimulus was applied three times before considering the response as negative at any one end-tidal halothane concentration (Figure 6.3). The procedure was repeated until movement response was detected (Figure 6.4).

In all animals, the surface temperature of the stimulated site (the ear pinna) was continuously measured and, recorded as explained above, using the thermocouple with CONTROL/FOCUS in the FOCUS position.

6.2.3: Data Analysis:

The mean, the standard deviation (SD) were calculated for all relevant parameters. Paired sample t-tests were used to test for significant differences between the normothermic and hyperthermic MAC values in the mechanically stimulated group and between the heating rates.

Figure 6.3: MAC determination in a hyperthermic rabbit (rectal temperature of 41 °C). At an end-tidal halothane concentration of 1.5% (no-response concentration) a consistent mechanical stimulus generated from a driving pressure of 275 Kpa (40 psi) was applied for three times before the head movement response was considered negative. Note the stimulus-related drop in blood pressure (cBP) and the increase in tidal volume (cTV).

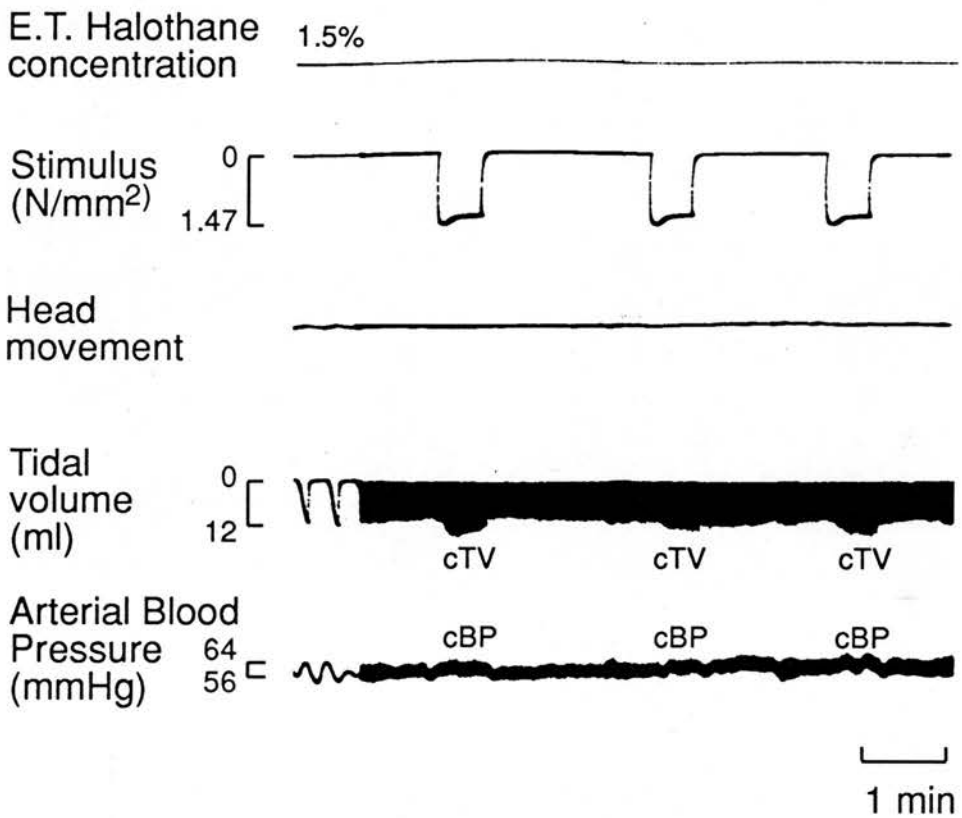
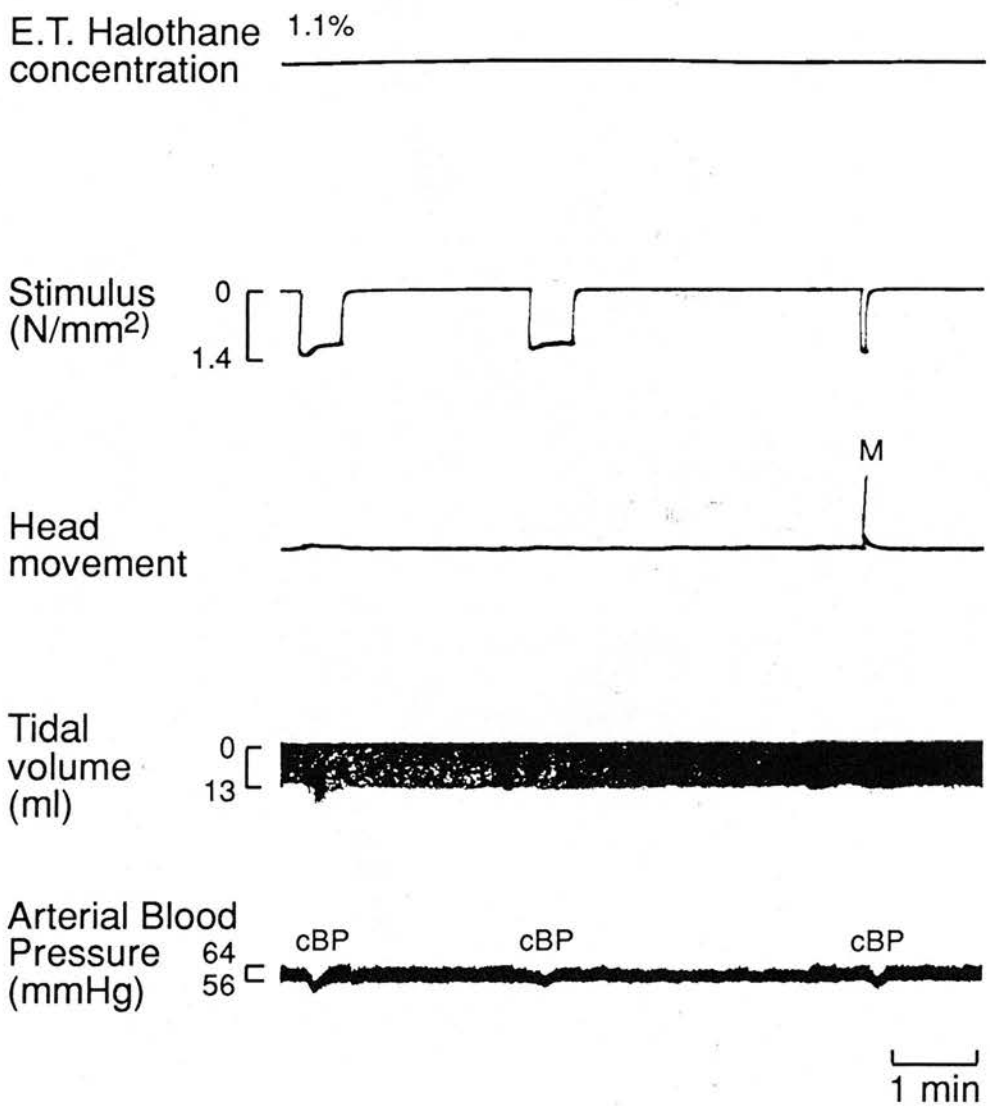

E.T. Halothane concentration
1.5%
Stimulus (N/mm2)
0
1.47
Head movement
Tidal volume (ml)
0
12
cTV
cTV
cTV
Arterial Blood Pressure (mmHg)
64
56
cBP
cBP
cBP
1 min

Figure 6.4: MAC determination in a hyperthermic rabbit (rectal temperature of 40.6 °C). At an end-tidal halothane concentration of 1.1% (response concentration), head movement, in response to a consistent mechanical stimulus generated from a driving pressure of 275 Kpa (40 psi), occurred at (M) after 1.2 seconds (response time) from the start of the third stimulus application. Note the stimulus-related drop in blood pressure (cBP).


E.T. Halothane concentration
1.1%
Stimulus (N/mm2)
0
1.4
M
Head movement
Tidal volume (ml)
0
13
Arterial Blood Pressure (mmHg)
64
56
cBP
cBP
cBP
1 min

6.3: Results:

A significant increase in the MAC of halothane was found with the mechanical stimulus while no change was obtained with the focal heat stimulation. A summary of results and calculations of MAC and related parameters for the individual rabbits are available in Appendix C.

6.3.1: Heat Stimulation:

The response and no-response as well as the normothermic values of MAC determined using the heat stimulator are shown in Table 6. 1. The MAC values ranged between 0.95 and 1.15 vol.% with a mean of 1.05 +/- 0.09 (SD) vol.%. The mean stimulus temperature applied was 54.37 +/-0.07 (SD) °C. The response time ranged between 9.6 and 21.6 seconds with a mean of 13.7 +/- 4.3 (SD).

The hyperthermic values of MAC (Table 6. 2) ranged between 0.85 and 0.95 vol.% with a mean value of 0.93 +/- 0.04 (SD) vol.% which is obviously not significantly different from the normothermic mean value of MAC. The mean stimulus applied was 54.35 +/- 0.09 (SD). The response time was between 7.2 and 21.6 seconds with a mean value of 17.4 +/- 4.6 (SD).

6.3.2: Mechanical Stimulation:

The normothermic MAC values determined using the mechanical stimulator are shown in Table 6. 3. Their values ranged between 0.85 and 1.15 vol.% with a mean value of 0.98 +/- 0.11 (SD) vol.%. The mean stimulus ranged between 1.31 and 1.42 with a mean value of 1.35 +/- 0.04 (SD) N/mm^2. The response time ranged between 1.2 and 2.4 with a mean value of 1.8 +/- 0.6 seconds.

The hyperthermic values of MAC (Table 6. 4) were between 1.15 and 1.35 vol.% with a mean value of 1.25 +/- 0.08 (SD) vol.% which is significantly higher ($P < 0.05$) than the normothermic mean value of MAC. The mean stimulus was between 1.33 and 1.43 with a mean value of 1.37 +/- 0.04 N/mm^2. The response time was between 1.2 and 2.4 with a mean value of 1.6 +/- 0.6 (SD) seconds.

The heating rates in the heat and mechanically stimulated animals are shown in Table 6. 5.

6.4: Discussion:

Stimuli for the determination of MAC includes tail clamping in animals (Eger et al., 1988; Lundeen et al., 1983), controlled mechanical stimulation in rabbits (Sobair et al., 1993), skin incision (mainly in man) (Saidman and Eger, 1964) and electrical stimuli in both man (Saidman and Eger, 1964) and animals (Steffey et al., 1977)).

Table 6. 1: The values of MAC of halothane determined in normothermic rabbits using controlled focal heat stimulation.

Animal No.	Rectal temp. °C.	Surface temp. °C	Stimulus °C	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.
6.I	39.5	30.9	54.4	1.1	1.2	1.15	14.4
6.II	39.0	33.8	54.4	0.9	1.0	0.95	9.6
6.III	39.0	30.6	54.4	1.1	1.2	1.15	10.8
6.IV	39.0	32.8	54.5	0.9	1.0	0.95	18.0
6.V	39..5	34.9	54.3	0.9	1.0	0.95	12.0
6.VI	39.5	30.8	54.3	1.1	1.2	1.15	21.6
6.VII	39.5	32.3	54.3	1.0	1.1	1.05	9.6
Mean	39.29	32.3	54.37	1.0	1.1	1.05	13.7
SD	0.25	1.7	0.07	0.09	0.09	0.09	4.3

Table 6. 2: The values of MAC of halothane determined at hyperthermic rabbits using controlled focal heat stimulation.

Animal No.	Rectal temp. °C	Surface temp. °C	Stimulus °C	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.
6.IV	41.0	36.8	54.3	0.9	1.0	0.95	7.2
6.V	42.0	35.6	54.3	0.9	1.0	0.95	21.6
6.VI	41.5	33.6	54.3	0.8	0.9	0.85	19.2
6.VII	42.0	36.4	54.5	0.9	1.0	0.95	21.6
Mean	41.63	35.6	54.35	0.88	0.98	0.93	17.4
SD	0.42	1.4	0.09	0.04	0.04	0.04	6.0

Table 6. 3: The MAC values of halothane determined at normothermia using controlled mechanical stimulation.

Animal No.	Rectal temp.°C	Surface temp.°C	Stimulus N/mm^2	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.
6.VIII	39.4	34.6	1.42	0.9	1.0	0.95	1.2
6.IX	38.3	29.0	1.31	1.1	1.2	1.15	2.4
6.X	38.7	32.9	1.34	0.9	1.0	0.95	2.4
6.II	38.4	35.1	1.33	0.8	0.9	0.85	1.2
Mean	38.7	32.7	1.35	0.93	1.03	0.98	1.8
SD	0.4	2.4	0.04	0.11	0.11	0.11	0.6

Table 6. 4: The MAC values of halothane determined at hyperthermia using controlled mechanical stimulation.

Animal No.	Rectal temp.°C	Surface temp. °C	Stimulus N/mm^2	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.
6.VIII	40.9	35.6	1.43	1.2	1.3	1.25	1.2
6.IX	40.2	33.9	1.35	1.3	1.4	1.35	2.4
6.X	40.6	33.8	1.33	1.1	1.2	1.15	1.2
6.XI	-	-	-	-	-	-	-
Mean	40.6	34.4	1.37	1.2	1.3	1.25	1.6
SD	0.29	0.83	0.04	0.08	0.08	0.08	0.6

Table 6. 5: The first (F.H.R.), second (S.H.R.) and average heating rates (°C./hour) calculated in the heat and mechanically stimulated rabbits.

Focal heat stimulation				Mechanical stimulation			
Animal No	F.H.R. °C	S.H.R. °C	A.H.R °C	Animal No	F.H.R. °C	S.H.R. °C	A.H.R °C
6.VIII	1.50	1.94	1.72	6.VIII	0.75	1.20	0.98
6.IX	1.33	1.88	1.61	6.IX	0.90	1.33	1.12
6.X	1.33	1.58	1.46	6.X	0.71	1.32	1.02
6.XI	1.20	1.88	1.54	6.XI	-	-	-
Mean	1.34	1.82	1.58		0.79	1.28	1.04
SD	0.11	0.14	0.10		0.08	0.06	0.06

On the other hand no attempt was made to use heat stimuli for the determination of MAC, despite the fact that the existence of heat receptors is well documented in sensory physiology (Raja et al., 1988). This is probably because it was reported that the use of a stimulus, regardless of its kind, with a supramaximal intensity results in a consistent and reproducible value of MAC (Eger et al., 1965a). However, the contradictory reports about the concept of the supramaximal stimulus (Tranquilli et al., 1983; Eger et al., 1965a; Lundeen et al., 1983 and Eger et al., 1988) and the results showed in Chapter 4 indicate that this is not the case. These factors suggest the need for a device that provides for the application of controlled stimuli for the determination of MAC without altering the receptor sensitivity (Sobair et al., 1993).

Another reason that was probably responsible for making previous investigators reluctant to use heat stimuli might be attributed to the fact that most of the then available heat stimulators might have been relatively complicated to provide for a controlled "press button" application of the stimulus required for MAC determination as it has been demonstrated with the heat stimulator used in this investigation.

The results shown in Table 6.1 and table 6.2 and the record tracing in Figure 6 2, indicate very clearly that the heat stimulator described and used in this section is capable of applying a heat stimulus that was reproducible in terms of amount and duration. Such reproducibility is statistically obvious from the negligible values of the standard deviation. Stimuli that are precise, reproducible were reported to be essential for comparative studies in the field of MAC research by removing or

minimising the variability in MAC that was related to the variation in such stimuli (Deady et al, 1981; Zavisca et al., 1993). The focal heat stimulus of 55 °C. was chosen on the basis of threshold values of heat stimuli available in the literature (Bessou and Perl, 1969) and the lack of any obvious burn injury or inflammatory response. In this investigation that level of stimulation was found to cause no apparent damage to the stimulated site and therefore is less likely to cause receptor sensitisation (Campbell et al., 1979).

Using the heat stimulator, the normothermic values of MAC (Table 6. 1) ranged between 0.95 and 1.15 vol.% with a mean value of 1.05 +/- 0.093. When compared with the previously obtained mean values of MAC in the rabbit, the mean value in this investigation is slightly higher than 0.82 +/- 0.25 (SD)% (Davis et al., 1975), but significantly lower than 1.39 +/- 0.23 (SD)% (Drummond, 1985).

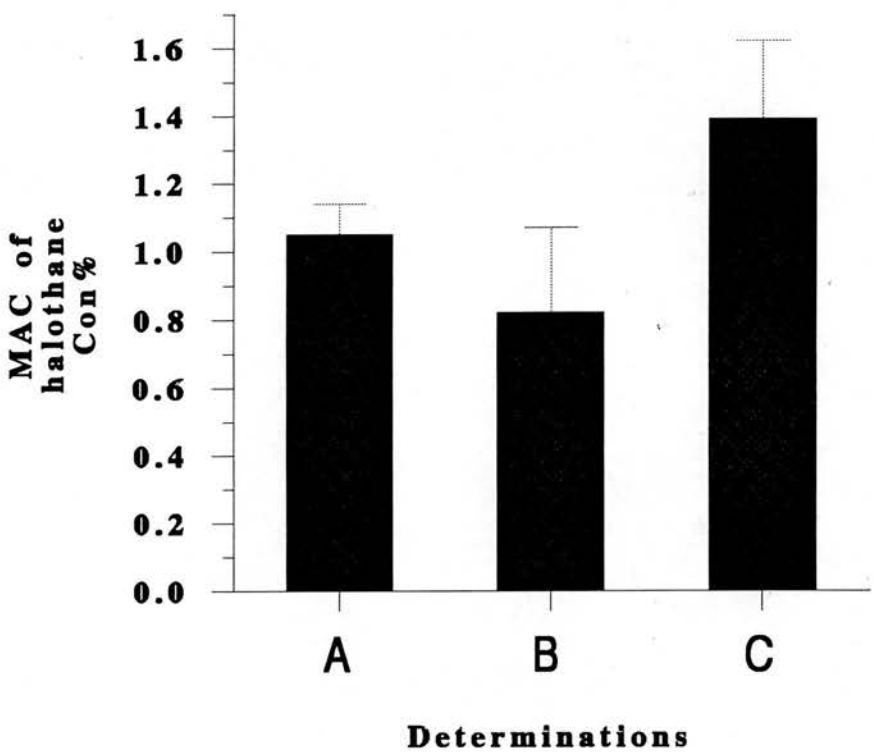
The reproducibility of MAC in this investigation compared with the previously reported values is readily apparent from the very small SD and it is graphically illustrated in Figure 6.8. However such comparison is obscured, mainly because of factors related to stimuli used in those other investigations as has been discussed in Chapter 4. On the other hand the normothermic values of MAC obtained in this section compared reasonably well with those obtained in Chapter 4 using the mechanical stimulator and those reported by Sobair et al. (1993). That might imply that at normothermia the responses to any kind of stimuli in the threshold nociceptive range, are obtunded by more or less the same level of anaesthesia. Such suggestion

might appear to be more sensible than a statement made by Merkel and Eger (1963) that both mild and intense stimuli are blocked by approximately the same anaesthetic concentration.

In the group of rabbits where MAC was determined using the mechanical stimulator, the normothermic mean value of MAC of 0.98 +/- 0.11 (SD) vol.% (Table 6. 3) is similar to the normothermic mean value of MAC of 1.05 +/- 0.09 (SD) vol.% determined using the heat stimulator and slightly higher than 0.82 +/- 0.25 (SD) vol.% obtained by Davis et al. (1975), but significantly lower than 1.39 +/- 0.23 (SD) vol.% reported by Drummond (1985). The hyperthermic values of MAC determined by using the heat stimulator (Table 6. 2) showed no significant difference from those obtained at normothermia in the same group of animals (Table 6. 1). In contrast, the hyperthermic mean value of MAC obtained by using the mechanical stimulator, 1.25 +/- 0.08 (SD) vol.% (Table 6. 4) was significantly higher ($P < 0.05$) than the normothermic mean value of 0.98 +/- 0.11 (SD) vol.% in the same group (Table 6. 3).

The increase in the value of MAC determined by mechanical stimulation under hyperthermic conditions is in agreement with similar findings reported in dogs (Steffey and Eger, 1974) and the gold fish (Cherkin and Catchpool, 1964). The MAC increasing effect of hyperthermia was probably related to the hyperthermia-associated lipid solubility decreasing effect at the site of action (the brain) (Steffey and Eger, 1974). The extent of such increase in this investigation was calculated to be 12.3 % per °C. rise in body temperature (Table 6. 4), compared with 8% per °C. in the dog (Steffey and Eger, 1974).

Figure 6.8: Graphical illustration of the MAC values obtained in this investigation using controlled heat stimulation (n = 7) (**A**), compared to those previously reported by Davis et al. (1975) (n = 11) (**B**) and Drummond (1985) (n = 8) (**C**). Note the comparatively small error bars (SD) in this investigation, indicating a small variability in the MAC values.


MAC of halothane Con%
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
B
C
Determinations

That difference might partially be explained on the basis of factors related to stimulus stability such as the type, amount and duration (see Chapter 4). Also the rate of heating might play a role in that variation. Elsewhere, the toxicity of whole body hyperthermia in man was reported to be influenced by the heating-up time (Vander Zee et al., 1990). In the investigation conducted by Steffey and Eger (1974), animals were heated, using heating lamps and thermal insulation, at a rate of 1 °C. per 45 or 60 minutes. The use of a controlled heating protocol allowed the calculation of two significantly different ($P < 0.001$) heating rates in either of the heat or mechanically stimulated groups (Table 6. 5). Those were "The first and second heating rates". The difference between the first and second heating rates could probably be explained on the same grounds as was discussed in Chapter 5.

The lack of a significant difference between the normothermic and hyperthermic values of MAC determined using the heat stimulus (Table 6.1 and Table 6.2) could probably be explained on a number of grounds. Firstly, the increase in body temperature might have blocked or affected the sensitivity of the heat receptors, thereby raising the pain threshold in a manner similar to distractions induced by interaction with somatosensory input such as a sound of a loud bell (Wolff and Wolf, 1958). In common practice mechanical stimulation could be used to distract the attention of the conscious animal to a simultaneously applied greater stimulus as is the case with the application of a twitch to the upper lip or ear of a horse.

Secondly, the rise in body temperature by 2-2.5 °C. could not have been significant enough to trigger a decrease in solubility of halothane to level that results in a measurable increase in MAC determined with the heat stimulus such as those reported when using mechanical stimulation (Steffey and Eger, 1974). This implies that the same magnitude of the temperature-related change in solubility might affect the MAC to a different extent, depending on the type of stimulus used for the determination of MAC.

Thirdly, raising the body temperature by 2-2.5 °C in the heat-stimulated group (Table 6. 2), brought the rectal temperature to 42 °C or closer to it which might have compromised cerebral oxygenation and metabolism (Nemoto and Frankel, 1970). At body temperature exceeding 42 °C, MAC of halothane was reported to decrease in dogs (Steffey and Eger, 1974). Also whole body hyperthermia of 42.5 °C. was considered to be the limit for survival in the rabbit (Hilling, 1987). Moreover, the heating protocol, designed in Chapter 5, showed that at rectal temperatures exceeding 42 °C., the body temperature continued to rise even after cessation of heating and the start of active cooling.

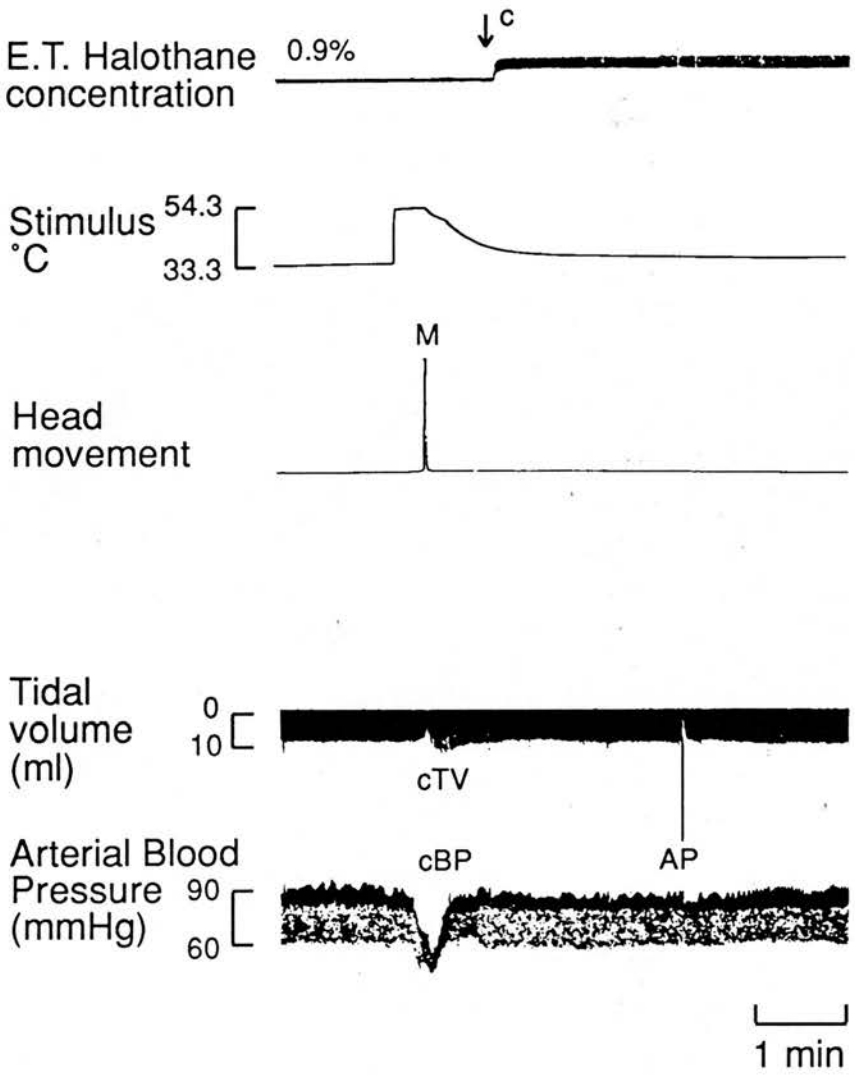
Therefore it appeared that a body temperature of 42 °C. represents a critical temperature above which detrimental effects of heat starts to take place in the brain and other sensitive organs in the rabbit, thereby affecting the normal physiological responses. Similar observations were reported in dogs by Steffey and Eger (1974).

In the light of these explanations, one might suggest that the species with a "narrow margin of heating" such as the rabbit, with normal temperature of upto 40 °C. (Haskins, 1987) are not ideal candidates for experiments that require high levels of hyperthermia. Verification of such suggestion requires the conduction of experiments employing hypothermia which is expected to be more tolerated by the rabbit than hyperthermia and that will be the subject of the next Chapter. In contrast the body temperature of species with a "wide margin of heating" such as the dog, could be raised by 5 °C . before reaching the critical temperature of 42 °C. (Steffey and Eger, 1974) and therefore could be considered as good subjects for experiments involving whole body hyperthermia.

The heat stimulator used in this investigation, beside providing for the application of a controlled heat stimulus, also has the advantage that both the stimulus output and the head movement response are simultaneously recordable (Figure 6.5). This provision made it easy to calculate the response time. It was found to have mean values of 13.7 +/- 4.3 (SD) seconds (n = 7) and 17.4 +/- 6 (SD) seconds (n = 4) at normothermia (Table 6. 1) and hyperthermia (Table 6. 2) respectively.

With the mechanical stimulator the response time was calculated to have a mean value of 1.8 +/- 0.6 (SD) (Table 6. 3) and 1.6 +/- 0.6 (SD) (Table 6. 4) at normothermia and hyperthermia respectively. As it is shown in Table 6. 1, all animals but one, responded within less than 20 seconds i.e. had the stimulus been applied for 20 seconds instead of the 30 seconds, the value of MAC could have differed in one single animal. On the other hand if a duration of 10 seconds had been chosen as it was suggested by Merkel and Eger (1963), different values of MAC could have been obtained in 5 out of the 7 animals (Table 6. 1).

Figure 6.5: MAC determination in a normothermic rabbit (rectal temperature of 39.5 °C) using a controlled focal heat stimulation. At an end-tidal halothane concentration of 0.9% (response concentration), head movement occurred at (M) after 18 seconds (response time) from the start of the first stimulus application after which the stimulus was interrupted and the halothane concentration was adjusted at (c). Note the stimulus-related drop in both arterial blood pressure (cBP) and the tidal volume (cTV) as well as the transitory apnoea that occurred approximately 2 minutes after cessation of stimulation.


E.T. Halothane concentration
0.9%
C
Stimulus °C
54.3
33.3
M
Head movement
Tidal volume (ml)
0
10
cTV
Arterial Blood Pressure (mmHg)
90
60
cBP
AP
1 min

Therefore it is now clear that the 30 second duration of stimulus used in this investigation is a better compromise between the short duration of 10 seconds (Merkel and Eger, 1963) and a duration of upto a minute (Eger et al.,1965a). A duration as short as 2 seconds was also used by some investigators (Shim and Andersen, 1972) such wide variability in the duration of the applied stimulus might have accounted, at least in part, for the different values of MAC obtained by the various investigators (Deady et al., 1981). The longer the duration, the more is the likelihood of tissue damage and associated change in sensitivity and the difficulty in the interpretation of results (Woolf, 1983, 1991; Dubner, 1991; Handwerker and Reeh, 1991; Zavisca et al., 1993).

As it is shown in Table 6.1, 4 animals responded at the first application of the stimulus with a mean response time of 13.2 seconds and 3 animals at the second application with a mean response time of 14.3 seconds. Obviously the difference between the two mean values is not significant and therefore one might conclude that, with the heat stimulator used in this investigation, the response time is not dependent on the number of applications of the stimulus, provided that the stimulus is within the nociceptive range. Also that might imply that no sensitisation took place, otherwise the response times at the second application would have been significantly shorter than those at the first application. Similarly the response time showed no correlation with the response concentration or the corresponding value of MAC or the rectal temperature. Therefore the variation in the response time might be related to some inherent features in the individual animals.

Other stimulus related responses included changes in blood pressure, apnoea and hyperpnoea. As it is shown in the results for the individual rabbits (Appendix C) the most common feature between those responses is their inconsistency. The arterial blood pressure in particular showed both quantitative (the extent of the pressor or depressor effects) and qualitative (pressor or depressor responses) (Figure 6.6 and Figure 6.7). moreover such responses quite often can occur spontaneously without stimulus application in a manner that was neither quantitatively or qualitatively indistinguishable from the stimulus related responses. With halothane, the nature of arterial blood pressure responses to noxious stimuli i.e. whether a pressor or a depressor was found to be dependent on the level of anaesthetic concentration in the rat (Gibbs et al., 1989) and the rabbit (Fukuanga et al., 1990). Inconsistency in these parameters was also demonstrated in chapters 5 and 7 and therefore, it might be concluded that such parameters could not be used as reliable end points for the determination of MAC as it was explained in Chapter 5. In the rabbit, at least, the results do not support the use of such responses to noxious stimuli as indices of anaesthetic depth (Prys-Roberts, 1987; Moffit and Sethna1986).

Figure 6.6: MAC determination in a hyperthermic rabbit using a controlled focal heat stimulation. At an end-tidal halothane concentration of 0.9% (response concentration), head movement occurred at (M) after 7.2 seconds from the start of the third stimulus application, after which the stimulus was interrupted. Note the change in arterial blood pressure (cBP), the transitory apnoea and the change in surface temperature (cST) as the thermocouple was moved to another stimulation site.

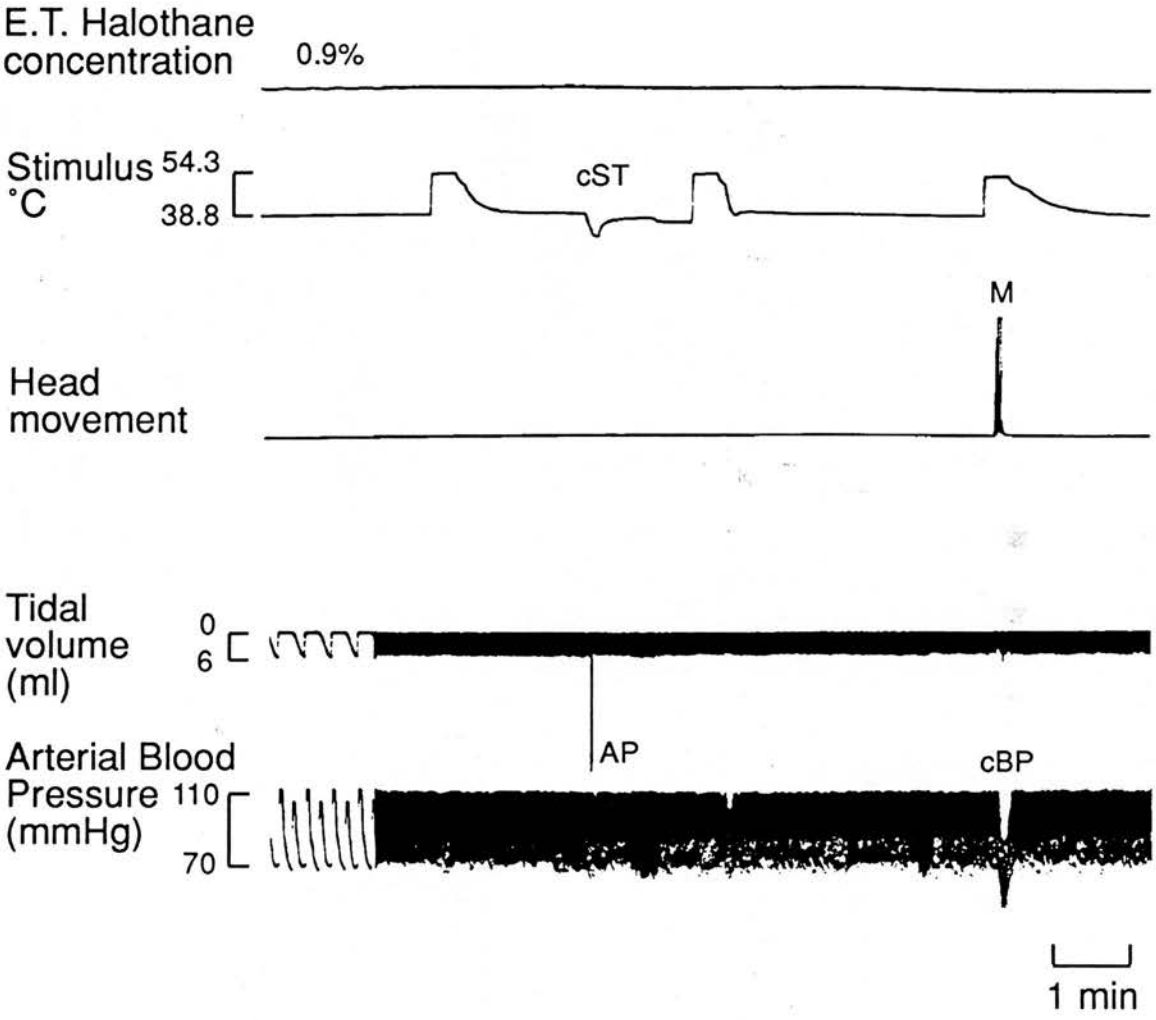
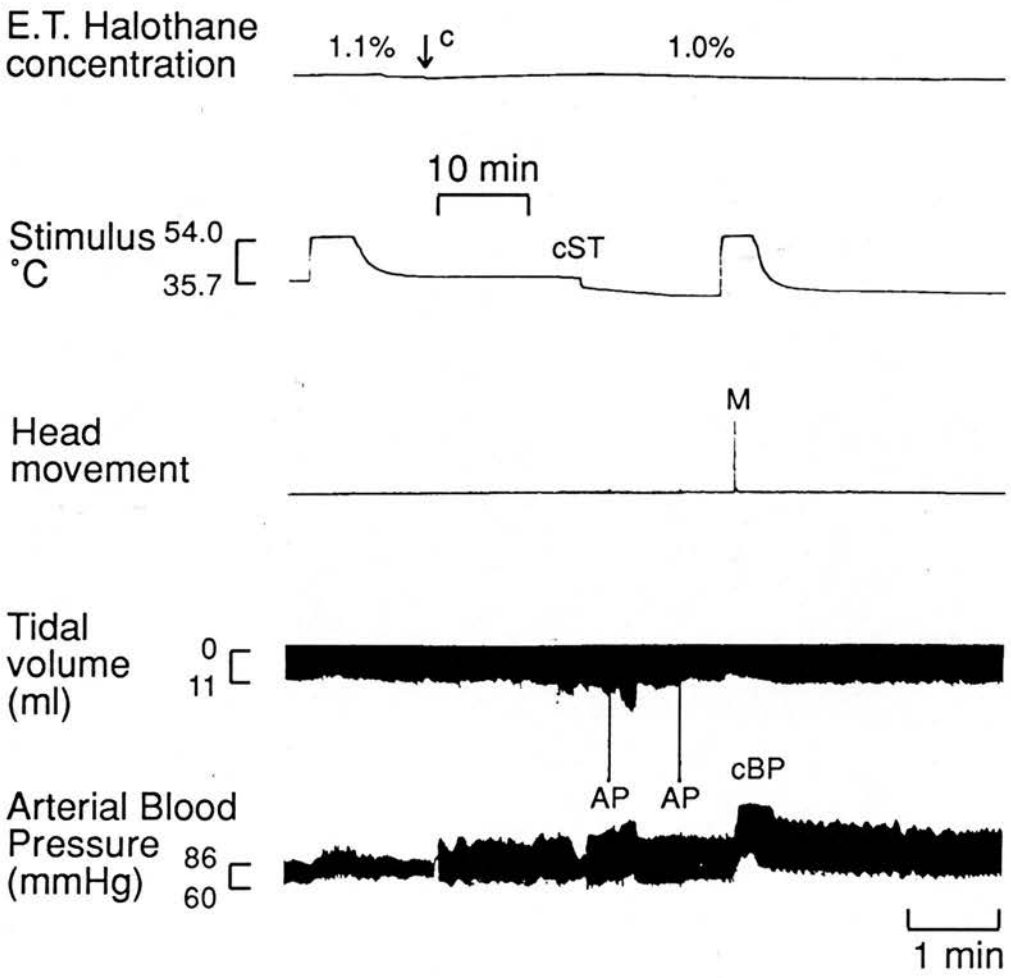

E.T. Halothane concentration
0.9%
Stimulus °C
54.3
38.8
cST
M
Head movement
Tidal volume (ml)
0
6
AP
Arterial Blood Pressure (mmHg)
110
70
cBP
1 min

Figure 6.7: MAC determination in a normothermic rabbit using controlled focal heat stimulation. At an end-tidal halothane concentration of 1.1% (no-response concentration), the third stimulus application provoked no head movement and the concentration was reduced at (c) to 1.0% (response concentration).


E.T. Halothane concentration
1.1%
C
1.0%
10 min
Stimulus °C
54.0
35.7
cST
M
Head movement
Tidal volume (ml)
0
11
AP
AP
cBP
Arterial Blood Pressure (mmHg)
86
60
1 min

CHAPTER 7

THE INFLUENCE OF HYPOTHERMIA ON MAC OF HALOTHANE DETERMINED USING MECHANICAL AND HEAT STIMULATORS

7.1: Introduction:

Hypothermia has been reported to decrease MAC of inhalation anaesthetics in rats (Vitez et al., 1974; Eger and Johnson, 1987; Munson, 1970) and dogs (Eger et al., 1965b; Regan and Eger, 1967). No reports are available on the influence of hypothermia on inhalation anaesthetic requirements in the rabbit. Also the stimulus used for the determination of MAC in those reports was predominantly tail clamping that has been shown to be scientifically unreliable and ethically unacceptable (Sobair et al, 1993).

The purpose of this section is to study the influence of hypothermia on MAC of halothane in the rabbit determined by using a controlled quantifiable mechanical stimulator (Sobair et al, 1993) and a heat stimulator described in the previous chapter.

7.2: Materials And Methods:

Unless stated other wise, all methods and procedures used in this section are performed as described in Chapter 3 (Materials and General Methods).

6 New Zealand White rabbits (3 males, 3 females, mean weight of 3.6 +/- 0.2 (SD) kg. and mean age of 5 +/- 0.4 (SD) month) were used.

7.2.1: Experimental Protocol:

Anaesthesia was induced and maintained with halothane in oxygen. Animals were instrumented with provision for the measurement, recording and or display of end-tidal anaesthetic concentration, stimulus, response (head movement), rectal temperature, arterial blood pressure and tidal volume. The mechanical stimulator was set-up at a gauge pressure of 275 KPa (40 psi), to deliver a controlled mechanical stimulus to one of the ear pinnas as described by Sobair et al.(1993) and demonstrated in Chapter 4. The heat stimulator described in Chapter 6 was set-up to provide for an abrupt application of controlled focal heat stimulus of 55 °C for 30 seconds to the pinna of the other ear.

In each animal, MAC of halothane was first determined at normothermia (initial rectal temperature) and then at hypothermia (4 °C. below the normothermic temperature) using either the mechanical or heat stimulator (in random sequence) as described in Chapter 4 and Chapter 6 respectively. At any one end-tidal concentration, the presence or absence of provoked movement response of head and or limbs to either stimulus was tested by applying the stimulus three times before adjusting the end-tidal concentration (Figures 7.1 and 7.2) and proceeding with the repetition of the procedure until movement response was detected (Figure 7.3).

Figure 7.1: MAC determination in a normothermic rabbit (rectal temperature of 39.0 ^{o}C) using consistent mechanical and focal heat stimulation. At an end-tidal halothane concentration of 1.0% (no-response concentration), both the mechanical and the heat stimuli, either of them applied three times, did not provoke head movement. The concentration was then decreased at (**c**) to 0.9% to proceed with the repetition of the procedure for MAC determination. Note the transitory apnoea (**AP**) and the slight head movement (**S**) that was probably associated with either swallowing or stiffening of the muscle of the neck.

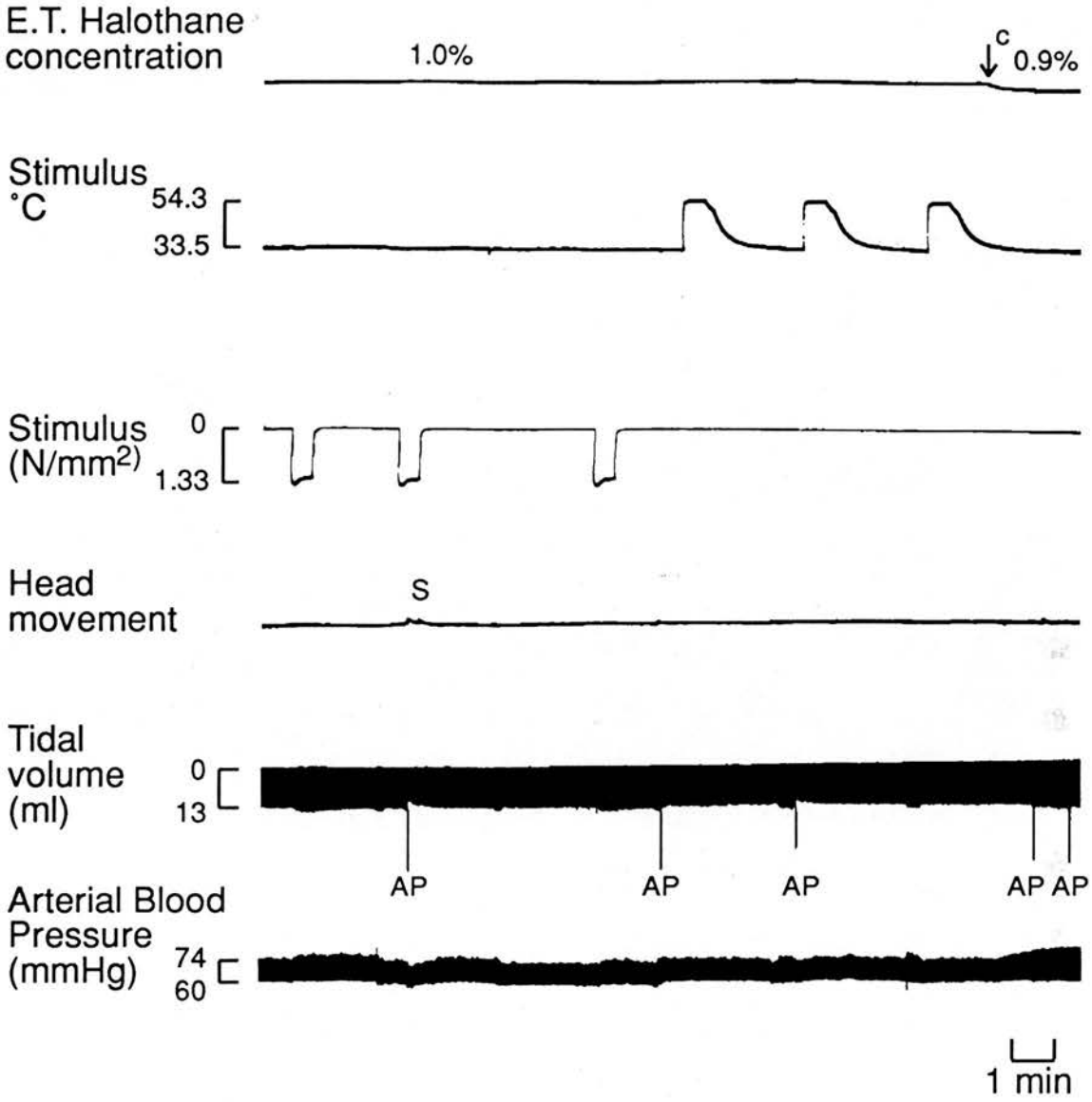

E.T. Halothane concentration
1.0%
C
0.9%
Stimulus °C
54.3
33.5
Stimulus (N/mm2)
0
1.33
Head movement
S
Tidal volume (ml)
0
13
AP
AP
AP
AP AP
Arterial Blood Pressure (mmHg)
74
60
1 min

Figure 7.2: MAC determination in a normothermic rabbit (rectal temperature of 38.6 ^{o}C) using consistent mechanical and focal heat stimulation. At an end-tidal halothane concentration of 0.7% (no-response concentration), a consistent heat stimulus was applied for three times and no provoked head movement was detected. The concentration was then adjusted at (**c**) to 0.6% at which head movement (**M**) was detected after 19.2 seconds from the start of the first mechanical stimulus generated from a driving pressure of 275 Kpa (275 KPa (40 psi)). Note the transitory apnoea (**AP**) and the slight head movement (**S**) that was probably associated with either swallowing or stiffening of the muscle of the neck.

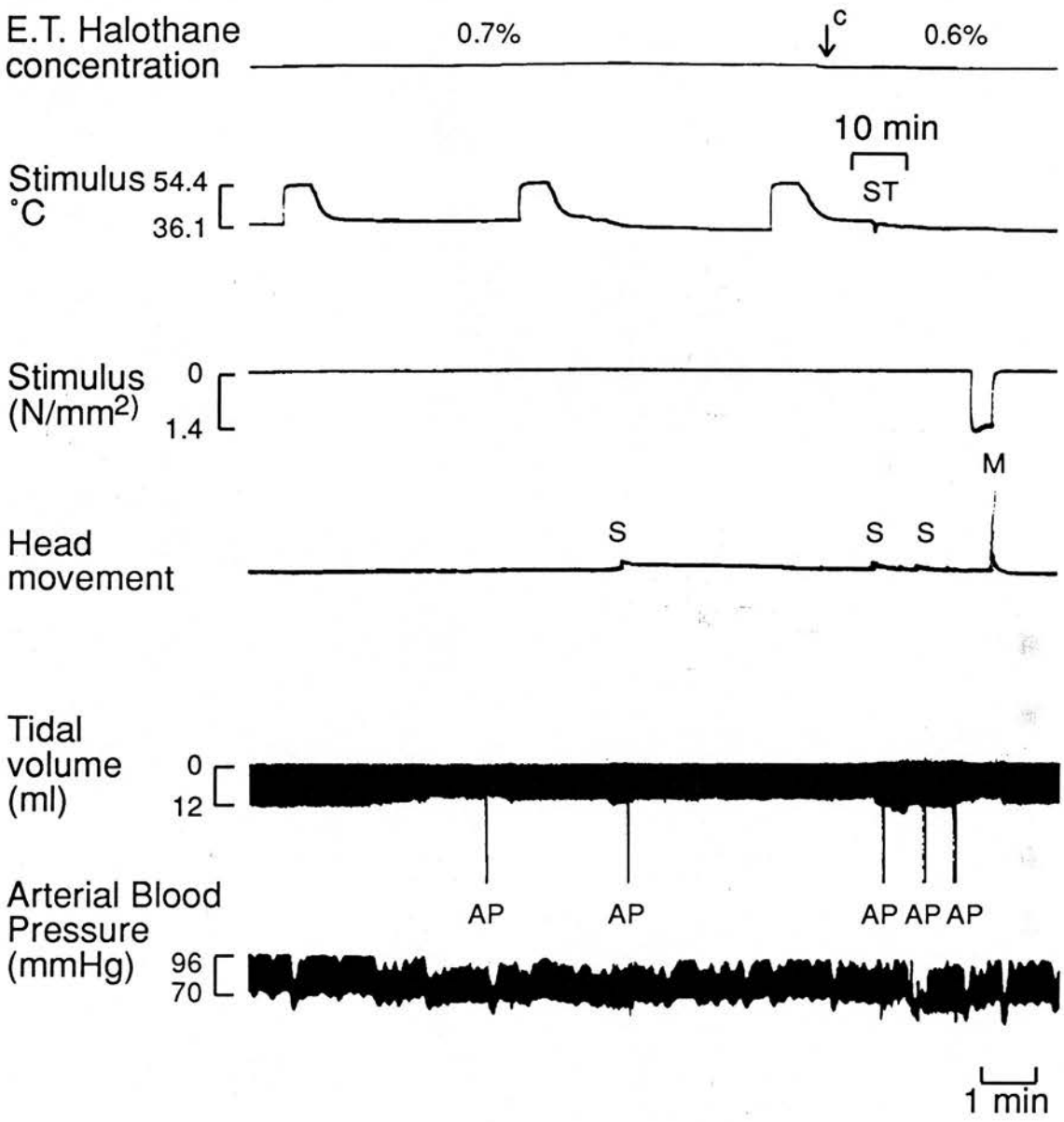
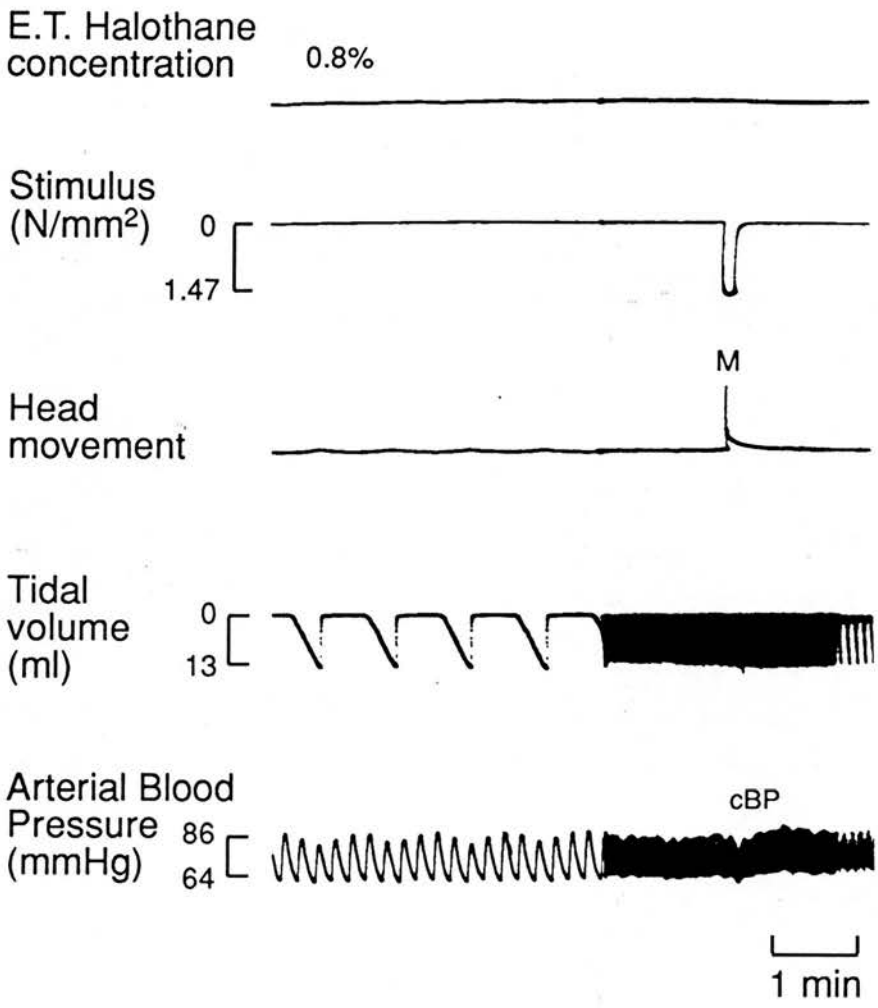

E.T. Halothane concentration
0.7%
C
0.6%
10 min
ST
Stimulus °C
54.4
36.1
Stimulus (N/mm2)
0
1.4
M
Head movement
S
S
S
Tidal volume (ml)
0
12
AP
AP
AP
AP
AP
Arterial Blood Pressure (mmHg)
96
70
1 min

Figure 7.3: Using a mechanical stimulus generated from a driving pressure of 275 Kpa (275 KPa (40 psi)) in a normothermic rabbit (rectal temperature of 38.0 °C), head movement (**M**) was detected after 2.4 seconds from the start of the first stimulus application. Note the stimulus-related drop in arterial blood pressure (cBP), also, the change in paper speed.


E.T. Halothane concentration
0.8%
Stimulus (N/mm²)
0
1.47
M
Head movement
Tidal volume (ml)
0
13
Arterial Blood Pressure (mmHg)
86
64
cBP
1 min

Hypothermia (4 °C. below the starting rectal temperature) was induced using the cooling protocol described in Chapter 5 and the cooling rates were calculated as the first, second, third and fourth cooling rates to denote the cooling rates during the first, second, third and fourth °C. drop in rectal temperature respectively.

7.2.2: Statistical Analysis:

Results are presented as mean +/- SD. Statistical analysis was performed by analysis of variance to test for significance ($P < 0.05$). The stimuli, animals and rectal temperatures were employed as co-variants for the analysis procedure. The statistical analysis was carried out using Genstat soft ware.

7.3: Results:

Hypothermia significantly ($P < 0.05$) reduced MAC of halothane determined by either of the mechanical or heat stimuli. The detailed results for the individual rabbits are available in appendix D.

7.3.1: Mechanical Stimulator:

At hypothermia (34.9 +/- 0.4 (SD) °C) MAC was 0.50 +/- 0.19 (SD)% (Table 7.2). It was significantly lower ($P < 0.001$) than 0.77 +/- 0.16 (SD)% (Table 7.1) obtained at

normothermia (38.8 +/- 0.2 (SD) °C). The mean value of the mechanical stimulus at normothermia was 1.41 +/- 0.03 (SD) N/mm^2 (Table 7.1). It was not significantly different from 1.39 +/- 0.05 (SD) N/mm^2 used at hypothermia (Table 7.2).

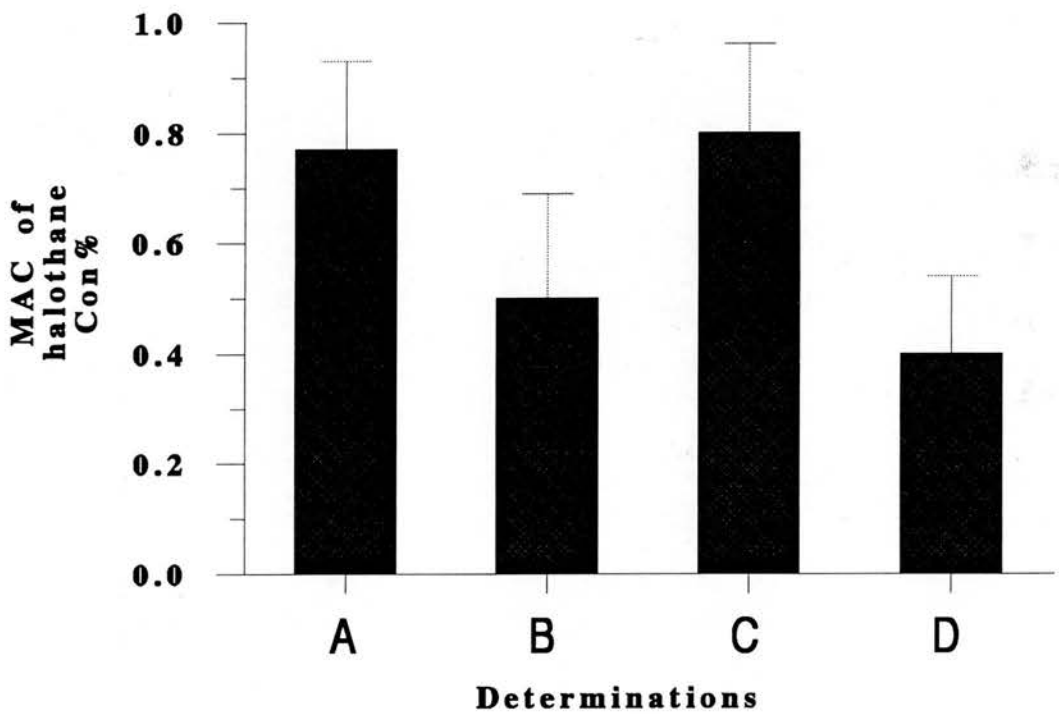
The response time showed a wide variability both at normothermia and hypothermia. It was 8.4 +/- 8.6 (SD) seconds at normothermia (Table 7.1) and 6.1 +/- 9.4 (SD) seconds at hypothermia (Table 7.2). The movement response occurred predominantly during the first application of the stimulus at both normothermia (Table 7.1) and hypothermia (Table 7.2).

7.3.2: Heat Stimulator:

At hypothermia (34.9 +/- 0.4 (SD) °C., MAC was 0.40 +/- 0.14 (SD)% (Table 7.4). That was significantly lower ($P < 0.001$) than 0.80 +/- 0.16 (SD)% obtained at normothermia (38.8 +/- 0.2 (SD) °C. (Table 7.3). The mean value of the heat stimulus at normothermia was the same as that at hypothermia with a value of 54.3 °C. (tables 7.3 and 7.4).

As with the mechanical stimulator the response time was widely variable, with a mean value of 15.0 +/- 6.0 (SD) seconds at normothermia (Table 7.3) and 12.0 +/- 6.6 (SD) seconds at hypothermia (Table 7.4). The movement response occurred during the first application of the stimulus in all animals at hypothermia (Table 7.4) and in just two animals at normothermia (Table 7.3). The mean values of the 4 cooling rates are similar with a "mean average cooling rate" of 1.05 +/-0.16 (SD) °C./hour (Table 7.5).

Figure 7.4: Graphical illustration of the mean normothermic and hypothermic MAC values of halothane determined using controlled mechanical and focal heat stimulation. (**A**) and (**B**) normothermic and hypothermic MAC respectively, determined using mechanical stimulation. (**C**) and (**D**) normothermic and hypothermic respectively, determined using heat stimulation. The vertical error bars represent standard deviation(SD)s.


1.0
0.8
0.6
0.4
0.2
0.0
MAC of halothane Con%
A
B
C
D
Determinations

The normothermic and hypothermic mean values of MAC of halothane, determined with either of the stimulators, are graphically illustrated in Figure 7.4.

Both the mechanical and heat stimuli were always accompanied by a widely variable and inconsistent changes in arterial blood pressure apnoea and hyperpnoea at both normothermia and hypothermia.

7.4: Discussion:

For any parameter which is repeatedly measured, standardisation of techniques to minimise the variability, is a paramount prerequisite for reproducibility and comparative study. The investigation of the influence of hypothermia on MAC is a typical example where there is a need for repeated measurements.

The development of a mechanical stimulator (Sobair et al., 1993) and a heat stimulator (Chapter 6) that provide for the application of a controlled non-destructive stimuli, helps in minimising the variability in the applied stimulus and thereby improving the chances of obtaining reproducible values of MAC. Such reproducibility was considered to be one of the advantages of MAC over other indices of anaesthetic potency (Quasha et al., 1980).

The reproducibility of both the mechanical and heat stimuli, at both normothermia and hypothermia is crucial and is clearly shown in tables 7.1, 7.2, 7.3 and 7. 4. Had there been a significant variation with either stimulus at normothermia and hypothermia, the difference in MAC values would not have been easily explained because there would have been more than one variable involved.

Table 7.1 : Normothermic values of MAC determined using mechanical stimuli.

Animal No.	Rectal temp. °C.	Surface temp °C	Stimulus N/mm2	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.	Resp.* applic. No.
7.I	38.5	33.8	1.36	0.7	0.8	0.75	1.2	1
7.II	39	35.8	1.46	1.0	1.1	1.05	1.2	1
7.III	38.5	36.3	1.40	0.6	0.7	0.65	19.2	1
7.IV	38.7	31.9	1.39	0.7	0.8	0.75	4.8	2
7.V	38.8	32.4	1.42	0.5	0.6	0.55	21.6	3
7.VI	39.0	33.8	1.42	0.8	0.9	0.85	2.4	1
Mean	38.8	34.0	1.41	0.72	0.82	0.77	8.4	
SD	0.2	1.6	0.03	0.16	0.16	0.16	8.6	

* Response application No.= Number of the application of the stimulus at which response occurred

Table 7.2 : Hypothermic values of MAC determined using mechanical stimuli.

Animal No.	Rectal temp.°C	Surface temp. °C	Stimulus N/mm^2	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.	Resp. applic. No.
7.I	34.6	30.2	1.29	0.7	0.8	0.75	1.2	2
7.II	35.5	28.5	1.37	0.7	0.8	0.75	1.2	2
7.III	34.4	26.5	1.41	0.4	0.5	0.45	1.2	1
7.IV	34.6	25.2	1.40	0.3	0.4	0.35	27.0	1
7.V	34.7	28.8	1.47	0.2	0.3	0.25	2.4	3
7.VI	35.3	27.8	1.40	0.4	0.5	0.45	3.6	1
Mean	34.9	27.8	1.39	0.45	0.55	0.50	6.1	
SD	0.4	1.6	0.05	0.19	0.19	0.19	9.4	

Table 7.3 : Normothermic values of MAC determined using focal heat stimuli.

Animal No.	Rectal temp.°C.	Surface temp °C	Stimulus °C.	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.	Resp. applic. No.
7.I	38.5	33.5	54.3	0.9	1.0	0.95	14.4	1
7.II	39.0	34.9	54.3	0.9	1.0	0.95	6.0	1
7.III	38.5	35.9	54.5	0.6	0.7	0.65	9.6	2
7.IV	38.8	32.9	54.2	0.7	0.8	0.75	19.2	3
7.V	38.8	33.0	54.2	0.5	0.6	0.55	24.0	2
7.VI	39.0	33.5	54.4	0.9	1.0	0.95	16.8	2
Mean	38.8	33.95	54.3	0.75	0.85	0.8	15.0	
SD	0.2	1.1	0.02	0.16	0.16	0.16	6.0	

Table 7.4 : Hypothermic values of MAC determined using focal heat stimuli.

Animal No.	Rectal temp.°C	Surface temp. °C	Stimulus °C	Response Conc.%	No-response Conc.%	MAC Conc.%	Response time/secs.	Resp. applic. No.
7.I	34.6	27.4	54.3	0.2	0.3	0.25	2.4	1
7.II	35.5	26.8	54.3	0.6	0.7	0.65	9.6	1
7.III	34.4	25.6	54.3	0.4	0.5	0.45	12.0	1
7.IV	34.6	24.5	54.3	0.3	0.4	0.35	19.2	1
7.V	34.8	29.1	54.4	0.2	0.3	0.25	7.2	1
7.VI	35.3	27.8	54.3	0.4	0.5	0.45	21.6	1
Mean	34.9	26.9	54.3	0.35	0.45	0.4	12.0	
SD	0.4	1.5	0.04	0.14	0.14	0.14	6.7	

Table 7.5 : Cooling rates for the induction of hypothermia expressed in °C. per hour.

Animal No.	1st cooling rate	2nd cooling rate	3d cooling rate	4th cooling rate	Average cooling rate
7.I	0.80	1.09	1.13	1.13	1.04 +/- 0.14
7.II	1.05	1.43	1.30	1.43	1.30 +/- 0.16
7.III	0.94	0.92	1.0	0.80	0.92 +/- 0.07
7.IV	0.86	0.91	1.02	1.03	0.96 +/- 0.07
7.V	1.20	1.50	1.20	1.07	1.24 +/- 0.16
7.VI	0.69	0.75	0.97	1.03	0.86 +/- 0.14
Mean	0.92	1.10	1.10	1.08	1.05
SD	0.17	0.28	0.12	0.19	0.16

The MAC values of halothane obtained with either stimulus, were significantly lower ($P < 0.001$) than those determined at normothermia . This finding is qualitatively in agreement with previous reports in rats (Vitez et al., 1974; Eger and Johnson, 1987; Munson, 1970) and dogs (Eger et al., 1965b; Regan and Eger, 1967). Most of those reports used decrements of 10 °C. in body temperature without mentioning the cooling rates, compared to a single decrement of 4 °C. at an average cooling rate of 1.05 +/- 0.16 (SD) °C./hour, which was obtained here (Table 7.5).

Therefore a meaningful quantitative comparison of the MAC values in this investigation with the previously reported values, is reasonable on the basis of calculating the reduction in MAC per one °C. drop in body temperature. This is by assuming a linear relationship i.e. same amount of reduction in MAC for each one °C. reduction in body temperature.

Using the mechanical stimulator the normothermic MAC value was significantly ($P < 0.001$) reduced from 0.77 +/- 0.16 (SD)% (Table 7.1) to 0.50 +/- 0.19 (SD)% at hypothermia (Table 7.2). That reduction correspond to a mean reduction in rectal body temperature of 3.9 °C. i.e. a reduction of MAC by 9% per one °C. decrease in body temperature. With the heat stimulator, calculations on similar grounds indicated a reduction in MAC value of halothane of 12.8% per one °C. drop in body temperature.

Reductions in MAC values with either stimulators, are significantly greater than reductions per °C. decrease in body temperature, obtained for the same anaesthetic, of 4.8% in the rat (Vitez et al., 1974); 5.3% in the dog (Regan and Eger, 1967). Such difference might have been brought about by a number of reasons. Firstly, the stimulus in the previous reports was applied using a haemostat that might have resulted in severe trauma and damage to the sensory pathways that eventually lead to a diminished sensitivity to subsequent stimuli for the determination of MAC (Handwerker and Reeh, 1991; Sobair et al., 1993). Secondly, in the previous reports the MAC reduction due to hyothermia was calculated after 10 °C. reduction in body temperature compared to 4 °C. in this investigation. In that case, if the reduction in MAC/1 °C. occurred at a decreasing rate, that might have "diluted" the effect of hypothermia during the first 4 °C. reduction in body temperature, with the result that a smaller reduction per 1 °C. was obtained by those investigators. Thirdly, animals in this investigation were cooled at an average cooling rate of 1.05 +/- 0.16 (SD) °C./hour (Table 7.5). Previous investigators made no mention of cooling rates (Regan and Eger, 1967; Vitez et al., 1974). However, the reduction in body temperature was far greater than in this investigation which may imply that more drastic cooling rates were used in those reports. Although there are no reports on the influence of cooling rates on hypothermia related reduction in MAC, in an invitro investigation, the adverse effects of pre-arrest hypothermia on immature hearts from neonatal piglets was reported to dependent on the cooling rate (Hosseinzadeh, Techervenkov, Quantz and Chiu, 1992).

The exact role played by hypothermia in reducing MAC, is still not fully understood. Early investigators suggested a direct relation between hypothermia and "cold narcosis" that was reported to develop when body temperature was reduced to 28 °C. in dogs (Bigelow et al., 1950) and 20 °C. in monkeys (Callaghan, McQueen, Scott and Bigelow., 1954). However, subsequent investigators argued that since fixed agents such as vinyl ether and ethyle ether were used in those studies, there was a good chance of getting residual anaesthetic effects at those levels of hypothermia (Eger et al., 1965b). Further more, the extent to which hypothermia reduces MAC was found to vary from one anaesthetic to the other. For instance, reducing the body temperature by 10 °C, was reported to decrease MAC of cyclopropane , ether, fluroxene, halothane and methoxyflurane by 19.6, 37.6, 42.2 and 53.2 respectively (Regan and Eger, 1967). That variability lead some workers to suggest that the hypothermia related reduction of MAC was not purely due to cold, otherwise such reduction for the same reduction in body temperature would have been similar for all anaesthetics (Eger et al., 1965b).

Another approach to explain that variability was based on the well established correlation between the extent of MAC reduction and the increase in lipid solubility of the individual anaesthetic agents (Regan and Eger, 1967). Using such an approach, a hypothermia-related reduction of halothane by 50% and that of cyclopropane by 25% was reported to parallel the hypothermia-associated decline of the solubility of the two agents by 4 and 2% respectively per 1 °C. drop in body temperature (Allott, Steward, Flook and Mapleson, 1973). However, more recent studies revealed that

with the anaesthetic I- 653, a derivative of isoflurane (Eger and Johnson, 1987), although it has a similar lipid solubility as cyclopropane, the hypothermia related decrease of its MAC was similar to that of halothane (42.2%) rather than that of cyclopropane (19.6%) (Regan and Eger, 1967). In this investigation, even for the same anaesthetic, halothane, the reduction in the MAC value over the same level of hypothermia varied with the stimulus used. It was 50% with the heat stimulus (Table 7.3 and 7.4), compared to 35.1% obtained with the mechanical stimulus (Table 7.1 and 7.2).

Very recently, the need for anaesthesia with isoflurane in goats was reported to be completely eliminated by hypothermia at 20 °C. and it was suggested that the decrease in MAC associated with hypothermia may result from the influence of solubility during the initial stages and hypothermia itself at latter stages (Antognini, 1993).

Assuming a linear relationship, and by extrapolation of the results in this section, the "anaesthetising hypothermia" in the rabbit would be at a rectal temperature of 31.0 °C. and 27.7 °C. for a heat stimulus of 54.3 °C. and a mechanical stimulus of 1.41 - 1.39 N/mm^2 respectively (tables 7.1, 7.2 , 7.3, and 7.4). Previously the "anaesthetising hypothermia" was reported to be at 28 °C. in dogs (Bigelow et al., 1950); 20 °C. in both monkeys (Callaghan et al., 1954) and goats (Antognini, 1993) and 18 - 21 °C. obtained by extrapolation in dogs (Regan and Eger, 1967).

At normothermia the value of MAC determined with the mechanical stimulator (Table

7.1) agreed reasonably well with previously reported values, using the same stimulator and stimulus (Sobair et al, 1993). Similarly, the normothermic mean value of MAC determined with the heat stimulator (Table 7.3) compares favourably with those obtained in the previous Chapter using the same stimuli. These findings further support the argument that the use of a controlled reproducible stimuli for the determination of MAC improves the chances of the reproducibility of MAC. The latter is crucial particularly in the field of comparative research (Zavisca et al., 1993; Sobair et al., 1993).

With both the mechanical and heat stimuli, the response time showed a wide variability. It was relatively shorter at hypothermia than at normothermia (tables 7.1, 7.2, 7.3 and 7.4). With the heat stimulator, the relatively shorter response time at hypothermia was probably related to the diminished efficiency of "the stimulus diluting mechanisms" brought about by the hypothermia-related vasoconstriction i.e. the latter might have interfered with the dissipation of the heat from the stimulus through the reduction in the blood flow to the site of stimulation.

The application at which the movement response occurred, also showed great inconsistency with either stimulators. However, with the heat stimulator at hypothermia (Table 7.4), all animals responded during the first stimulus application probably due to "stimulus concentration" as a result of the hypothermia-related vasoconstriction.

Other stimulus related responses (Appendix D) included arterial blood pressure changes, apnoea and hyperpnoea (Figures 7.2, 7.5 and 7.6). As mentioned in the previous chapter, the occurrence of such responses demonstrated obvious quantitative inconsistency. However, the arterial blood pressure was qualitatively consistent i.e. in all 5 animals where blood pressure was monitored, the stimulus-related changes were constantly reductions with both the mechanical and the heat stimulators. This is in contrast with a consistent increase in blood pressure when similar mechanical stimuli were applied to the nostril of a rabbit (Sobair et al., 1993). These observations might indicate a link of some sort between the nature of the change in arterial blood pressure and the site of stimulation e.g. the ear versus the nostril. Elsewhere inspired concentrations of halothane of 2.25 - 2.5% were reported to convert the pressor of arterial blood pressure into depressor effects in rabbits (Fukunaga et al., 1990).

Figure 7.5: MAC determination in an hypothermic rabbit (rectal temperature of 35.5 °C) using focal heat stimulation. At an end-tidal halothane concentration of 0.4% (response concentration), the application of a focal heat stimulus of 54.3 °C, provoked head movement (M) after 21.6 seconds (response time) from the start of the first stimulus application. Note the drop in arterial blood pressure (cBP) and apnoea (AP).

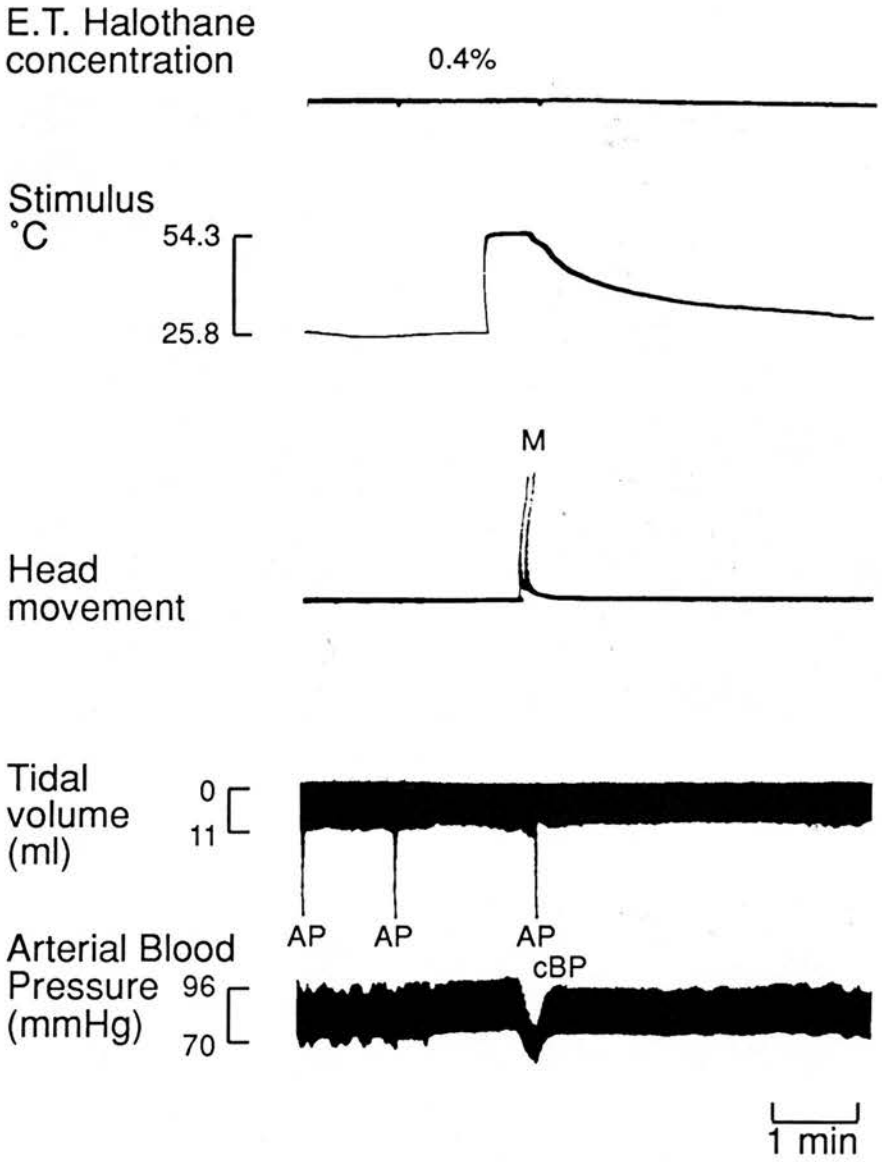

E.T. Halothane concentration
0.4%
Stimulus °C
54.3
25.8
M
Head movement
Tidal volume (ml)
0
11
AP
AP
AP
cBP
Arterial Blood Pressure (mmHg)
96
70
1 min

Figure 7.6: An illustration of a mechanical stimulus-related apnoea (AP) and hyperpnoea demonstrated by running the chart paper at a faster speed (25 divisions per second) for few seconds that allowed the identification of the individual spikes of the respiratory cycle (Tidal volume tracing). At both stimulus applications, there was a deep breath (gasp) followed by apnoea (AP) and then hyperpnoea. In this hypothermic rabbit (rectal temperature of 35.5 °C), there was also provoked movement and a stimulus-related drop in arterial blood pressure (cBP).

Veterinary Research Communications, 17 (1993) 375–385

A MECHANICAL STIMULATOR FOR THE DETERMINATION OF THE MINIMUM ALVEOLAR CONCENTRATION (MAC) OF HALOTHANE IN THE RABBIT

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ABSTRACT

Sobair, A.T.H., Cottrell, D.F. and Camburn, M.A., 1993. A mechanical stimulator for the determination of the minimum alveolar concentration (MAC) of halothane in the rabbit. *Veterinary Research Communications*, **17** (5), 375–385

The minimum alveolar concentration (MAC) of halothane was determined in New Zealand White rabbits. Tracheal anaesthetic concentrations were measured using a Siemens Servo Gas Monitor. A stimulator was used to deliver precisely controlled mechanical stimuli for the determination of MAC. Movement of the rabbit's head was recorded using a force transducer attached to the teeth. Evidence is presented that for the determination of MAC a precise nociceptive threshold is preferable to the so-called supramaximal stimulus used in clinical anaesthesia and in determinations of anaesthetic potency. We conclude that techniques for the determination of MAC which disregard either sensitization of sensory mechanisms by producing tissue inflammation or the possibility of nerve compression by severe mechanical stimuli are of questionable value. The use of the mechanical stimulator described, or a similar device, would help in the standardization of the determination of MAC in all species by facilitating the application of a force of controlled amplitude, duration and velocity, thereby removing some of the variables which confound comparative studies of MAC.

Keywords: anaesthesia, efficacy, minimum alveolar concentration, rabbit, stimulus, technique

Abbreviations: DC, direct current; ID, internal diameter; MAC, minimum alveolar concentration

INTRODUCTION

It is important to set levels of clinical anaesthesia at which the animal is unconscious. The minimum alveolar concentration (MAC) is described (Merkel and Eger, 1963) as 'the minimal anaesthetic concentration in the alveolus required to keep a dog from responding by gross movement of the head and/or limbs to a painful stimulus'. The stability, reproducibility and relative ease of measuring MAC in animals (Eger *et al.*, 1965) and man (Saidman and Eger, 1964) are among the important factors that have led to its becoming a widely accepted measure of anaesthetic potency. MAC is measured when the inspired, alveolar, arterial and cerebral anaesthetic concentrations are assumed to be equal. Therefore it represents a partial pressure and not a concentration as its name implies (Merkel and Eger, 1963). MAC has been regarded as a reliable measure for comparing the pharmacodynamic effects of inhalation anaesthetics, using multiples of the MAC values (Merkel and Eger, 1963; Regan and Eger, 1967) to replace the use of clinical signs, which differ widely from one drug to another (Cullen *et al.*, 1972).

The three components of MAC determination described by Merkel and Eger (1963) were: (1) the measurement of the end-tidal anaesthetic concentration, (2) the application of the stimulus, and (3) the seeking of a response. The stimuli used are often extreme, causing actual or potentially damaging injury to the skin. These include skin incision in man, tail clamping and a rotating chamber that tests for the righting reflex in animals, and electrical stimulation in both man and animals (Miller, 1986). Tail clamping is the most widely used stimulus for MAC determination in animals. In rabbits this has been performed using a 25-cm (10-inch) rubber-shod haemostat (Drummond, 1985) or a 6.25-cm (2.5-inch) Johns Hopkins arterial clamp (Davis *et al.*, 1975). Electrical stimulation of the oral mucous membrane (Muir *et al.*, 1992) has been used for MAC determination in horses. The variability of stimuli used may in part explain the differences in the values of MAC obtained (Eger *et al.*, 1965).

We decided to attempt to define a consistent stimulus, taking into consideration modern concepts in sensory physiology, and to establish the MAC at the nociceptive threshold. Such an approach should minimize the effect of sensory sensitization by inflammatory mediators and, by accurately recording the forces used, should allow meaningful comparison between investigations.

MATERIALS AND METHODS

Anaesthetic induction and maintenance

Halothane anaesthesia was induced without premedication in 9 non-fasting New Zealand White rabbits, weighing 3–4 kg, using a rubber mask and a modified Ayre's T-piece circuit. Halothane was delivered in 100% oxygen at 2 L/min (Flecknell, 1987) from a Drager vaporizer, with inspired concentrations of up to 4.5 vol% (Hall and Clarke, 1991) for induction, later adjusted to maintenance levels. Tracheostomy was performed and a bevelled plastic tube (4–5 mm ID) was inserted and connected through a plastic Y-tube to the breathing circuit. The Y-tube also allowed the end-tidal sampling.

Animal instrumentation

The femoral artery was catheterized and the blood pressure was measured using a Statham transducer, recorded on an 8-channel recorder (Lectromed Ltd., Jersey) and displayed on an oscilloscope. The arterial line was filled with heparinized saline and calibrated with a mercury manometer. The rectal temperature was measured continuously using a thermistor probe in the rectum and displayed on heat-sensitive paper using a DC amplifier. Calibration of the thermistor was carried out using water baths of known temperature and a mercury thermometer. The rectal temperature was maintained within the normal range for rabbits (39–40°C) by a heating blanket with a thermistor feedback (homeothermic blanket control CFP 8140, C.F. Palmar, London).

Measurement of the end-tidal concentration of halothane

The end-tidal concentration was considered to be the minimum concentration of halothane at the end of the expiratory phase measured from the tracheal tube. The end-tidal concentration of halothane was measured using a Siemens Servo Gas Monitor 120, which was calibrated using certified gas concentrations (1.52% isofluorane in 35.5% oxygen; Siemens-Elema). Calibration with isofluorane is equivalent to calibration for halothane (Siemens-Elema instruction manual). Gas sampling was continuous at 300 ml/min through a 1 mm ID catheter inserted through an airtight rubber cap and down the free limb of the Y-tube into the trachea. The sampling tube was connected to the gas monitor through 0.8 μm micropore filters (Siemens-Elema AB), which served to remove condensation. The concentration of halothane was continuously displayed on a scale and recorded on heat-sensitive paper.

The mechanical stimulator

The mechanical stimulator consisted of a pincer driven by compressed air, and was operated from a control panel with a manual trigger (Figure 1). The compressed air was delivered via a pressure regulator (range 0–415 kPa; 0–60 psi) to an electronic valve (12 V DC) and a flow valve to control the rate of change of force of the pinch applied. Before applying the stimulus, the gap between the pincers (A) was adjusted to suit the thickness of the stimulus site, by turning the knurled ring (C). Air was fed to the stimulator (Figure 1) through the line (D), forcing the cylinder (B) to move forward so that the pincers (A) closed. The stimulus intensity was controlled by a preselected driving pressure. At the end of the stimulus, the air pressure was released and the tips were forced open by a return spring (F).

A foil strain transducer (120 ohm; Showa Measuring Instrument Co., Tokyo) was mounted on the lower arm of the pincers so as to enable the force applied to the target site to be measured. The signal in the line (E) was displayed on a chart

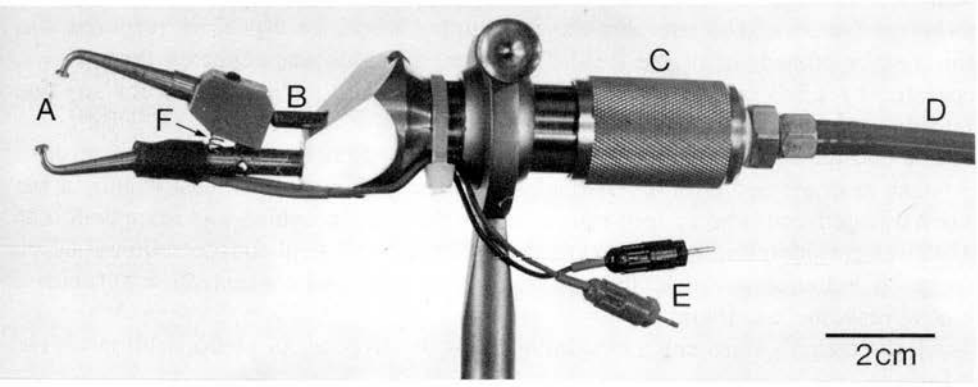


Figure 1. The air-operated mechanical stimulator (pincher) used for the application of a controlled and recordable stimulus. A, two pinching tips; B, cylinder; C, knurled ring; D, line through which air is fed to the pincher; E, line for pincher output; F, return spring

recorder and oscilloscope. The stimulator was triggered to apply a precise stimulus at a preselected pressure, rate of change of force and duration. Calibration of the force applied was carried out by applying gram weights to the lower arm of the pincers and reading the voltage deflection from the recording paper. The force applied was calculated after measuring the surface area of the circular pinching disc (3.8 mm^2) and converted to newtons per unit surface area (4.90 N represents the force from a mass of 0.5 kg). For this investigation the stimulus was kept steady, a mean force of approximately 1.35 N/mm^2 being applied for 30 s by the stimulator at a driving pressure of 275 kPa (40 psi) and a rate of change of force of 1.6 N/s. The velocity of displacement was calculated from a storage oscilloscope.

Determination of the response

The response was defined as the observed provoked movement of the head which was detected by force transducers connected by cotton thread to the upper incisor teeth. The signal was amplified and continuously recorded on heat-sensitive paper and an oscilloscope. Head movement was selected for recording because it occurred more often than limb movement in response to ear stimulation. Movement artefacts, due to respiration, were of lower amplitude and frequency than evoked responses, which were of high frequency and amplitude and coincided with the application of the stimulus.

Experimental protocol

Anaesthesia was maintained for at least 15 min at an end-tidal concentration of halothane that was believed to approximate to MAC. A range of forces was used on the basis of known nociceptor thresholds (Iggo, 1985; Livingstone *et al.*, 1992). The force provided by a driving pressure of 275 kPa (40 psi) was also chosen on the basis of a pilot study. This stimulus force could just be tolerated when applied to the human thumb web and did not cause an inflammatory response. The pincer force was applied to the ear for 30 s. The stimulus was interrupted whenever a positive response was indicated by movement of the head. The same stimulus was repeated three times, separated by 1.5–3 min, before considering the response to be negative for any one end-tidal concentration.

The end-tidal concentration was then adjusted by 0.1% (increased after a positive response or decreased after a negative one) and maintained for at least 15 min at the newly adjusted end-tidal concentration before the same stimulus was reapplied. The MAC was considered to be the average of the lowest end-tidal concentration at which a negative response was detected and the highest end-tidal concentration at which a positive response was found.

All the animals were killed by administering an overdose of pentobarbitone at the end of each study.

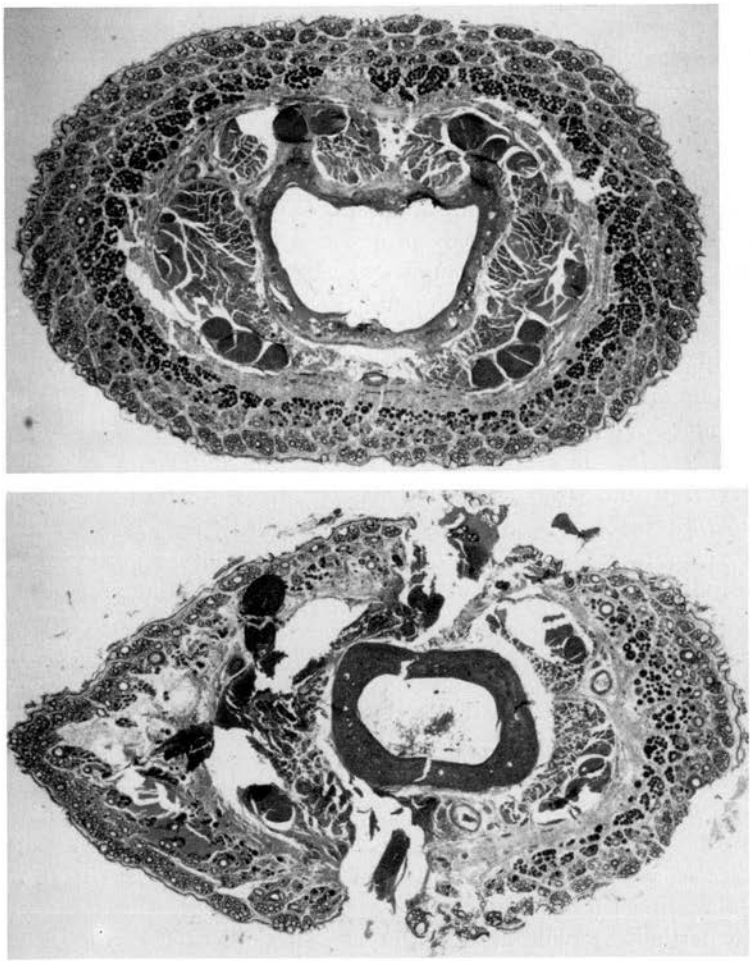
Data analysis

The mean and the standard deviation were calculated for all relevant measurements.

RESULTS

Pilot study

A pilot study was conducted to determine the MAC of halothane in two rabbits using a padded artery forceps haemostat as a mechanical stimulus by clamping the tail and ear. With this technique the data demonstrated that there was altered tissue sensitivity when the haemostat was applied to the ear of one of the rabbits. During the initial applications of the haemostat the MAC value was calculated to be 1.65% (an average of a response concentration of 1.6% and a no-response concentration of 1.7%).



(a)

(b)

Figure 2. The result of the application of a padded arterial haemostat to the rabbit's tail. (a) A transverse section of the tail proximal to the areas of compression with normal histological appearance; (b) a transverse section coinciding with an area of compression, revealing gross tissue disruption including fracture of the caudal vertebra, areas of haemorrhage and muscular contusion

During a second attempt to determine MAC of halothane using the same ear, a positive response was still being detected at an end-tidal halothane concentration of 1.9%.

Alternatively, the damage to underlying nerves might be so severe as to make them incapable of conducting impulses (Wolff and Wolf, 1958; Sunderland, 1978) as was apparently the case in the other rabbit, when the haemostat was applied to the tail. During this trial MAC for halothane was initially calculated at 1.5% (an average of responses at 1.4% and no response at 1.6%). When the haemostat was applied later to a previously used site, there was no response at 0.9% halothane. This implied that the sensory conduction pathway was probably severed. When we studied the resultant pathology of tails in fully anaesthetized animals, we found that repeated application of the haemostat caused severe trauma and lacerations (Figure 2). We considered that the extent of these lacerations and contusions, even in the absence of actual nerve damage, would alter the sensitivity of the sensory nerve terminals either directly or by the release of inflammatory mediators (Willoughby, 1987), which might have been the mechanism for the alteration of MAC when the ear was used.

The findings of altered sensitivity and physical damage in the pilot studies prompted us to look more carefully at the techniques involved in the determination of MAC. In particular, we wished to devise a mechanical stimulator that would accurately deliver a measured stimulus that would allow knowledge of the thresholds required to activate mechano-nociceptors (Iggo, 1985; Waterman *et al.*, 1992) to be taken into consideration. In the only previous quantitative study on mechanical noxious stimuli, normal conscious sheep were shown to have a pedal withdrawal to pressure from a blunt-pin (of unreported surface area) of 4.35 N on the skin overlying the lower end of the radius (Waterman *et al.*, 1992).

In addition, the pilot studies involved the use of the air-operated mechanical stimulator on a third trial rabbit. Driving pressures greater than 207 kPa (30 psi) applied to the tail caused an unacceptable level of tissue damage, which increased with the driving pressure used. A driving pressure of 207 kPa (30 psi) applied to the tail produced a similar force to the mean value of the stimulus from 275 kPa (40 psi) applied by the stimulator to the ear (1.35 ± 0.07 (SD) N/mm^2). This finding demonstrates the contribution of the site of application to the reaction force measured, i.e. the stimulus pressure against the bone of the tail produced a greater force than the same pressure across the cartilage of the ear. The requirement to measure the actual force applied for each stimulus application is further demonstrated with reference to Table I. It is seen that even when the closing pressure and velocity are constant and the pincer diameter remains the same, the actual force applied varies with the nature of the tissue being compressed, even in a relatively uniform tissue like a rabbit's pinna. We conclude that the absence of accurate measurements in other studies will have led to differences in the stimulus applied, which may partially account for the different values of MAC reported.

Main investigation

The mechanical stimuli and movement responses at various halothane end-tidal concentrations for the individual rabbits are shown in Table I. The concentrations at which either a response or no response was obtained are shown in Table II.

TABLE I
Response to stimulus

Animal	End-tidal halothane conc. %	Applied stimulus (N/mm^2)	Response	Animal	End-tidal halothane conc. %	Applied stimulus (N/mm^2)	Response
1	0.9	1.42	–	4	1.2	1.39	–
	0.9	1.42	–		1.2	1.39	–
	0.9	1.42	–		1.2	1.34	–
	0.8	1.42	+		0.9	1.24	+
Mean		1.42		Mean		1.34	
SD		0		SD		0.06	
2	0.9	1.38	–	5	0.9	1.29	–
	0.9	1.38	–		0.9	1.38	–
	0.9	1.46	+		0.9	1.20	–
	1.0	1.29	–		0.8	1.46	–
	1.0	1.29	–		0.8	1.38	–
	1.0	1.38	–		0.8	1.29	+
Mean		1.36		Mean		1.33	
SD		0.056		SD		0.08	
3	0.9	1.29	–	6	1.0	1.43	–
	0.9	1.29	–		1.0	1.29	–
	0.9	1.20	–		1.0	1.29	–
	0.8	1.29	–		0.9	1.43	–
	0.8	1.29	+		0.9	1.35	+
Mean		1.27		Mean		1.36	
SD		0.04		SD		0.06	

Response (head movement) to stimulus generated from a pre-set driving pressure of 275 kPa (40 psi) applied to the ear for a duration of 30 s at a rate of change of force of 1.6 N/s. At any one end-tidal concentration the stimulus was applied for three times before considering the response as negative

The concentrations of halothane that allowed a response ranged between 0.80% and 0.90% with a mean value of 0.85 ± 0.05 (SD) % ($n=6$). The concentrations of halothane that prevented a response ranged between 0.90% and 1.20% with a mean value of 0.98 ± 0.11 (SD) % ($n=24$).

The MAC values (Table II), calculated as the average of the halothane concentrations that allowed either a response or no response, ranged between 0.85% and 1.05% with a mean value of 0.92 ± 0.07 (SD) % ($n=6$).

The mean value of the stimulus for the 30 applications in Table I was a force of 1.35 ± 0.07 (SD) N/mm^2 and the mean of the mean values was 1.35 ± 0.05 (SD) N/mm^2. This small standard deviation indicates how reproducible the applied force can be with this mechanical stimulator. An example of movement in response to an applied stimulus is shown in Figure 3.

The response time, defined as the time from the application of the stimulus until the animal responded, was between 1.2 and 12 s, with a mean value of 7.4 ± 4.5 (SD) s ($n=6$). The rectal body temperature shown in Table II ranged between 39°C and 40°C, with a mean value of 39.5 ± 0.3 (SD)°C ($n=6$).

Other effects of the stimulus included a sudden drop in blood pressure (Figure 3) and transitory apnoea.

TABLE II
Mean stimulus generated from a driving pressure of 275 kPa (40 psi) applied to the ear and MAC values calculated as the average of the response and no-response concentration

Animal	Stimulus force (N/mm^2)	Response conc. (%)	No-response conc. (%)	MAC conc. (%)	Rectal temperature (°C)	Response time (s)
1	1.42	0.8	0.9	0.85	39.5	12
2	1.36	0.9	1.0	0.95	40.0	4.8
3	1.27	0.8	0.9	0.85	39.2	9.6
4	1.34	0.9	1.2	1.05	39.5	4.8
5	1.33	0.8	0.9	0.85	39.0	12
6	1.36	0.9	1.0	0.95	39.7	1.2
Mean	1.35	0.85	0.98	0.92	39.5	7.4
SD	0.05	0.05	0.11	0.07	0.3	4.5

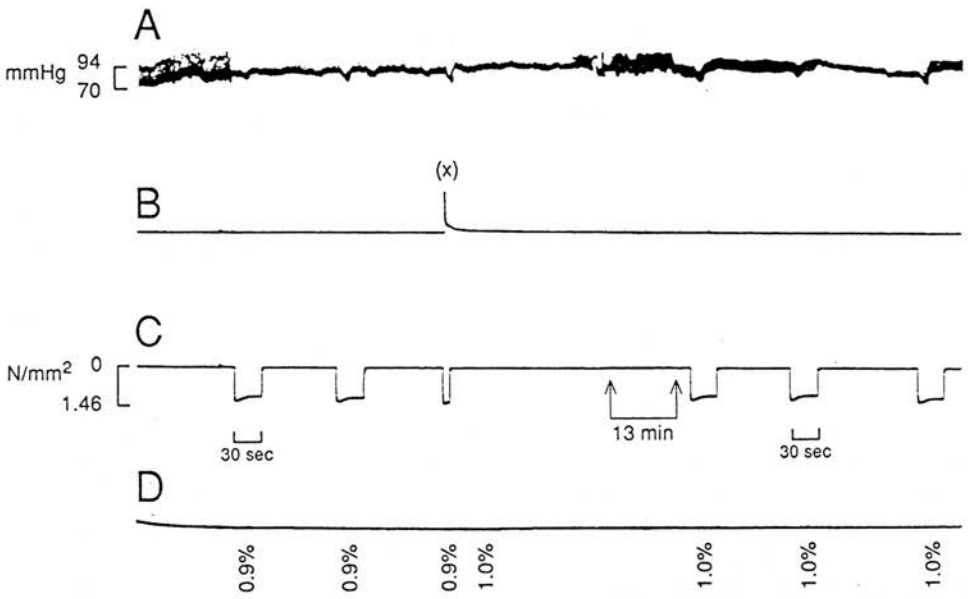


Figure 3. The response to controlled application of force during two values of end-tidal halothane concentrations (animal 2 in Tables I and II). (A) Systemic blood pressure. Note the drop in blood pressure when the stimulus is applied. (B) Movement of the head (X) at the third stimulus application at 0.9% end-tidal halothane concentration. (C) The application of driving pressure of 40 psi (producing a stimulus of 1.29 – 1.46 N/mm^2) for 30 s. At (X) head movement occurred and the force was removed after 4.8 s. (D) End-tidal halothane concentration

DISCUSSION

Of the aspects of MAC determination, it is the stimulus applied that appears to have been least quantified by investigators. The stimulus required to determine MAC must be substantial but it must not be of sufficient intensity to either desensitize or sensitize the tissues, and the anaesthetist must be confident that no unnecessary pain is inflicted. We were anxious about the intensity of the stimulus used in establishing MAC in relation to present knowledge of nociceptor sensitivities (Iggo and Kornhuber, 1977; Iggo, 1985). In terms of sensory physiology it is conceptually difficult to interpret what 'supramaximal', a term often used by anaesthetists, means – does it mean exciting a discrete population of nociceptors maximally, or all somatosensory nociceptors maximally? Furthermore, there are conflicting reports regarding the reliability of the supramaximal stimuli that are used. For instance, tail clamping is considered to be a supramaximal stimulus by some investigators (Eger *et al.*, 1988; Lundeen *et al.*, 1983) but not by others (Tranquilli *et al.*, 1983; Eger *et al.*, 1965). Also, skin incision has been shown not to be a supramaximal stimulus in the dog (Eger *et al.*, 1965) and pig (Lundeen *et al.*, 1983; Tranquilli *et al.*, 1983) but it is a standard stimulus in man (Saidman *et al.*, 1967). No investigators have accurately quantified the mechanical stimuli used for MAC determination.

Our own pilot study demonstrated that a stimulus such as a haemostat to the ear or tail caused severe trauma and lacerations that produced an altered sensitivity to stimulation and, in the tail, may even have prevented sensory impulse conduction (Woolf, 1983, 1991; Handwerker and Reeh, 1991; Dubner, 1991). Elsewhere, chronic inflammatory disease of the hoof significantly reduces the flexor reflex threshold in conscious sheep from control values of 4.35 N to 2.49 N (Waterman *et al.*, 1992). We were therefore reluctant to continue using such a stimulus even in non-recovery work, and consider that its use is unacceptable for the determination of MAC. When the extent of tissue trauma produces inflammation, sensitization or desensitization, this makes scientific interpretation of MAC data impossible.

Other workers have obviously been aware of this, for some have tried to circumvent or at least minimize the harm caused by the various clamping devices, for instance, by using a rubber-shod haemostat (Drummond, 1985) or a haemostat with taped jaws (Doorley *et al.*, 1988) or by always stimulating the tail proximal to an earlier test site – i.e. avoiding repeating the stimulus at the same site (Stone *et al.*, 1992). On the basis of our pilot study in the rabbit, we concluded that these precautions would not have succeeded in solving the problem of tissue damage provoking altered somatosensory sensitization.

The MAC values (as a volume percent) obtained in our investigation employing nociceptive threshold techniques ranged between 0.85% and 1.05%, with a mean value of 0.92 ± 0.07 (SD)%. This is similar to the value obtained by Davis *et al.* (1975) of 0.82 ± 0.25 (SD)%. It is significantly lower than the 1.39 ± 0.23 (SD)% obtained by Drummond (1985). However, the comparison of our MAC values with those investigations is confounded because of a number of factors, mainly related to the stimulus. Firstly, the stimulus used in our investigation was different from theirs, which was a rubber-shod haemostat (Drummond, 1985) and a Johns Hopkins arterial clamp (Davis *et al.*, 1975). Secondly, they applied the stimulus for an unspecified duration of up to 30 s (Davis *et al.*, 1975) or for 60 s during which time the haemostat was continuously moved (Drummond, 1985). Neither of these investigators reported

the velocity of application of the clamps and they may not have been in a position to do so because of the difficulty in controlling the application of clamps. Both the velocity of application and the duration of the stimulus have been found to affect the degree of firing of somatosensory nociceptors (Iggo and Kornhuber, 1977; Iggo, 1985) and are therefore intimately involved in eliciting any movement response. In this investigation, we have used a specific force applied at a rate of change of force of 1.6 N/s and for a constant duration of 30 s. Thirdly, we used the ear for the application of the stimulus rather than the tail (Davis *et al.*, 1975; Drummond, 1985). This was because the ear, unlike the small tapering tail, offers a large, flat surface area of more or less uniform thickness, so that there is less chance of repeating the stimulus at the same test site. Also, the stimulus was easily and precisely applied to the ear without modifying the existing pincer tips, as would be required for the tail. These factors could account both for the differences between our results and those of the previous investigators and also for the greater variability in the MAC values reported by Davis *et al.* (1975) and Drummond (1985).

In one rabbit the estimated MAC value was unexpectedly high (1.05, the underlined value in Table II). This was probably accounted for by the larger anaesthetic increments (0.3%) used to determine the response on that occasion. The true MAC value for that rabbit could have had any value between 0.90 and 1.20 vol%.

As shown in Table II, positive responses occurred within 1.2 – 12 s after the start of the stimulus, with a mean value of 7.4 ± 4.5 (SD) s, indicating wide variability. Had we applied the stimulus for no more than 10 s, as used by Merkel and Eger (1963), two of the positive responses would have been reported as negative and a different value obtained for MAC. For similar reasons, Eger *et al.* (1965) suggested that the stimulus should be applied for at least 30 – 40 s, usually for one minute. However, our results suggest that the stimulus need not be applied for longer than 30 s, which will minimize the tissue damage.

In addition to movement, other responses to the stimulus included a sudden drop in blood pressure as seen in the 'playing dead' reaction (Folkow and Neil, 1971). In one of the rabbits, for comparison, we applied the stimulus with the same force to the nostril. This location consistently produced an increase of blood pressure as part of the positive response, as was previously reported to be a reaction to stress in human subjects (Wolf *et al.*, 1948). The site at which the stimulus is applied is therefore important when autonomic reflexes are tested and requires further study.

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