ACID-BASE HOMEOSTASIS AND MYOCARDIAL AND CEREBRAL PRESERVATION DURING AND AFTER INDUCED HYPOTHERMIA

A Thesis submitted for the Degree of Doctor of Philosophy

Yi Yi Huang

M.B., B Med Sc., M. Sc (Medicine)

Department of Physiology, University of Tasmania,
Hobart, Tasmania, Australia

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I dedicate this thesis with love and deep gratitude to my parents in Kunming, Yunnan, CHINA

ABSTRACT

Two acid-base strategies, pH-stat and alpha-stat, are used in hypothermic cardiac pulmonary bypass (CPB) although wide spread practice has not clarified which is the better (Swan, 1984; Takao, 1991; Aoki et al, 1994). pH-stat exists among the hibernators, which tends to preserve normothermic pH and PCO_2 values as body temperature falls (Malan, 1982). Alpha-stat, a term used to describe the responses of ectotherms and heterotherms, which tends to maintain an optimal function in all organ systems at all temperatures with a lower PCO_2 and a higher pH, but no change in plasma HCO_3^- or CO_2 stores (Reeves, 1972). Previous investigations have compared pH-stat and alpha-stat and postulated that a more alkaline pH would be even more effective in preserving myocardial and cerebral function during hypothermia (Buckberg, 1985; Becker et al, 1981).

In this study the effects of pre-alkalinization and pre-acidification on cerebral and cardiovascular function during and after hypothermia were investigated in 37 fentanyl-anaesthetised New Zealand rabbits.

Animals were divided into four groups: a normothermic (Nom, n=6) and 3 hypothermic groups a) hypothermic control (HCo, n=9); b) hypothermic acidosis (HAc, n=10); c) hypothermic alkalosis (HAl, n=12). Alkalosis was induced by injection of carbicarb (0.33 M Na_2CO_3 and 0.33 M $NaHCO_3$, 2 ml kg^{-1}, Rhee et al, 1993) and acidosis by NH_4Cl (2 M, 2.5 ml kg^{-1}) before reducing body temperature (Tb). Animals were sedated with fentanyl (4.2 $\mu g\ kg^{-1}\ min^{-1}$) during surgical operation and 2.1 $\mu g\ kg^{-1}\ min^{-1}$ during monitoring and ventilated with 35% O_2 in N_2. The experiment was divided into 5 phases: 1. normothermic control; 2. cooling; 3. profound hypothermia (Tb = 25±1°C for one hour); 4. rewarming and 5. normothermic recovery. Blood samples were taken for PO_2, PCO_2 and pH analysis. Heart rate (HR), mean aortic pressure (MAP), central venous pressure (CVP), carotid arterial blood flow (CrdBF), electrocardiograph (ECG) and electroencephalogram (EEG) were monitored. Cerebral oxygen delivery (DO_2), oxygen consumption ($\dot{V}\ O_2$) and oxygen extraction (ER) were calculated from oxygen content (CtO_2) and CrdBF.

pH increased when Tb was reduced. All pHa and pHv values were higher in HAl than those in other groups ($P<0.01$). During the one hour of profound hypothermia (phase 3), pHa was constant within the three hypothermic groups (HCo: 7.52 ± 0.07 to 7.52 ± 0.08; HAl: 7.69 ± 0.06 to 7.67 ± 0.06; HAc: 7.43 ± 0.05 to 7.40 ± 0.05). After rewarming, only pHa of HAl in hypothermic group was 7.37±0.09 and not different from the value of Nom (7.38±0.04). The other two groups were significantly lower, with values of 7.17±0.08 for HCo and 7.06±0.06 for HAc ($P < 0.05$).

ECG records from HAl often showed conduction disturbances during cooling and hypothermia but returned to normal after rewarming. Conduction disturbances and arrhythmias were most severe and frequent in HAc during rewarming, and persisted after the Tb had returned to normal. HR fell during cooling and hypothermia in all animals but recovered better in HAl after rewarming. Also in HAl, MAP was higher, and stayed at a relatively constant level during hypothermia and returned to normal after rewarming.

CrdBF and DO_2 decreased when Tb was reduced and were slightly higher in HAl than in HAc. ER was lower in HAl than in HAc during rewarming ($P<0.05$). Suppression of EEG started earlier in HAc and HCo than in HAl during cooling and recovered faster in HAl than in the other two groups.

The results showed 1) a normal pH was attained after rewarming in HAl; 2) Pre-alkalinization has beneficial effects on cerebral and myocardial performance; 3) the rabbit provides a suitable small animal model for induced hypothermia, and has been successfully tested for experiments over 6 or more hours duration.

This work suggests that alkalinization before cooling could reduce or eliminate anaerobic metabolism and subsequent acidosis, and thus could be the optimal acid-base strategy during induced hypothermia and during rewarming.

Statement of Sources

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Yi Yi Huang

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What is past is prologue

— Shakespeare: The Tempest

Table of abbreviations

AVB	Atrioventricular block
$AVDO_2$	Arteriovenous O_2 content difference
CABG	Coronary artery bypass grafting
CBF	Cerebral blood flow
CI	Cardiac index
$CMRO_2$	Cerebral oxygen consumption
CNS	Central nervous system
CO	Cardiac output
CPB	Cardiopulmonary bypass
CPP	Cerebral perfusion pressure
CrdBF	Carotid blood flow
CSI	Isoelectric suppression
$CtaO_2$	Arterial blood oxygen content
CtO_2	Blood oxygen content
$CtvO_2$	Venous blood oxygen content
CVP	Central venous pressure
CVR	Cerebral vascular resistance
DO_2	Oxygen delivery = Blood flow (Q) × Oxygen content in arterial blood (CaO_2)
dTc	d-Tubocurarine
ECG	Electrocardiograph
EEG	Electroencephalogram
ER	Oxygen extraction ratio ($ER=\dot{V}O_2 \div DO_2$)
HAc	Hypothermic acidotic group
HAl	Hypothermic alkalotic group
HCo	Hypothermic control group
HIA	High voltage with irregular electric activity

HR	Heart rate
I_{Ca}	Inward calcium current
I_{Na}	Inward sodium current
IVB	Intra-ventricular Block
LDH	Lactate Dehydrogenase
MAP	Mean aortic blood pressure
MRP	Membrane resting potential
Nom	Normothermia
$M\dot{V}O_2$	Myocardial oxygen consumption
ODC	Oxyhaemoglobin dissociation curve
PCO_2	CO_2 partial pressure
PO_2	O_2 partial pressure
Tb	Body temperature
TCO_2	Total CO_2
VF	Ventricular fibrillation
VFT	Ventricular fibrillation thresholds
$\dot{V}O_2$	Tissue oxygen uptake

Chapter 1

Introduction

Hypothermia has a long history in medicine, and has been used as a remedy for diseases. In ancient times, Hippocrates described the use of ice for controlling haemorrhage, a treatment still in frequent use. "Refrigeration" was used by Baron Lary, Napoleon's surgeon, to carry out surgical interventions on soldiers. Local cooling by evaporating ether for local anaesthesia was already in use in the middle of the last century and the same principle is still employed in the form of ethyl chloride spray. Total body cooling was first applied as a form of shock therapy in psychotic patients (Talbott, 1941).

Hypothermia is used widely in cardiac and cerebral surgery to reduce metabolism, (Swan 1985; Hickey & Andersen, 1987; Spetzler et al, 1988; Magovern, 1991) so that circulation can be reduced, or even stopped, for the time needed to perform the intended surgery. Despite the prevalence of its use in clinical practice, there appears to be no clear answer to the questions that were raised approximately forty years ago when hypothermia was introduced as a new approach to surgery in humans: Should acid-base management still be the same during hypothermia as during normothermia? What is proper acid-base management during hypothermia (Swan, 1984; Takao, 1991; Aoki et al, 1994)?

Research in comparative physiology has suggested that the acid-base responses of vertebrates to cooling can be broadly described by two differing strategies — pH-stat and alpha-stat.

1.1. Alpha-stat

Histidine-imidazole is the predominant non-bicarbonate buffer group of enzymes. When temperature falls, the imidazole moiety of histidine, unlike bicarbonate or phosphate, undergoes a pK change with temperature almost exactly parallel to that of pKw. Thus it retains its buffering capacity at all temperatures and is responsible for the $\Delta pH/^{\circ}C$ of blood when TCO_2 is held constant.

The portion of histidine imidazole that has lost a proton in its dissociation is designated as alpha imidazole (Somero, 1981), which typifies or represents the charge state, not only

of histidine, but also of all proteins. When alpha imidazole remains constant with changing temperature, the over-all protein charge state likewise remains constant because histidine is to a greater part bound in proteins and dipeptides (Edsall, 1958; Rahn et al, 1975).

Reeves (1972) coined the term alpha-stat to describe the responses of ectotherms (or poikilotherm or heterotherms, whose peripheral tissues are cooler than their core temperature) to changes in body temperature. This acid-base regulation strategy maintains an optimum function in all organ systems at lower temperatures, with a lower PCO_2 and a higher pH, but little or no change in plasma HCO_3^- or CO_2 stores. With this alpha-stat strategy, buffering properties of body compartments are dominated by imidazole-type compounds; $\Delta pH/\Delta t$ follows the change in $\Delta pK/\Delta t$ of histidine-imidazole; There is no relevant transfer of transepithelial and transmembrane acid-base with changes of temperature.

Reeves' hypothesis was that appropriate $\Delta pH/\Delta t$ values were maintained in all body compartments simultaneously by rapid adjustment of $\Delta pH/\Delta t$ to the $\Delta pK/\Delta t$ of free imidazole-histidine via changes in PCO_2. Change in the ratio of CO_2 production /ventilation, which determines PCO_2 in blood, was accordingly the only parameter to be regulated in order to maintain optimum pH in all body compartments even with extreme rates of temperature changes. An important point of the alpha stat hypothesis is that the dissociation of imidazole may be maintained constant by regulation of PCO_2, i.e. by changes in convective gas exchange, therefore it is presumed that all the functions of proteins which depend upon charge state, particularly enzymatic function, will act constantly (Siggaard-Andersen, 1979; Severinghaus, 1965). Animals without any regulatory device in this respect (e.g. lung-less and gill-less salamanders) are accordingly a *priori* incapable of alpha-stat regulation (since gas exchange is diffusion-limited) because of the lack of any regulatory ability. However, as will be seen below there are significant problems with this alpha-stat concept although it provides a useful (if

simplistic) way to categorise acid-base strategies of a number of organisms including heterotherms.

Histidine, as the main biological imidazole-containing compound occurs as a free amino acid in some tissues only (mainly of invertebrates), but is to a greater part bound in proteins and dipeptides. For instance, Matthew et al (1979) has measured the pK values for a variety of specific residues in haemoglobin and indicated that human haemoglobin has 19 histidine residues, and all except eight are buried in the protein and are nontitratable. These eight have pK values ranging from 7.3 to 8.2, depending on ionic strength and Cl^- binding. In another report, pK values were from 6 to about 9.2 and $\Delta pK/\Delta t$ ranged from -0.018 to -0.024 U/°C (Edsall & Wyman). Furthermore, the ΔH values for individual histidine residues of at least one protein have been shown to be as variable as the pK, so the change in the net protein charge (Z) with temperature is impossible to predict from the ΔH for a single imidazole compound. That is why it is always difficult to evaluate $\Delta pH/\Delta t$ values determined in body compartments of heterothermic animals regarding to the imidazole hypothesis (alpha-stat) because of the uncertainties about the applicability of physiol-chemical constants of isolated imidazole compounds to biological fluid systems. This is also the reason that the pK values of histidine and other free imidazole compounds may vary in the literature concerning the imidazole alpha-stat hypothesis (Heisler, 1986b).

To complete the alpha-stat theory Cameron (1989) suggested perhaps a more appropriate model would be a "Z-stat" model, that is one predicting that a constant net protein charge would be maintained as temperature changed. Numerical predictions of $\Delta pH / \Delta T$ would then have to be based on the shift in the relationship between Z and pH with temperature change:

$$Z = k\ (pH\text{-}pI)$$

Where pI is the isoionic point for the protein mix present, and k the slope of the Z-versus-pH curve.

However in many cases the distribution of the average values determined in individual studies on the $\Delta pH / \Delta T$ scale (Fig 1, Edsall & Wyman, 1958) indicates that the $\Delta pH / \Delta T$ values were definitely smaller than the range listed for $\Delta pK / \Delta T$ of imidazole and imidazole compounds. The highest class average is found in amphibians ($\Delta pH / \Delta T$ = -0.015). By comparison, the average for heterothermic species is lower ($\Delta pH / \Delta T$ = -0.011). The average of hibernators ($\Delta pH / \Delta T$ = -0.0036) is close to the $\Delta pK / \Delta T$ of inorganic phosphate ($\Delta pK / \Delta T$ = -0.0027 U/°C) which has virtually no temperature dependence at all (Heisler, 1986b).

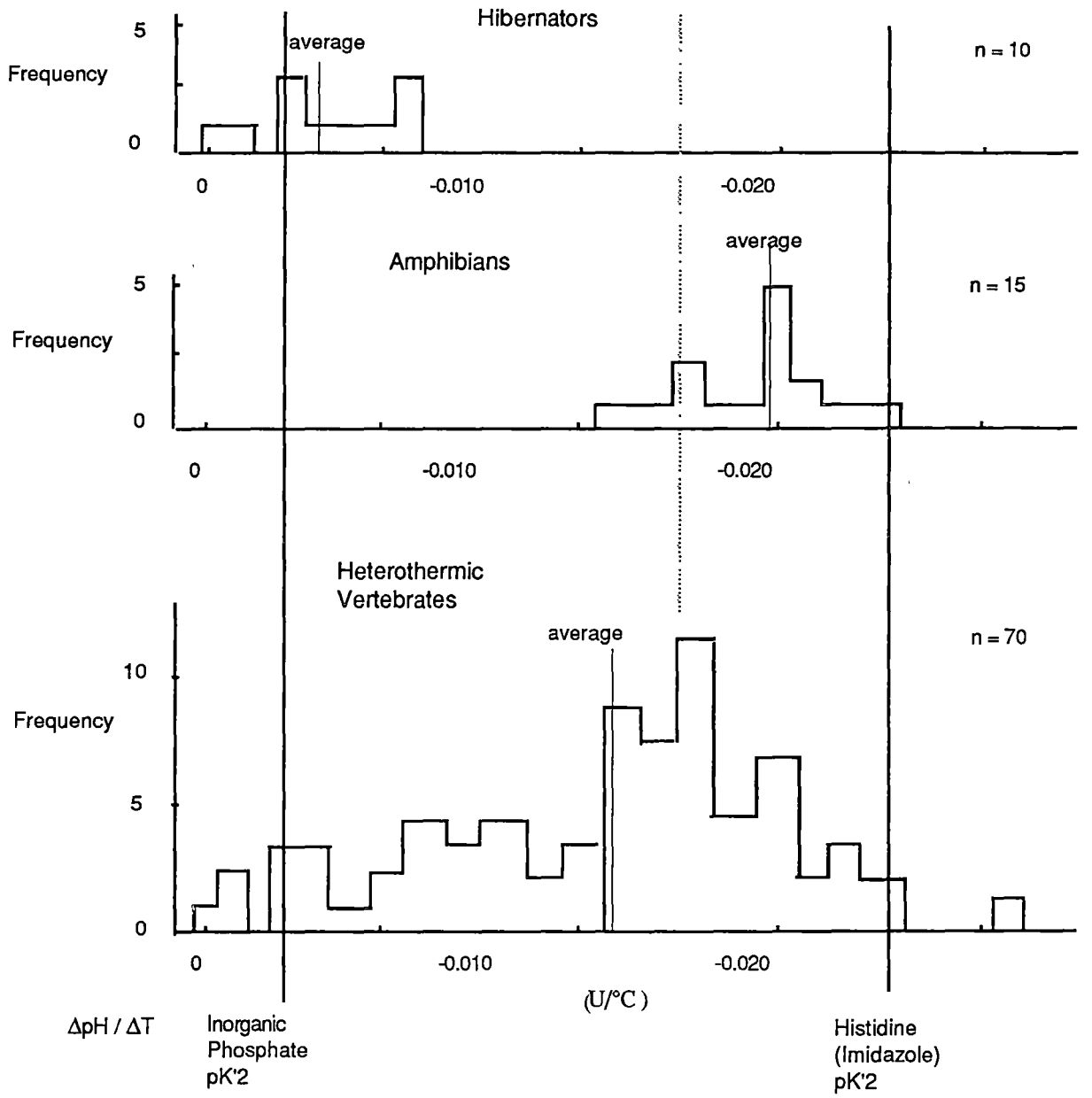

Fig 1. Histogram Adapted from Heisler (1986b) and Edsall and Wyman (1958). The changes in plasma pH with changes of body temperature ($\Delta pH / \Delta T$) for various classes of heterothermic animals, hibernators and amphibians. Also indicated are the $\Delta pK / \Delta T$ values for inorganic phosphate and the imidazole group of free histidine (temperature range 10-30 °C)

Obviously, evaluation of the acid-base behaviour with changes of temperature on the basis of the model of a semi-closed buffer system (a semi-closed buffer system is one, which is closed for ionic species, but open for CO_2) is therefore rather questionable without exact knowledge of the compartment non-bicarbonate buffering properties (Heisler, 1986b).

1.2 pH-stat

The term pH-stat has been used to describe the acid-base strategy of hibernators (Malan, 1982), which tend to provide "normothermic" pH and PCO_2 values as body temperature falls. A reduction in ventilation relative to metabolism leads to an increase in CO_2 store in blood and tissues but a relatively constant PCO_2 of about 40 mmHg and pH of about 7.4, and an increase in Donnan ratio and shift of water into cells (Malan et al, 1973; Malan, 1982; Reeves, 1972; Rodeau & Malan, 1979). It has been suggested that this "relative respiratory acidosis" functions to inhibit metabolism during entry into hibernation and maintain metabolic suppression during the course of hibernation (Malan et al, 1973; Snapp & Heller, 1981).

1.3 Induced hypothermia

In clinical practice, both the pH-stat and alpha-stat strategies are commonly used although there is still no clear answer about which of these commonly used acid-base strategies is the better option. A pH-stat strategy may be achieved by the addition of carbon dioxide to the gas flow to the oxygenator, breathing CO_2 or by hypoventilation. If successful, the strategy exactly follows the pH isopleth so that the PCO_2 remains about 40 mmHg irrespective of temperature (Howell & Rahn, 1976; Jackson, 1971). By contrast no CO_2 need be added to the oxygenator gas to achieve alpha-stat.

Whichever acid-base strategy is chosen it should allow an adequate hypothermic metabolism and limit the negative side effects of hypothermia, which are (Gravlee et al, 1993):

* Cellular swelling due to altered Donnan equilibrium of Cl and inhibition of Na-K-ATPase;

* Increased myocardial inotropic state and $M\dot{V}O_2$ per beat;

* Heart fibrillation;

*. Impaired vascular autoregulation;

*. Inhibition of sarcoplasmic reticulum sequestration of Ca^{2+} in myocardium.

*. Decreased rate of repair of tissue or organs.

Considerable evidence supports better enzymatic, cellular, and organ function with alpha-stat management during hypothermia (White, 1981). Swan (1984), Rahn (1974) and Rahn et al (1975) also indicated that, during alpha-stat hypothermia, cellular metabolism may remain more "normal" i.e., the oxygen consumption may be higher compared with pH-stat hypothermia. This is supported by a study in 33 patients who were undergoing coronary artery bypass grafting or valve replacement, or both, where a positive correlation ($r = 0.52$, $p < 0.01$) between arterial pH and systemic oxygen uptake during hypothermic CPB was demonstrated. This suggested that the systemic oxygen uptake is higher with an alpha-stat approach than with a pH-stat approach (Tuppurainen et al, 1989) On the other hand, the oxygen delivery may be increased during pH-stat hypothermia due to right displacement of the oxyhaemoglobin dissociation curve (ODC). P_{50} for humans is 14.30 and 11.28 mmHg with the pH-stat and alpha-stat approach at a body temperature of 25 °C, respectively (Gothgen et al, 1988). Swain et al (1990) compared the difference of haemodynamics and oxygen consumption between alpha-stat and pH-stat groups during hypothermia and found that neither systemic oxygen consumption nor the Q10 was different. They concluded that the alpha-stat pH scheme does not result in an oxygen consumption higher than that of the pH-stat scheme.

Table 1 shows the theoretical advantages and disadvantages of the pH-stat and alpha-stat strategies on cardiac, cerebral and other functions.

Table 1. Comparison of the effects of different acid-base strategies on cardiac, cerebral and other functions

Acid-base state		Advantages	Disadvantages
Alpha-stat			
	Left ventricular function	Greater blood flow and lactate consumption Higher preload peak (per mmHg) and greater contractility	Higher O_2 consumption
	Cerebral	Better preserved autoregulation and flow-metabolism coupling	Less efficient cooling (probably due to lower cerebral blood flow)
	Other	Optimal for enzyme activity, better cellular and organ function	Less reduction in O_2 consumption
pH-stat			
	Left ventricular function	reduced O_2 consumption	Lower blood flow Reduced lactate consumption Lower preload peak (per mmHg)
	Cerebral	Greater blood flow, better cooling	Loss of autoregulation, greater risk of microembolism
	Other	Greater reduction in O_2 consumption, reduced reperfusion injury, higher oxyhaemoglobin	Reduced cellular and orgar function

From Aoki et al (1994) and McConnell et al (1975)

So far many studies have suggested that the problem of hypothermic acid-base balance is considerably more complex than a simple choice between alpha-stat and pH-stat management (Heisler, 1986b; Hickey & Hansen, 1989).

Becker et al and Buckberg (1981 and 1985) postulated that a more alkaline acid-base state would be even more effective in preserving hypothermic circulation although it has not been observed in any naturally occurring physiological systems. This could be achieved by vigorous hyperventilation or infusion of alkali. Swan (1984) suggested that the most desirable acid-base status for myocardial preservation would probably be

respiratory alkalosis. A state of myocardial alkalosis prior to either aortic clamping or total circulatory arrest during reperfusion would help restore myocardial function by combating the accumulated myocardial acidosis due to the period of myocardial ischaemia. Furthermore, because it would not be harmful to the brain or any other organ system and would probably have a beneficial effect upon myocardium subjected to ischaemia, a significant degree of respiratory alkalosis might be the management of choice for the conduct of hypothermia during cardiac operations. In another report Swan (1985) again stressed that since acidosis is myotoxic itself, prevention of acidosis by deliberate alkalosis before circulatory arrest, treatment by buffered alkaline cardiopreservative perfusion, and instant restoration with pH 7.8 reperfusion, are the keys to preservation and restoration of myocardial function. However, few studies have been done on the haemodynamic and metabolic effects of an alkaline regime (Sinet et al, 1984; Hering et al, 1992) although various reports agree as to the influence of hypothermia itself on haemodynamics and metabolism.

1.4 The aim of the present study

This investigation aims to explore the effects of acid-base state on cardiac and cerebral function. The acid-base states in anaesthetised rabbits were adjusted by inducing both alkalosis and acidosis.

In this study a rabbit model for studying the effects of hypothermia was also established. This involved the use of fentanyl citrate (currently used in cardiac surgery), as the anaesthetic agent, and a muscle relaxant, vecuronium bromide, both of which have negligible cardio-vascular side effects. Neither fentanyl nor vecuronium has previously been used on hypothermic rabbits. In particular, there is little information about the use of vecuronium bromide on rabbits. Therefore, the effects of fentanyl and vecuronium have been investigated in this study as well.

Chapter 2

Acid-base chemistry and hypothermia

2.1 Introduction

Acid-base regulation in mammals refers to those chemical and physiological processes that maintain the hydrogen ion (H^+) concentration in body fluids at levels compatible with life and proper functioning, i.e., good health. Complete aerobic metabolism in mammals converts the constituent hydrogen, carbon and oxygen to water and carbon dioxide (CO_2). Adult humans prduce about 10-20 mol of CO_2 daily. This CO_2 may combine with water to form carbonic acid, which dissociates giving hydrogen ions. These hydrogen ions are removed from the body when the CO_2 is expired:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

In addition, our metabolic processes release a net amount of about 5×10^{-2} to 1 mol of hydrogen ion as non volatile acids daily into about 15 to 20 litres of extracellular fluid. Homeostatic mechanisms are so efficient that the normal extracellular hydrogen ion concentration is only about 4×10^{-5} mol/L (pH 7.4), and varies little despite changing loads.

Three mechanisms are involved in maintaining a fairly constant H^+ concentration (Masoro & Siegel, 1971a):

* The chemical buffer systems of the body fluids;
* The capacity of alveolar ventilation to eliminate CO_2 as quickly as it is formed, plus its ability to alter the rate of CO_2 elimination in a compensatory fashion relative to H^+ concentration of the body fluids;
* The capacity of the kidneys to restore buffer levels by generating or excreting bicarbonate.

2.2 pH and hydrogen ion concentration

Water is partially ionised into H^+ and OH^- ions. Ionisation of water is usually written as:

$$H_2O \underset{K_2}{\overset{K_1}{\Leftrightarrow}} H^+ + OH^- \qquad (2.2\text{-}1)$$

The rate constants can be combined into one equilibrium constant:

$$Keq = [H^+][OH^-]/[H_2O] \qquad (2.2\text{-}2)$$

Because the concentration of undissociated water is so large in comparison with the ions, it can be considered to be a constant itself, and combined with Keq to give a new constant Kw, and a simpler equation:

$$Kw = [H^+][OH^-] \qquad (2.2\text{-}3)$$

As pH is defined as the negative logarithm of the actual number of hydrogen ions (H^+) in this solution equation (2-2) can be shown in logarithmic form as:

$$pKw = \log [H^+] + \log [OH^-] \qquad (2.2\text{-}4)$$

Expressed in term of pH, the equation (2-3) can be rewritten as:

$$pKw = pH + pOH \qquad (2.2\text{-}5)$$

The dissociation constant of water, pKw, is a function of temperature. When water dissociates, its dissociation into OH^- and H^+ ions allows one to calculate the pH of chemical neutrality, i.e. pH must = pOH. Thus the neutral pH, pN, at any temperature is equal to 0.5 pKw. As water cools pKw increases Thus the neutral pH, pN, must also increase as temperature falls. Fig 2 shows pN changes from 6.8 at 37.5 °C to 7.4 at 3 °C.

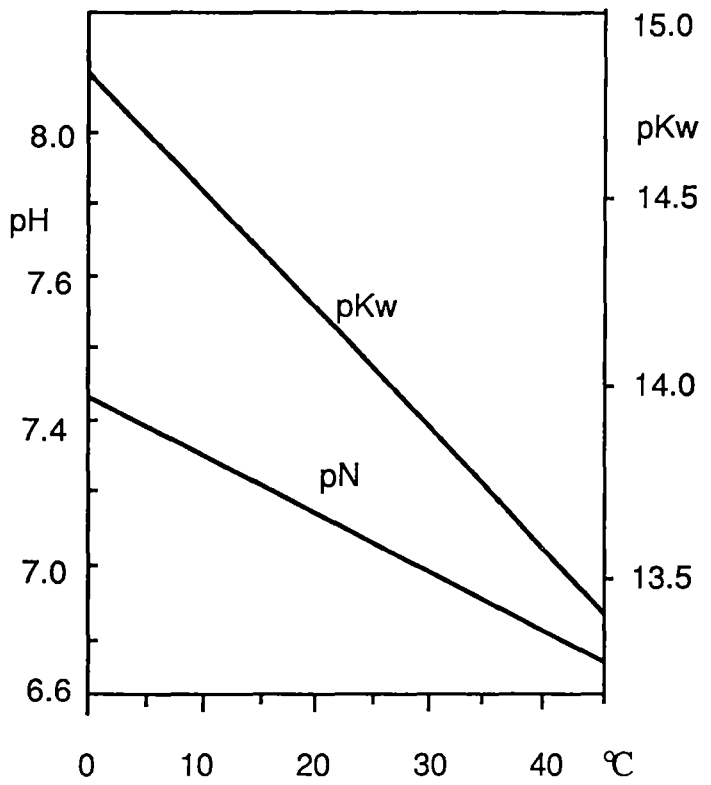

Fig 2. Changes in pKw and pN with temperature. Adapted from Rahn, 1967 and 1985

Because neutral pH changes with temperature, description of the acid-base state becomes complex in an animal whose temperature varies. When measured at body temperature the blood pH of ectotherms changes in manner similar to that of water. This has been described as maintaining "constant relative alkalinity" (Rahn, 1966). However in blood the major sources of H^+ ions are not water but the major buffers: bicarbonate, phosphate and imidazole.

2.3 Temperature correction of blood-gas and pH measurements

Assessing the acid-base status of an animal whose temperature varies becomes even more complex if pH and blood gases are measured at a temperature significantly different from body temperature. Rosenthal (1948) measured pH and blood gases in both human and laboratory animal blood. He described the change of the pH and blood gases as an aid to correct the measuring of blood pH *in vivo* when the glass electrode measuring system is not thermostated at body temperature. This practical observation soon led to a series of

empirical temperature correction factors for blood pH (Adamsons et al, 1964; Austin et al, 1964; Kelman & Nunn, 1966) and PCO_2 (Bradly et al, 1956; Nunn et al, 1965) for human blood warmed or cooled at constant carbon dioxide content.

In many clinical situations, blood gas analyzers allow the primary measurements to be automatically corrected to the patient's actual body temperature by use of various correction formulae. However, in these circumstances the blood in the syringe and measuring electrode is a closed system. The changes of blood gases and pH via temperature will be different in such a closed system to these in an open system (*in vivo*) i.e. to a patient.

2.3.1 Temperature-induced changes in pH

Many investigators correct blood pH versus temperature data by fitting a linear regression and characterizing the dependence by the slope of the regression line, i.e. values reported for $\Delta pH / \Delta T$ have ranged from -0.010 to -0.021 U /°C (Howell & Rahn, 1976). However calculating pH as a linear function of temperature *in vivo* analysis may be inappropriate. Calculated $\Delta pH / \Delta T$ values decrease significantly as temperature increases. If acid-base regulation *in vivo* is based on regulation of alpha imidazole values, a single $\Delta pH / \Delta T$ will neither describe the regulation observed nor assist in comparing one system with another (Reeves, 1977).

For example, the "anaerobic" change of blood pH with respect to temperature is a change that occurs within a closed system and constant pressure. By definition, a closed system permits no mass exchange with the environment. Energy exchange and chemical reactions alone determine the physical state of the system. The temperature, therefore, can vary. Under these closed-system conditions, the pH increases when blood is cooled, and dpH /dT for such a system has been measured and predicted by many investigators. For simplicity, consider the changes taking place in a closed system consisting of an ideal solution of a freely dissociable compound AH. At any given temperature, there is an equilibrium between AH, A^-, and H^+, which is defined by a dissociation constant, K:

$$AH = A^- + H^+ \qquad (2.3.1\text{-}1)$$

$$K = [A^-]\,[H^+]\,/[AH] \qquad (2.3.1\text{-}2)$$

K is a function of temperature, and for ideal substances in a solution its change is predicted by the Gibbs-Helmholtz's equation (Castellan, 1971):

$$dpK/dT = -\Delta H^\circ/(2.303\,RT^2) \qquad (2.3.1\text{-}3)$$

Where ΔH° is the heat of reaction at 25 °C, R is the ideal gas constant, and T is the absolute temperature. If the reaction is exothermic, then ΔH° is negative and K will decrease with increasing temperature. If the reaction is endothermic, K will increase with increasing temperature. If we assume that the total concentration of AH and A^- is much greater than that of H^+, then any change in pK will be reflected as a change in pH only:

$$dpH/dT \cong dpK/dT \qquad (2.3.1\text{-}4)$$

Much work has been done in measuring dpH /dT for whole human blood or plasma. The values vary from -0.007 to -0.01915 /°C (Rosenthal, 1948; Austin et al, 1964. Adamsons et al, 1964; Greenburg & Moulder, 1965; Patterson & Sondheimer, 1966; Reeves, 1976; Castaing & Pocidalo, 1979). Most of these investigators measured pH at two temperatures and then calculated the temperature effect from:

$$dpH/dT = \Delta pH/\Delta T \qquad (2.3.1\text{-}5)$$

However this method assumes that the function is linear with respect to temperature. Several investigations (Greenburg & Moulder, 1965; Reeves, 1976; Castaing & Pocidalo, 1979) reporting pH measurements at multiple temperatures showed that dpH /dT is not linearly related to temperature. Brewin et al (1955) suggested that mathematically correcting pH for temperature was not appropriate, because the observed changes are taking place in an open system. They recommended measuring pH at the body temperature of the patient by adjusting the temperature of the electrode. This method

method corrects for all of the confounding variables but requires that clinical laboratories have a stable, calibrated pH electrode at the patient's temperature.

2.3.2 Temperature-induced changes in PCO_2

The *in vitro* change of PCO_2 with respect to temperature is a function of the pK of the various blood buffers and the solubility of carbon dioxide. At any temperature, PCO_2 can be determined from the Henderson-Hasselbalch equation:

$$pH = pK_1' + \log ([HCO_3^-] / \alpha CO_2 PCO_2) \qquad (2.3.2\text{-}1)$$

Where pK_1' is the negative logarithm of the apparent first dissociation constant of carbonic acid and α (in mmol /L per mmHg) is the solubility of carbon dioxide in plasma, which is also called the solubility coefficient.

The derivative of this equation with respect to temperature is (Burnett & Noonan, 1974):

$$d\log(PCO_2)/dT = (dpK_1/dT) - (dpH/dT) + (d\log[HCO_3^-]/dT) - [d\log(\alpha CO_2)/dT] \qquad (2.3.2\text{-}2)$$

Therefore, $d\log(PCO_2)/dT$ for a closed system can be calculated from knowledge of the closed-system derivatives.

Bradley et al (1956) calculated this change using $dpH/dT = -0.0147$ /°C (Rosenthal, 1948) and $dpK_1'/dT = -0.005$ /°C (Severinghaus et al, 1956a). They assumed the temperature dependence of the solubility factor, αCO_2, for blood paralleled the corresponding changes seen for water (Severinghaus et al, 1956b) and used -0.00964 /°C for the slope of $\log (\alpha CO_2)$ vs temperature. Using these values and the assumption that total CO_2 was not a function of temperature, they calculated:

$$d\log(PCO_2)/dT = 0.0185 \qquad (2.3.2\text{-}3)$$

Therefore the effect of the bicarbonate term is -0.00084 /°C. They measured dlog(PCO_2) /dT for 16 whole-blood samples. The mean value determined was 0.013 /°C (SD, 0.028).

Since then more expressions have been reported. Siggaard-Andersen (1963) calculated dlog(PCO_2) /dT to be 0.021 /°C and reported that the value varies with plasma protein concentration, haemoglobin concentration, and PO_2.

Brewin et al (1955) and Astrup et al (1963) recommended plotting a carbon dioxide titration curve for each sample. They measured the initial pH. The patient's blood pH at body temperature was determined by either measuring at body temperature or measuring at 37 °C and correcting mathematically to body temperature. Then they tonometered the patient's blood to two different tensions of CO_2 and measured the pH at each. Their studies demonstrated that the titration curve was independent of temperature. They suggested that the PCO_2 at body temperature should be estimated by using a plot of log(PCO_2) vs pH.

2.3.3 Temperature-induced changes in PO_2

The PO_2 of whole blood is a function of oxygen solubility and haemoglobin affinity for oxygen, both of which are temperature dependent. If red blood cell metabolism is prevented, dPO_2 /dT would be a function of oxygen solubility only as haemoglobin becomes fully saturated. The affinity of haemoglobin for oxygen at 0 °C is 22-times that at 37 °C, but the solubility of oxygen (in water) is only doubled. As blood is cooled the solubility of oxygen increases (with decreasing temperature), the PO_2 would decline. The lower the temperature the more readily haemoglobin binds oxygen, which causes the PO_2 to further decrease. This shift of dissolved oxygen to bound oxygen is small compared with the amount of oxygen previously bound. Therefore the degree of saturation does not change significantly.

Bohr (Hedley-White et al, 1965) measured the solubility of hydrogen in water at 15 and 38 °C and in human blood at 38 °C. The value for blood was 92% of the value for water. He assumed that the solubility of oxygen in blood was proportional to that of hydrogen.

From these assumptions he calculated the solubility of oxygen in blood at 15 and 38 °C. The solubility of oxygen at a given temperature is α_t. From these solubility measurements, it is possible to calculate $d\log(PO_2)/dT$ for fully saturated whole blood:

$$d\log(PO_2)/dT = \log(\alpha_{t_1}/\alpha_{t_2})/(t_2 - t_1) \qquad (2.3.3\text{-}1)$$

Many investigators have verified Bohr's calculations for whole blood. The theoretical temperature corrections of Severinghaus (1966; 1979) and the experimental correction of Kelman and Nunn (1966) have been recommended by several investigators (Burnett & Noonan, 1974. Andritsch et al, 1981; Porter, 1979; Burnett, 1978). These theoretical equations are based upon the assumption that the change in degree of saturation is with change of temperature at a given pH (dsat /dpHT). Reeves (1978) presented data that contradicted this assumption. Data experimentally verifying the above formulae at less than full saturation are also found in some papers (Nunn et al, 1965; Marshall & Gunning, 1962; Thomas, 1972).

2.4 Clinical assessment of acid-base status during hypothermia

2.4.1 Measurement of hypothermic blood gases and pH

In clinical situations it is not practical to vary the temperature of the blood gas analyser so that it corresponds to the patients temperature. Usually, the instrument allows the primary measurements to be automatically corrected to the patient's actual body temperature, by use of various correction formulae given by different types of blood gas analysers (Table 2).

2.4.2 Interpretation of blood gas and pH measurements during hypothermia

It is important to understand that: a) the results of blood gas and pH obtained from a modern blood gas analyser are automatically "corrected" results at a standard measuring temperature of 37 °C; b) depending upon which acid-base strategy being used, interpretation of an "ideal" acid-base state could be very different between the applications of pH-stat and alpha-stat strategies. With application of pH-stat during hypothermia, the

temperature-corrected results will show a pH lower than 7.4, PCO_2 higher than 40 mmHg (White, 1981; White & Somero, 1982). By comparison, with alpha-stat, the temperature-corrected result should be around a pH of 7.4 and a PCO_2 of 40 mmHg (Reeves, 1972).

Table 2. Temperature-correction formulae used by some blood gas analysers

(Summarised from the relevant analyser manuals)

Analyser		Formulae used
Radiometer ABL-3	**pH**	$pH = pHm + [0.0146 + 0.0065\ (7.4 - pHm)]\ (t - 37)$
	PCO_2	$PCO2 = PCO2\ m\ 10^{0.021\ (t-37)}$
	PO_2	$PO2 = PO2m\ 10^{[(0.0252/(0.0243\ (PO2/100)^{3.88})+1)+0.00564]\ (t-37)}$
Corning 178, 170,168 & 158	**pH**	$pH = pHm - 0.015\ (t - 37)$
	PCO_2	$PCO2 = PCO2\ m\ 10^{0.019\ (t-37)}$
	PO_2	$PO2 = PO2m\ 10^{[(0.0052+0.27\ (1-10^{-0.13(100-Sat)}))]\ (t-37)}$
Instrumentation 1301 &1303	**pH**	$pH = pHm + [(-0.0147 + 0.0065\ (7.4-pHm)]\ (37 - t)$
	PCO_2	$PCO2 = PCO2\ m\ 10^{0.019\ (37-t)}$
	PO_2	$PO2 = PO2m\ 10^{[(5.49 \times 10^{-11}\ PO2^{3.88})+0.071)\ /\ (9.72\times10^{-9}\ PO2^{3.88})+2.3)}$
Instrumentation 813	**pH**	$pH = pHm - 0.0146\ (t - 37)$
	PCO_2	$PCO2 = PCO2\ m\ 10^{0.019\ (t-37)}$
	PO_2	Sat>=95% $PO2 = PO2m\ 10^{0.031\ (t-37)}$ Sat>=95% $PO2 = PO2m\ (10^{0.032 - 0.0268\ e^{(0.3\ Sat - 30)}})\ (t-37)$

Chapter 3

Material and Methods

All procedures were in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and approved by the University Ethics Committee (Animal Experimentation) of the University of Tasmania.

3.1 Animals

New Zealand rabbits of both sexes weighing 1.8-3.9 kg were used in this study. The animals were obtained from the animal house of the university.

3.2 Methods

3.2.1 Surgical preparation and recording techniques

Animals were sedated with an intramuscular injection of Innovar-vet (Fentanyl and Droperidol, 0.4 mg and 20 $mg \cdot ml^{-1}$ respectively, Smith Kline Animal Health products) at a dose of 0.3 $ml \cdot kg^{-1}$. After the induction of anaesthesia, the trachea was intubated. Animals were ventilated with 35% O_2 and 65% N_2 under positive pressure ventilation (10 cm H_2O, 10 $breaths \cdot min^{-1}$) with a SRI small animal respirator, and infused with fentanyl citrate (0.5 $mg \cdot 10\ ml^{-1}$, Astra Pharmaceutical Pty Ltd North Ryde N S W, Australia) at a dose of 4.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during the operation and 2.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ after the surgical procedure. Muscle relaxation was maintained by infusion of vecuronium bromide at a dose of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ after a bolus injection at a dose of 100 $\mu g \cdot kg^{-1}$.

The animals' temperatures (Tb) were monitored throughout the study by a thermocouple in the oesophagus. Normal Tb was maintained with two heating lamps under the operating table.

An electromagnetic flow transducer (International Biomedical, INC, Texas, USA) of 1 mm diameter was placed on the carotid trunk to measure the carotid arterial blood flow (CrdBF). Catheters were inserted into the aorta and vena cava through the femoral artery and vein respectively. Pressure transducers on the catheters were connected to a chart

recorder (Grass 7D Polygraph, Grass Instrument Co, USA), allowing monitoring of mean aortic blood pressure (MAP), heart rate (HR) and central venous pressure (CVP). Recordings were made every 30 minutes. Blood samples could also be withdrawn from the catheters for pH, PO_2, PCO_2 and oxygen content (CtO_2) analysis. Oxygen delivery (DO_2), oxygen consumption ($\dot{V}O_2$), oxygen extraction (ER) and $AVDO_2$ (oxygen content difference between arterial and venous blood = $CtaO_2$ -$CtvO_2$) were calculated from CtO_2 and CrdBF (DO_2 = CrdBF × $CtaO_2$; $\dot{V}O_2$ = CrdBF × $AVDO_2$; ER = $\dot{V}O_2/DO_2$). A butterfly needle was fixed in the caudal ear vein of the rabbit for injection and infusion of anaesthetics, muscle relaxant or other agents with an infusion pump (Model 255-1, SAGE Instruments, New York).

Three EEG electrodes were anchored into the skull and over the pia mater with acrylic dental cement. The electrode on the frontal site was earthed and connected individually with each of the remaining two electrodes on parietal sites, which formed a bipolar system. Cerebral electrical activity was monitored throughout the experimental period. Recording paper speed was 25 mm·second^{-1} and the sensitivity of the recorder set so that a calibration signal of 50 μv produced a 6 mm deflection (Sadove et al, 1967).

The bipolar standard lead II was used to record the ECG (7P4G, Grass 7D Polygraph, Grass Instrument Co, USA). Recording paper speed was 25 mm·second^{-1} (Goldman, 1986b) and a calibration signal of 0.33 mv gave a 10 mm deflection.

3.2.2 Experimental groups

The animals were divided into four groups: One normothermic group (Nom, n = 6) and three hypothermic groups (H, n = 31):

Normothermic group (n = 6) Body temperature was maintained at normal levels (38 ± 1°C) over an experimental period of 6 hours.

Hypothermic groups (n = 31) Animals were cooled to a body temperature of 25 ± 1°C and then exposed to hypothermia for 1 hour. Three hypothermic groups were: hypothermic control, hypothermic alkaline and hypothermic acidosis groups.

Hypothermic control group (HCo n = 9, pH = 7.4 ± 0.5). The control group for hypothermic alkaline and acidosis groups.

Hypothermic akaline group (HAl, n = 12, pH > 7.45). Animals were infused with carbicarb (0.33 M Na_2CO_3 and 0.33 M $NaHCO_3$) at a dose of 2 ml·kg^{-1} before cooling started.

Hypothermic acidosis group (HAc, n = 10, pH < 7.35). Animals were infused with ammonium chloride (2M NH_4Cl) at a dose of 2.5 ml·kg^{-1} (5-10 mmol·kg^{-1}) before cooling started.

Animals in the hypothermic group were studied through five distinct phases:

* phase 1: control — the normothermic period before cooling (Tb = 38 ± 0.5°C);

* phase 2: cooling — over the period of reducing body temperature;

* phase 3: steady hypothermia — body temperature was maintained at 25°C±1 for one hour;

* phase 4: rewarming — over the period of increasing body temperature;

* phase 5: after rewarming — when the body temperature had returned to 38°C±0.5.

Four main aspects of the physiology were studied:

* haemodynamics and myocardial function: MAP, CrdBF, HR, CVP, ECG

* acid-base status and blood gases: Arterial and venous pH, PO_2, PCO_2

* cerebral electrical activities: EEG

* oxygen consumption and delivery: CtO_2, DO_2, $\dot{V}O_2$, ER

3.2.3 Hypothermic protocol

Two small incisions were made in the lateral ventral region, and a 5 mm diameter needle guided intraperitoneally through to the opposite side of the peritoneal wall where the needle was pushed through the wall to the outside. A silastic tube was threaded through the needle, and the needle was then withdrawn so that the tip was in the peritoneal cavity again. Four metres of tubing were then pushed into the peritoneal cavity to form a heat exchange system.

The animal was cooled to 25 ± 1°C by pumping the ice water through the silastic coil system. During rewarming body temperature was warmed to 38 ± 0.5°C by pumping warm water through the silastic coil heat exchanger.

3.2.4 Analysis of blood gases

A total of 5 blood samples were taken during each experiment. Sample 1 was taken before any treatment during normothermia. Sample 2 was taken after the acid and alkaline treatment, during the precooling, normothermic control period. Samples 3 and 4 were taken at the beginning and end of hypothermia, respectively. Sample 5 was taken when Tb had returned to normal. Blood samples taken at the normal body temperature (38 ± 0.5°C) were analysed for PO_2, PCO_2 and pH on a Corning pH / Blood blood gas analyser (165/2, Corning Scientific Instruments Medifield, Massachusetts USA) calibrated and held at 38 ± 0.5°C. Blood samples obtained at low body temperature (25 ± 1°C, samples 3 and 4) were analysed for PO_2, PCO_2 and pH using a Radiometer BMS3 blood gas analyser (Radiometer Copenhagen) set to the actual body temperature, and calibrated at that temperature.

The Corning Medical 165/2 pH blood gas analyser can only be operated over a narrow range of sample temperatures (37-38 °C). The pH electrode was calibrated with phosphate buffer solutions, the pH of which were 6.838 and 7.382 at 38 °C (Corning

Medical 165/2 pH Blood gas analyser instruction manual). Oxygen and CO_2 electrodes were calibrated with precision gas mixtures. The actual partial pressure of the calibrating gases depends on the ambient barometric pressure, the temperature of the humidifiers (37 °C in the 165/2), and the percent composition of the gas in the tank. Corning gases were used Calibration point of gas electrodes was determined from the following formula (Corning Medical 165/2 pH Blood gas analyser instruction manual):

Calibration value = (Gas mole percentage) × (Barometric pressure - Water vapour pressure)

Sample 1,2 and 5 taken during normothermia with Tb ranged from 36 — 39 °C were measured on Corning at 38 °C without any temperature correcting because the difference between the actual body temperature and the sample chamber temperature was always less than ± 2 °C.

With BMS3 blood gas analyser, the temperature of the sample chamber was set at 25°C. Precision buffer solution types S1500 and S1510 were used for calibration of the pH electrode. At 25 °C these buffers have pH values of 6.865 and 7.410, respectively. Gas electrodes were calibrated with precision gas mixtures and determined from the same formula shown above.

The correlation coefficients of whole blood pH, PCO_2 and PO_2 measurements between Corning 165/2 and BMS3 are 0.960, 0.993 and 0.999 respectively (Corning Medical 165/2 pH Blood gas analyser instruction manual). This indicates that it is possible to compare the measured results from BMS3 and Corning 165/2 at a measured temperature of 37 °C.

3.2.5 Oxygen content (CtO_2)

CtO_2 of arterial and venous blood was measured using an OxyCon oxygen content analyser (Physiology Department, University of Tasmania, Australia) during the experimental period. DO_2, $\dot{V}O_2$ and ER were calculated (see Chapter 10).

All values are reported as mean ± SD (standard deviation) of the mean and analysed by means of Multiple-factor ANOVA (*Excel version 4.0 and Statistica*). A P value less than 0.05 was considered significant.

Chapter 4

Validation of fentanyl-vecuronium anaesthesia in rabbits

4.1 Introduction

Sedation, tranquillisation, pharmacological restraint, or general anaesthesia are frequently required in research animals. It is important that the anaesthetic regime is appropriate to the type of research procedure and animals to be used. For instance, cardiovascular physiological studies in anaesthetised animals may be confounded by the haemodynamic actions of the anaesthetic agents themselves. The rabbit is one of the most commonly used animals in biomedical research, but it is generally considered to be the most difficult research animal to anaesthetise safely (Walden, 1990; Sadgwick, 1986; Kaplan, 1979; Wesbroth, 1979).

Anaesthesia of rabbits presents three major problems:

(a) The respiratory centre of the rabbit is very sensitive to the depressant action of anaesthetics.

(b) The range between anaesthetic and lethal doses is extremely narrow.

(c) The variability between rabbits of susceptibility to the depressant action of conventional anaesthetics is so great that doses for surgical anaesthesia virtually have to be individualised for each animal (Walden, 1990; Sadgwick, 1986)

The aim of this part of the investigation was to establish whether the rabbit, anaesthetised with fentanyl-vecuronium, provides a useful model for cardiovascular experiment over at least 6 hours. The reasons for using this combination of anaesthetic/muscle relaxant were:

(a) Fentanyl citrate is a safer choice of all anaesthetics in cardiovascular research on humans and probably it is so on rabbits (Guerreiro & Page, 1987) although there is little experimental information on rabbits;

(b) Of the commonly used muscle relaxants vecuronium bromide has little or no cardiovascular effect (Larach et al, 1991; Paulissian et al, 1991; Chen et al 1991) in human beings. However, no reports of its effect on rabbits were found.

4.2 Results

4.2.1 General aspects

After being given a 0.3 $ml \cdot kg^{-1}$ intramuscular injection of Innovar-vet, animals (n = 6) showed a light narcotic state. Both corneal and palpebral reflexes still existed. The respiratory pattern became slow and shallow. After a subcutaneous injection of local anaesthetic (2% Procaine) animals were tracheotomized and ventilated artificially. During the infusion of fentanyl citrate at a dose of 4.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and vecuronium bromide at a dose of 8 $\mu g \cdot kg^{-1} \cdot min^{-1}$ animals achieved a stable level of anaesthesia. Both corneal and palpebral reflexes disappeared. No spontaneous movement such as paradoxical respiration was observed. EEG was 5—10 $waves \cdot second^{-1}$ and of constant amplitude. Body temperature (Tb) ranged from 36.5 to 39.5°C throughout the experiment (Fig 4).

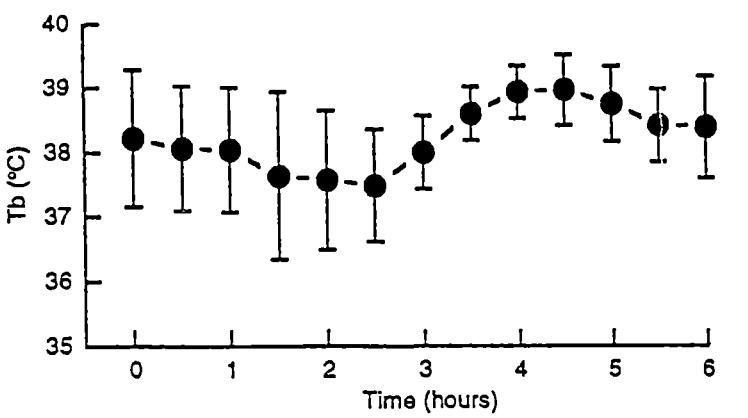

Fig 4. Changes of body temperature of adult rabbits during normothermia under fentanyl-vecuronium anaesthesia (Mean ± SD, n=6)

4.2.2 Cardiovascular response and blood gas changes under fentanyl-vecuronium anaesthesia

As is shown in Table 4.1, heart rate (HR), mean aortic pressure (MAP), carotid blood flow (CrdBF) and central venous pressure (CVP) remained constant and within normal ranges. PO_2, PCO_2 and pH remained within the normal range. There were no significant differences in HR, MAP, CrdBF, CVP, PO_2, PCO_2, pH among the three blood samples ($P>0.05$).

Table 4.1 Cardiovascular and blood gas changes during 6 hours of fentanyl-vecuronium anaesthesia in normothermic rabbits (Mean ± SD, n=6)

	Cardiovascular functions				Blood gases		
Time (hours)	HR (beats·min^{-1})	MAP (mmHg)	CrdBF (ml·min^{-1})	CVP (mmHg)	PaO_2/ PvO_2 (mmHg)	$PaCO_2$/ $PvCO_2$ (mmHg)	pHa/ pHv
1	264±21	100±7	46±20	2.3±1.0	110±25/ 50 ± 6	39± 2/ 47± 2	7.40±0.04/ 7.34±0.04
3	271±19	93±9	39±12	1.9±1.0	94± 29/ 34 ± 10	42± 6/ 56± 4	7.38±0.04/ 7.41±0.20
6	273±20	95±8	35±12	1.5±0.8	114± 60/ 35 ± 8	41± 3/ 50±5	7.39±0.05/ 7.34±0.05

4.3 Discussion and conclusion

In this study fentanyl citrate was used as the anaesthetic agent, rather than a number of other agents such as halothane, urethane, barbiturates and ketamine, all of which have been more frequently used for rabbits. The reasons for choosing fentanyl are outlined in the brief discussion of anaesthetic agents which follows.

4.3.1 Anaesthetics commonly used for small animals

4.3.1.1 Halothane (Fluothane, 2-bromo-2-chloro-1,1,1-trifluoro-ethane)

Halothane alone or in combination with nitrous oxide is administered to produce the required depth or plane of anaesthesia. Maintenance rate is usually about 0.5-1% halothane with an oxygen flow rate of 1 litre /minute (Stoelting, 1991). Administration of

halothane is characterised by a dose-dependent reduction of arterial blood pressure. Hypotension results from two main effects. First, the myocardium is depressed directly and cardiac output is decreased; second, the normal baroreceptor-mediated tachycardia in response to hypotension is blunted (Marshall, 1990).

4.3.1.2 Urethane (ethyl carbamate, ethyl ester of carbamic acid).

Urethane produces prolonged basal narcosis in dogs, cats and rabbits after i.v. injection at 1.0g-1.75g/kg. Except in rabbits, cardiopulmonary effects are very minimal (Walden, 1990). It can adversely affect blood and vessels in rabbits. The superficial vessels can become greatly dilated and haemolysis of blood may occur up to a few hours after injection. Blood clotting time greatly increases for a few hours (Bree, 1965). A temperature-depressing effect of urethane anaesthetic was reported by O'Reilly and Zak (1992).

Urethane is unsuitable when recovery from anaesthesia is anticipated. Some evidence showed that urethane becomes mutagenic and carcinogenic, with a high incidence of lung tumours occurring in various animal species regardless of the route of administration. There are substantial risks to personnel as a result of exposure to urethane during laboratory use. Because of these risks and the availability of alternative anaesthetics, the use of urethane is not recommended (Walden, 1990).

4.3.1.3 Barbiturates

Barbiturates are used intravenously to produce or supplement hypnosis during anaesthesia. Barbiturates are highly lipid-soluble molecules and follow many of the distribution characteristics of the inhaled anaesthetics. Barbiturates are hypnotics only and do not possess analgesic or muscle relaxant activities except with gross overdose. Pentobarbital sodium (Nembutal) is one of the commonly used barbiturates, and has been used extensively in research into animal anaesthesia in a great variety of species. However, it alone does not provide very substantial analgesia, except at high doses that cause significant respiratory depression. Induction and particularly recovery from

pentobarbital anaesthesia can be unstable (Kaplan, 1979). Pentobarbital can cause cardiac dysfunction (Hughes, 1981). Lang et al (1992) investigated the effects of pentobarbital, fentanyl and morphine chloralose on myocardial mechanics by assessing the relation between the end-systolic wall stress, which is a measure of ventricular systolic fibre force or afterload and the rate-corrected mean velocity of fibre shortening. This relation has been previously shown to be a sensitive measure of contractility (Borow, 1988). Lang et al (1992) observed that pentobarbital caused a significant reduction in the afterload-corrected mean velocity of fibre shortening, indicating a decrease in left ventricular contractility. They concluded that pentobarbital was a potent negative inotropic agent, which profoundly depressed systolic performance. Kaplan (1979) showed there is a narrow margin of safety between the anaesthetic dose and the lethal dose of pentobarbital in rabbits. He indicated that pentobarbital is a poor analgesic for rabbits. Only a light surgical plane of anaesthesia should be expected with this agent at safe dosage levels. Anaesthesia usually will persist for less than 20 minutes and repeated injections of pentobarbital in rabbits is associated with an unacceptably high mortality.

4.3.1.4 Ketamine

Ketamine hydrochloride [2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone]

Ketamine is a non-barbiturate general anaesthetic for intravenous or intramuscular use. As well as producing general anaesthesia, it produces profound somatic analgesia, enhanced muscle tone, cardiovascular stimulation and occasionally mild respiratory depression. High dosages of ketamine (44 mg /kg i.m.) provide good restraint but very little analgesia in rabbits. Muscle rigidity rather than relaxation occurs. Some seizure activity may also occur when ketamine is administered without sedatives (Charles, 1986). The haemodynamic effects of ketamine were studied for the anaesthetic induction of 20 patients with cardiomyopathies undergoing cardiac transplantation by Gutzzke and coworkers (1989). They observed that ketamine caused progressive increases in mean arterial pressure (28%), mean pulmonary artery pressure (56%), central venous pressure (109%), and pulmonary capillary wedge pressure (84%) over time, whereas the cardiac

pentobarbital anaesthesia can be unstable (Kaplan, 1979). Pentobarbital can cause cardiac dysfunction (Hughes, 1981). Lang et al (1992) investigated the effects of pentobarbital, fentanyl and morphine chloralose on myocardial mechanics by assessing the relation between the end-systolic wall stress, which is a measure of ventricular systolic fibre force or afterload and the rate-corrected mean velocity of fibre shortening. This relation has been previously shown to be a sensitive measure of contractility (Borow, 1988). Lang et al (1992) observed that pentobarbital caused a significant reduction in the afterload-corrected mean velocity of fibre shortening, indicating a decrease in left ventricular contractility. They concluded that pentobarbital was a potent negative inotropic agent, which profoundly depressed systolic performance. Kaplan (1979) showed there is a narrow margin of safety between the anaesthetic dose and the lethal dose of pentobarbital in rabbits. He indicated that pentobarbital is a poor analgesic for rabbits. Only a light surgical plane of anaesthesia should be expected with this agent at safe dosage levels. Anaesthesia usually will persist for less than 20 minutes and repeated injections of pentobarbital in rabbits is associated with an unacceptably high mortality.

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index, stroke volume index, and stroke work index remained unchanged or decreased. Plasma noradrenaline significantly increased (31%) in the ketamine group as well. They suggested that ketamine may not be appropriate for a patient with a cardiomyopathy undergoing noncardiac surgery. Lehot et al (1992) observed 26 adults undergoing elective cardiac surgery and reported the systemic vascular resistances was 27% lower in the ketamine group than in the control group. The blood reservoir level was 37% higher in the ketamine group than in the control group, suggesting a decreased venous capacitance. They concluded that ketamine leads to venous constriction, and probably arterial dilation.

4.3.2. Fentanyl Citrate

Fentanyl belongs to the opioid family. It is a synthetic compound with action that mimics morphine but is even more potent. It is a synthetic opioid related to the phenylpiperidines. As an analgesic, it is estimated to be 80 times as potent as morphine. There are few reports of its application in rabbits. Its respiratory depressant effect is of shorter duration than that of meperidine; its analgesic and euphoric effects are antagonised by opioid antagonists, but are not significantly prolonged or intensified by droperidol, a neuroleptic agent with which it is usually combined for use. The major adverse reactions associated with fentanyl are respiratory depression, apnoea and muscle rigidity (Becker et al, 1976; Badewit, 1991; Stoeckel et al, 1979; Marshall, 1990).

Fentanyl shows little evidence of negative cardiovascular effects in humans and other species (Stanley, 1992; Lang et al, 1992). However, its effects in ventilated rabbits have not been reported.

The results shown in Table 4.1 in the present study indicated that fentanyl did not cause significant changes to cardiovascular functions. Under fentanyl-vecuronium anaesthesia heart rate ranged from 264 to 273 beats per minute and mean aortic pressure ranged from 93 to 100 mmHg ($P>0.05$). HR and MAP were stable and within normal ranges compared to the data from Harris (1994), who measured cardiovascular parameters in

normal New Zealand rabbits. He reported that the heart rate of a spontaneous breathing rabbit is 150-300 beats per minute; arterial blood pressure, systolic is 90-130 mmHg and diastolic is 60-90 mmHg, mean arterial blood pressure is 90-120 mmHg. There is little information of CVP and carotid blood flow on rabbits. My results show that central venous pressure and carotid blood flow were stable at 1.5-2.3 mmHg and 35-46 ml·min^{-1}, respectively.

Previous investigations showed that fentanyl has positive influences on the cardiovascular system in human and other species although its mechanisms have not yet been clarified. Presumably it may act similarly in rabbits. The possible effects could be:

(a) Enhancement of myocardial contractility — In experiment with dogs, fentanyl showed no effect on heart rate but a positive inotropic effect on myocardial contractility ($P<0.05$). It also tended to increase afterload with the net result that overall systolic performance remained unchanged during three hours (Lang et al, 1992). Other studies have demonstrated a relatively stable haemodynamic profile in dogs during fentanyl anaesthesia without detectable changes in myocardial contractility (Freye, 1974; Ostheimer et al, 1975; Stanley & Webster, 1978)

(b) Improvement of myocardial circulation — There are few data available on the effects of fentanyl on coronary blood flow, myocardial oxygen balance, and regional distribution of blood flow. Hirsch et al (1993) investigated the effects of fentanyl on coronary blood flow distribution and myocardial oxygen consumption in dogs and found that fentanyl at a dose of 50 μg·kg^{-1} resulted in a heart rate decrease of 30% at 5 minutes after administration, and 29% at 20 minutes. Mean arterial pressure fell by 20% and 22% at 5 minutes and 20 minutes respectively. Myocardial oxygen consumption and regional coronary blood flow decreased significantly at 20 minutes post-fentanyl.

(c) Stabilization of haemodynamics — Changes in cardiovascular dynamics of patients with induction doses ranging from 8 to 30 μg·kg^{-1} consisted of small decreases in heart rate and arterial blood pressure. All other cardiovascular variables studied, including

cardiac output, remained unchanged, even with additional doses up to 100 $\mu g \cdot kg^{-1}$ (Stanley, 1992). Guerreiro and Page (1987) observed the effects of fentanyl and droperidol on rabbits in which ventilation was unassisted. They took hourly measurements of blood gases, lung mechanics, mean arterial blood pressure and heart rate, and showed that the animals breathed spontaneously and their cardiovascular and respiratory systems were stable throughout the monitoring period. The results from Fujita and his colleagues (1992), who measured the cardiovascular effect of anaesthetic doses of fentanyl in 25 patients for open heart surgery, showed that the administration of fentanyl intravenously at a dose of $30\mu g \cdot kg^{-1}$ plus an additional fentanyl infusion at a rate of 100 to 200 μg /hr did not have a significant effect on heart rate, systolic blood pressure, diastolic blood pressure and rate pressure product. Fentanyl at a dose of 75 $\mu g \cdot kg^{-1}$ was able to suppress the epinephrine level that increased during cardiopulmonary bypass. This dose of fentanyl caused small decreases in heart rate and arterial blood pressure.

(d) Lowered effectiveness of cardiac automaticity and conduction — Fentanyl at a dose of 100 mg /kg produced a statistically significant prolongation of cycle length, sinoatrial conduction time, antegrade block point and antegrade effective refractory periods of the A-V node and ventricles ($P<0.01$) (Alvarez et al, 1992). 9.5% — 13.8% of patients with coronary artery disease and valvular diseases had frequent premature beats (single forms), but changes in heart rate and ventricular arrhythmias occurring were infrequent and not severe (Poveda et al, 1992).

Compared to other species, particularly humans (Stanley, 1992; Fujita et al, 1992; Hirsch et al, 1993), I noticed that rabbits require large doses of fentanyl, 252 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ during surgery and 126 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ after the surgery compared to the human dose (50-100 $\mu g \cdot kg^{-1}$) but these doses did not cause significant changes to HR, MAP, CrdBF, CVP and blood gases.

The known major adverse reactions associated with fentanyl are respiratory depression, apnoea and muscle rigidity (Badewit, 1991; Marshall, 1990). In the experiments reported

here, rabbits were also given vecuronium and artificially ventilated with a 65%N_2 — 35% O_2 gas mixture. The blood gas data indicates that there was no significant impairment of gas exchange. PO_2, PCO_2 and pH were maintained close to the normal values, which were PaO_2/PvO_2 , 94-114/34-50 mmHg; $PaCO_2/PvCO_2$, 39-42/47-56 mmHg and pHa/pHv, 7.38-7.40/7.34-7.41 (Table 4.2-3). The fluctuations of PCO_2 were insignificant ($P>0.05$). The blood gas results reported by Kaplan (1979) and Wesbroth (1979) were PCO_2, 35-46 mmHg, pH, 7.35 and PO_2, 100 mmHg (Gonzalez, 1986). The blood gas and pH results of the present study suggested that the muscle relaxant effect of vecuronium was appropriate and rabbits had a stable metabolic state during 6 hours of observation. Due to the use of vecuronium, the rigidity resulting from fentanyl became undetectable, with all skeletal muscle being relaxed. Although I have found no reports on the use of vecuronium bromide in rabbits, the references of other studies from either humans or other animals may help to understand its effect on cardiovascular function in this study.

Vecuronium is a short-acting neuromuscular blocking drug. It has a steroid nucleus like pancuronium, which is commonly used in cardiac surgery, differing only in that the nitrogen at position 2 is tertiary rather than quaternary. Despite the small structural difference, vecuronium has distinctly different pharmacological properties. It has little or no cardiovascular effect. All the other currently used nondepolarizing muscle relaxants produce cardiovascular effects (Miller, 1989; Larach et al, 1991), many of which are mediated by autonomic and histamine receptors.

Narita et al (1992b) investigated the blocking effects of vecuronium and pancuronium on the negative chronotropic and dromotropic responses to stimulation of the parasympathetic nerves in the anaesthetised, open-chest dog. The results suggested that the blocking effect of vecuronium on the negative cardiac responses to parasympathetic stimulation was about one tenth as potent as pancuronium. Paulissian et al (1991) compared vecuronium and pancuronium during high-dose fentanyl anaesthesia for coronary artery bypass grafting surgery in 48 patients. They found that in the

pancuronium group (n=26), heart rate, cardiac index, and rate-pressure product were increased after induction of anaesthesia and following intubation. 11 patients displayed ischaemic ST segment changes. 4 patients in this group developed tachycardia and hypertension to an extent requiring pharmacological intervention. Vecuronium-treated patients (n=22) displayed no increases in HR, mean arterial pressure, and a decrease in CI. Only one patient in this group developed evidence of ischaemic ECG changes by comparison to 11 patients in the pancuronium group. Another report (Rathmell et al, 1993) showed the same result, from patients undergoing coronary artery bypass surgery, namely that pancuronium increased heart rate (from 68± 4 beats /min before induction to 76 ± 5 beats /min only 5 minutes after intubation). Chen et al (1991) reported there were no significant differences in any haemodynamic parameter measured during different doses of vecuronium. High doses of vecuronium of up to 0.4 mg /kg may be administered to patients with coronary artery disease with few haemodynamic changes. The chronotropic and inotropic effects of vecuronium bromide and its interaction with the autonomic nervous system were investigated in the isolated, cross-circulated right atrial and left ventricular preparations of the dog (Narita et al, 1992a).

The results indicated that the reasons vecuronium caused less negative effects on cardiovascular system than other muscle relaxants, could be:

* vecuronium has a positive inotropic effect which is mediated by nonadrenergic mechanisms and beta-adrenoceptors;

* vecuronium blocks ganglionic and presynaptic nicotinic and postsynaptic muscarinic receptor-mediated responses similarly;

In conclusion, the present study is the first to demonstrate that anaesthesia with a combination of fentanyl citrate and vecuronium bromide caused no significant changes to the cardiovascular system and blood gases on ventilated rabbits throughout a monitoring period of 6 hours. Although this study cannot evaluate the mechanisms of fentanyl and vecuronium in rabbits, there seemed to be even more favourable effects of fentanyl and

vecuronium in rabbits compared to some other mammals. Therefore, this anaesthetised animal model will be a useful tool for research on cardiovascular function.

Chapter 5

Ventilated rabbit model

5.1 Introduction

It is very important to select proper ventilating parameters to enable animals to sustain physiological homeostasis through a long experimentation period. It is also very difficult to do so because the data from conscious and anaesthetised rabbits show that the respiratory rate varies greatly with body weight, species and experimental conditions. Guyton (1947) and Wright (1934) reported that the respiratory rate of rabbits is 32 to 60 breaths·minute^{-1}, tidal volume 19.3 to 24.6 ml and minute volume 0.37 to 1.14 litres·minute^{-1}. According to Stahl's equation (Stahl, 1967), tidal volume is very nearly proportional to the body size ($Vt = 7.69\ Mb^{1.04}$). For a 1 kg animal, the tidal volume at rest will be about 8 ml, and for larger and smaller animals the proportion relative to body size will remain almost the same, as the exponent is not significantly different from 1. Maskrey and Trenchard (1989) reported frequency is 46.6±14.8 breaths·minute^{-1} and tidal volumes 25.3±2.6 ml in pentobarbital-sodium anaesthetised New Zealand rabbits, which weighed from 2.5-3.0 Kg. There are no reports in which rabbits have been artificially ventilated for a period as long as 6 hours. The goal of this experiment was to establish the optimum ventilatory parameters for a long term anaesthesia of over 6 hours.

5.2 Results and discussion

The animals formed three groups according to ventilating rate and positive driving pressure. Group 1 (n=4) was set at a ventilatory frequency (f) of 10·minute^{-1} and driving pressure (P) of 10 cmH_2O. Group 2 (n=3) was set at f of 13·minute^{-1} and P of 10 cmH_2O. Group 3 (n=4) was set at f of 20·minute^{-1} and P of 60 cm H_2O.

Table 5. shows the relationship between ventilator setting and the values of animals' blood gases and pH.

Table 5. Blood gases and pH with different ventilating frequencies (f) and pressures (P), mean ± SD

Group (f × P)	PaO_2 (mmHg)		$PaCO_2$ (mmHg)		pHa	
	Hour 1	Hour 6	Hour 1	Hour 6	Hour 1	Hour 6
1 (n=4) (10 × 10)	103±10	103±33	34±4.0	43±2.0	7.42±0.02	7.40±0.06
2 (n=3) (13 × 10)	118±3.0	81±11	40± 2.0	38±4.0	7.50±0.08	7.42±0.02
3 (n=4) (20 × 60)	98 ±4.0	84±7.0	26±1.0*	26±3.0*	7.53±0.03	7.56±0.05

* $P<0.05$ — The PCO_2 in Group 3 is significantly different from those in Group 1 and 2.

An inappropriate ventilation could result in either overventilating or underventilating. The PCO_2 is the more important consideration in defining the adequacy of ventilation under experimental conditions because the PO_2 can easily be altered by changes in the concentration of oxygen in the inspired gas and is, therefore, less dependent on the minute volume (Nunn, 1988). However, there is no consensus of opinion on the optimum PCO_2 during anaesthesia. Whereas it might appear reasonable to keep the PCO_2 within the normal range of a spontaneously breathing rabbit (35-46 mmHg, Kaplan, 1979; Wesbroth, 1979). Nunn (1988) suggested that changes of blood gases under anaesthesia are related to the changes of ventilation/perfusion relationships, alveolar dead space and shunt. In humans, the dead space includes physiological and anatomical dead spaces. Anatomical dead space is about 150 ml at a tidal volume above 350 ml. The physiological dead space is about a third of tidal volume over a wide range of tidal volumes. Nunn (1958) first observed that the physiological dead space during anaesthesia was increased which resulted in a decrease of the alveolar ventilation and a high ventilation/perfusion ratio. Furthermore, this decrease of alveolar ventilation causes an increase of blood PCO_2 and a decrease of blood PO_2. The reduction in PaO_2 can be avoided by using a higher oxygen mixture instead of ambient air. In my experiment I

used a mixture of 35%O_2 and 65%N_2 during the experiment (Nunn suggested that some 30-40% inspired oxygen is usually adequate. 1988) since a high concentration and/or a long term supply of oxygen also has irritant properties which lead to bronchial irritation (Hunter, 1972).

In the conscious healthy subject, the shunt or venous admixture amounts to only 1-2% of cardiac output and this results in an alveolar/arterial PO_2 gradient of less 7.5 mmHg. During anaesthesia, the alveolar/arterial PO_2 difference is usually increased to a value of about 10 mmHg. There is evidence for the development of areas of relative as well as absolute underventilation during anaesthesia (Nunn, 1988), which leads to low ventilation/perfusion ratios. In the present study, as is shown in Table 5, there were no significant differences between group 1 and group 2 for PaO_2, $PaCO_2$ and pH although the PaO_2 and $PaCO_2$ values seem higher in group 1 than that in group 2. Within group 2 the initial $PaCO_2$ was 40 ± 2 mmHg and 38 ± 4 mmHg at the end of experiment. pH did not show a significant difference between the 1st hour and the 6th hour of the experiment. In group 2 acid-base state remained in the normal range, which was described by Kaplan (1979), Wesbroth (1979) and Gonzalez (1986). They reported that the normal range of PCO_2, pH and PO_2, are 35-46 mmHg, 7.35 and 100 mmHg respectively. Therefore, my results suggested that the ventilation settings for animals in group 1 and 2 were more appropriate than those in group 3.

Fig 5.1 shows the change of PaO_2 among three groups. There were some small fluctuations during the entire monitoring time and ranged from 100-150 mmHg in group 1; 80-130 mmHg in group 2 and 80-120 mmHg in group 3. There was no significant difference among the three groups, which suggested that PaO_2 was not affected significantly by ventilating animals with frequency of 10-20 breaths per minute and driving pressure of 10-60 cmH_2O. Another report showed that rabbits ventilated at rates of 30, 60, 90 and 120 breaths per minute with driving pressure 17 cmH_2O had no significant changes in arterial blood gases but with significant increases in both tracheal positive end-expiratory pressure and functional residual capacity (Gonzalez, 1986).

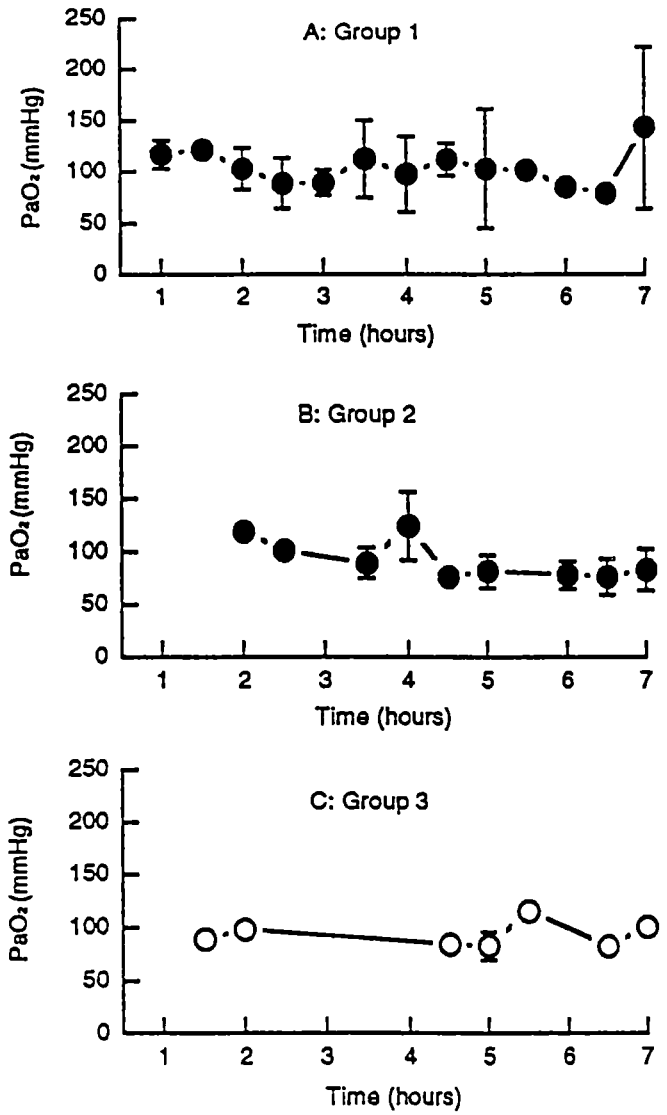


Fig 5.1 PaO_2 of ventilated anaesthetised rabbits during normothermia (Mean ± SD)

Fig 5.2 shows the change of $PaCO_2$ in the three groups. $PaCO_2$ in group 1 increased gradually over 6 hours with a range of 30-42 mmHg. $PaCO_2$ in group 2 was maintained within a range of 35-45 mmHg. There was no significant difference between groups 1 and 2. Animals in group 3 showed a constant lower $PaCO_2$ level compared to the other two groups ranging from 15-28 mmHg, indicating that this group animals were over ventilated (Moran Campbell et al, 1984). It is well known that hyperventilation due to increase of respiratory rate and driving pressure leads to hypocapnia. Observed values extend from the normal range down to about 18 mmHg in the course of routine anaesthesia (Nunn, 1988). An arterial PCO_2 of about 34 mmHg is considered to be appropriate (Gonzalez, 1986) although it has been difficult to demonstrate that hypocapnia does any significant harm during anaesthesia (Nunn, 1988). In my study however, hypothermia was to be applied on one of those ventilated models and hypothermia per se may result in severe hypocapnia unless further hyperventilation is prevented. On the other hand, an increased positive pressure tends to increase the ventilation/perfusion ratio. Therefore an increased ventilation/perfusion ratio leads to a decreased blood PCO_2. It has also been noted that the application of a positive pressure as high as 30 mmHg to the airways expands the human lungs to barely 70 % of the preoperative total lung capacity, which implies a reduced overall compliance. This consequently reduces lung volume, which could be due to a decreased surfactant activity in the lung under hyperventilation (Nunn, 1988).

Fig 5.3 shows the pattern of pHa in the three groups. Group 1 and group 2 ranged from 7.35-7.5 and 7.4-7.5 respectively. Group 3 animals showed a respiratory alkalosis due to over ventilation and pH ranged from 7.5-7.6. Usually the arterial blood pH begins to rise in 15 to 20 seconds after hyperventilation begins and becomes maximal in 10 to 15 minutes. The plasma HCO_3^- level drops along a similar time course (Masoro & Siegel, 1971b).

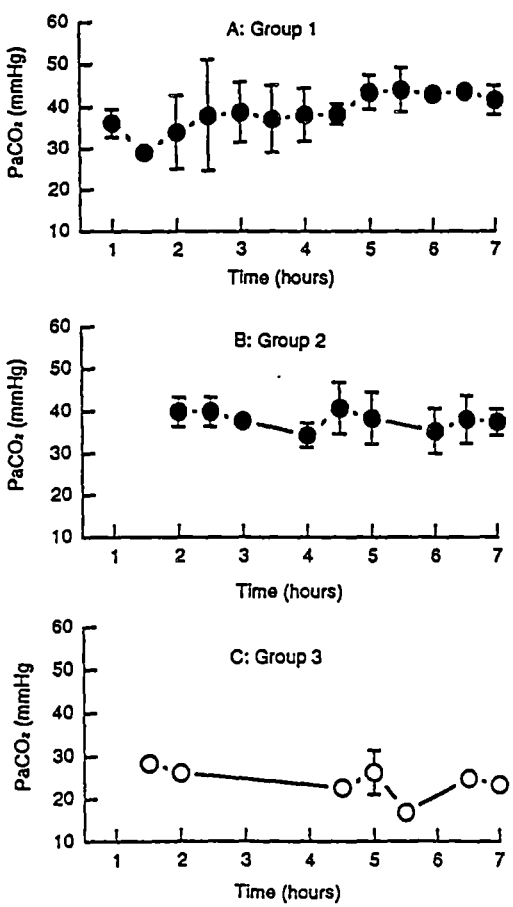


Fig 5.2 $PaCO_2$ of ventilated anaesthetised rabbits during normothermia (Mean ± SD)

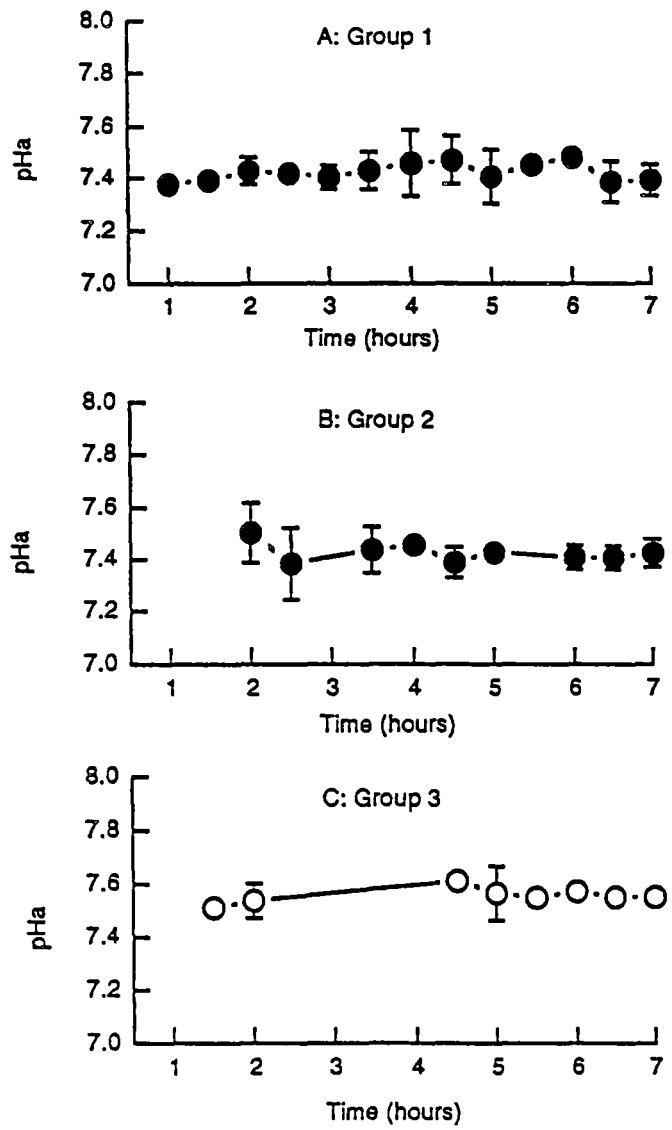


Fig 5.3 pHa of ventilated anaesthetised rabbits during normothermia (Mean ± SD)

In conclusion, this experiment investigated the effects of differing ventilatory frequencies and pressures on blood gases. The results here show that the changes in blood gases are the most reliable indication whether a proper ventilating rate and pressure are selected under anaesthetic monitoring. Of the combinations used, I found a pressure of 10 cmH_2O and a frequency of 10 — 13 breaths per minute produced the best results in terms of blood gases and pH under fentanyl-vecuronium anaesthesia during 6 hours or more. A pressure of 60 cmH_2O and frequency of 20 breaths per minute produced hyperventilation.

Chapter 6

Acid-base responses and blood gas status during and after induced hypothermia

6.1 Introduction

It has been stressed for decades that the acid-base status plays a crucial role as it influences essentially all other physiological processes during hypothermia. The critical question is what is the actual acid-base status during hypothermia? There is no satisfactory answer as to what would represent homeostasis of acid-base status as temperature changes (Williams, 1982; Ream et al, 1982; Heisler, 1986a; Swain, 1988). The present study was designed to assess the effects of pre-alkalinization and compare it to pre-acidification on the acid-base status of rabbits. There still is little known of the effects of pre-alkalinization and pre-acidification on acid-base status in intact animals during and after induced hypothermia although a few studies suggested that alkalinity protects the isolated heart from ischaemic insult and improves its performance (Poole-Wilson & Langer, 1975 & 1979). The hypothesis on which this study was based was that pre-alkalinization could not only protect the myocardium from acidotic ischaemia but would preserve cerebral function by enhancing cardiac function and improving the oxygen metabolism during and after induced hypothermia.

6.2 Results

6.2.1 Normothermic group

Arterial and venous pH were within normal ranges. Partial pressures of oxygen and carbon dioxide in arterial and venous blood were also within normal ranges. There was no significant change of any of the parameters with time ($P>0.05$). (Table 6.1).

Table 6.1. Blood gases and pH (mean ± SD, n = 6) in Nomorthermic Group

Time (hr)	pHa	pHv	PaO_2 (mmHg)	PvO_2 (mmHg)	$PaCO_2$ (mmHg)	$PvCO_2$ (mmHg)
1	7.40±0.03	7.34±0.04	109.7±25.4	50.1±6.2	39.2±2.3	47.7±2.3
3	7.38±0.04	7.41±0.24	93.6±28.5	34.9±10.1	42.1±6.3	56.1±3.9
6	7.39±0.04	7.34±0.05	114.1±60.1	35.9±7.7	40.5±2.9	50.1±4.6

6.2.2 Hypothermic groups

6.2.2.1 pH

There was no significant difference between any of the groups before treatment of NH_4Cl or carbicarb. Overall mean values for pretreatment pH were 7.35 ± 0.06 for arterial blood (mean ± SD, n=31) and 7.30 ± 0.09 for venous blood (mean ± SD, n=22, no record for HCo group). Following administration of NH_4Cl arterial pH fell from 7.35 ± 0.04 to 7.15 ± 0.01 while venous pH fell from 7.32 ± 0.04 to 7.06 ± 0.06. By contrast, following administration of carbicarb arterial and venous pH increased from 7.38 ± 0.03 to 7.53 ± 0.06 and from 7.36 ± 0.03 to 7.58 ± 0.09, respectively. pHa and pHv increased when the body temperature was reduced (HAl > HCo > HAc, $P < 0.01$). HAc had the largest increment from a post-treatment pHa value of 7.15 ± 0.01 at normothermia to 7.43 ± 0.05 at 25.5 ± 0.5°C (an increase of 0.28 units) and a pHv value of 7.06 ± 0.06 to 7.36 ± 0.06 (an increase of 0.3 units). By comparison, HAl showed less increase in pH than HAc when Tb fell. HAl had a post-treatment pHa value of 7.53 ± 0.06 and a pHv value of 7.58 ± 0.09 before cooling. The pHa and pHv values increased to 7.69 ± 0.06 and 7.66 ± 0.1. The increments were 0.16 and 0.11, respectively. HCo had a similar pHa increment (+0.14 units) to the HAl group. After rewarming, pH values in three groups declined as Tb increased. The decreases of pH values from the end of the hypothermia phase to normothermia after rewarming were 0.35 in HCo, 0.33 in HAc and 0.30 in HAl. However only pH in HAl reached the same value (pHa, 7.37 ± 0.09 and pHv, 7.33 ± 0.1, n=6) as that in Nom (pHa, 7.39 ± 0.04 and pHv, 7.34 ± 0.04, n=6). Animals in HCo and HAc had significantly lower pH (HCo: pHa, 7.17 ± 0.08 and pHv, 7.21 ± 0.09, n=4; HAc: pHa, 7.07 ± 0.06, pHv, 6.99 ± 0.08, n=3. $P<0.01$) (Fig 6.2.1).

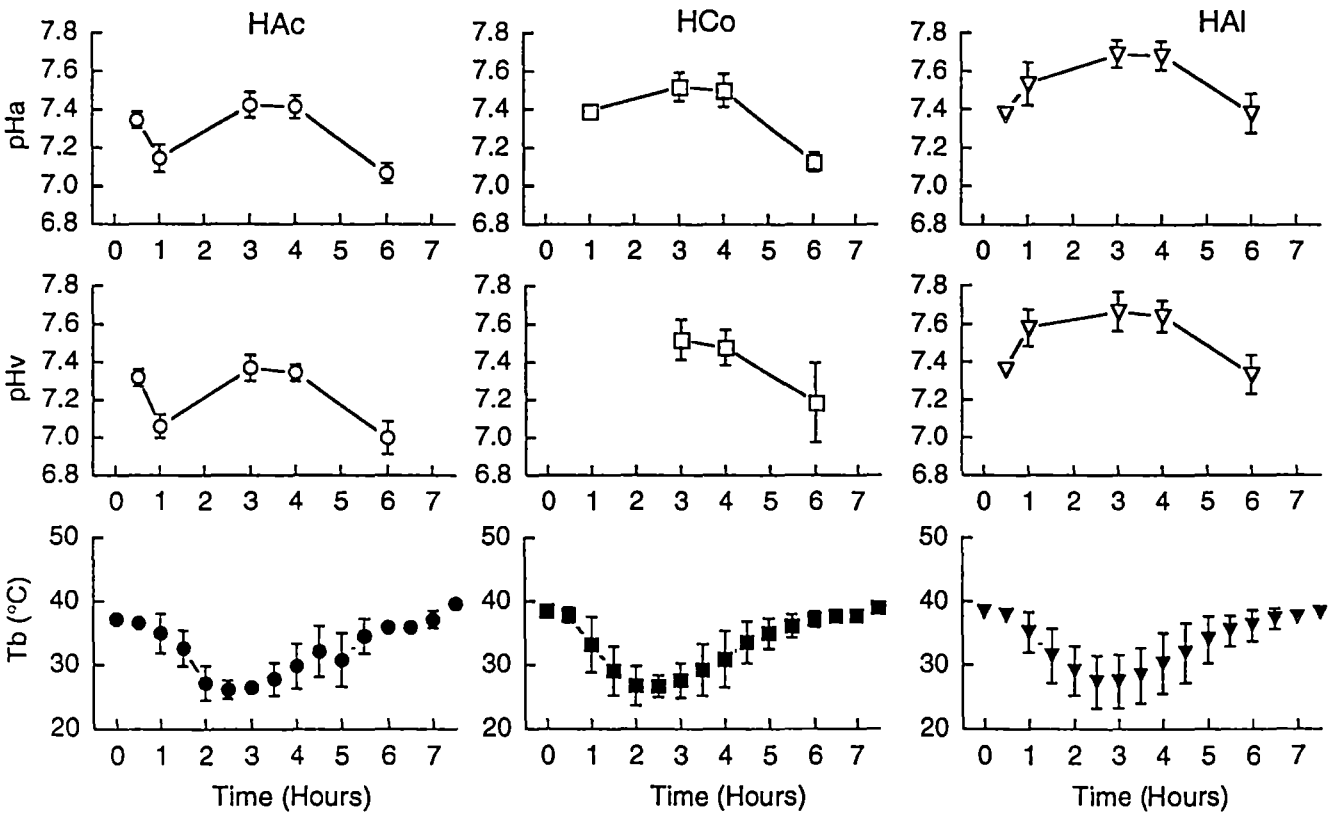


Fig 6.2.1 pH changes in the three treatment groups before, during and after hypothermia. The first pH values in HAc and HAl are the values before carbicarb or NH_4Cl treatment pH values given are those measured at actual body temperature. Values shown are mean ± SD.

6.2.2.2 Carbon dioxide partial pressure (PCO_2)

There was no significant difference between any of the groups before treatment of NH_4Cl or carbicarb. $PaCO_2$ changes following the administration of carbicarb and NH_4Cl were from 32.7 ± 9.9 to 36.3 ± 9.9 in HAl group and from 40.9 ± 7.0 to 48.6 ± 11.6 in HAc group. There were no significant differences between the $PaCO_2$ values before and after pre-treatment within both groups. In all hypothermic groups, $PaCO_2$ and $PvCO_2$ showed similar trends — a decline during cooling and an increase during rewarming. The initial and final values in HAl were significantly lower than the other two groups. However, during cooling, the net PCO_2 decrease in HAc was greatest. At the end of the experiment $PvCO_2$ in HCo and HAc was higher than in the HAl ($P<0.05$) (Fig 6.2.2 and Table 6.2). The difference in $PvCO_2$ between HAl and Nom was not significant ($P>0.05$).

6.2.2.3 Oxygen partial pressure (PO_2)

PaO_2 showed a non-significant increase when the body temperature was reduced. Thereafter it decreased significantly in all hypothermic groups after rewarming ($P<0.01$ in HCo and HAc, $P<0.05$ in HAl).

HAl had a higher initial value of PaO_2, which stayed at a higher level than in the other groups throughout the cooling and rewarming periods ($P<0.05$). At the end of the experiment, HAl had the highest value of the three hypothermic groups but this difference was not significant ($P=0.094$). Although PvO_2 appeared to fall during cooling and increased progressively during rewarming in all hypothermic groups, these changes were not significant. (Fig 6.2.3 and Table 6.2).

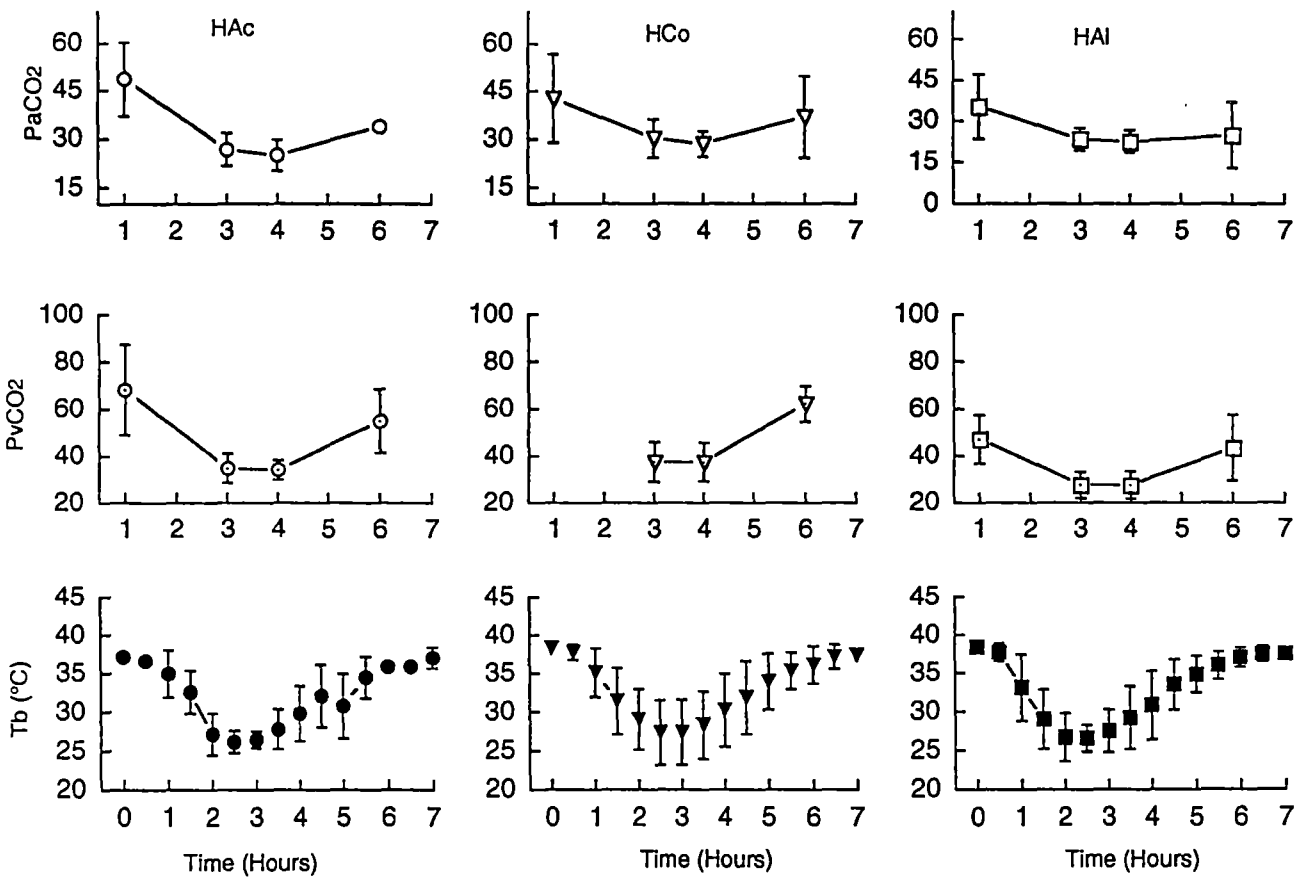


Fig 6.2.2 PCO_2 changes in the three treatment groups before, during and after hypothermia (mean ± SD)

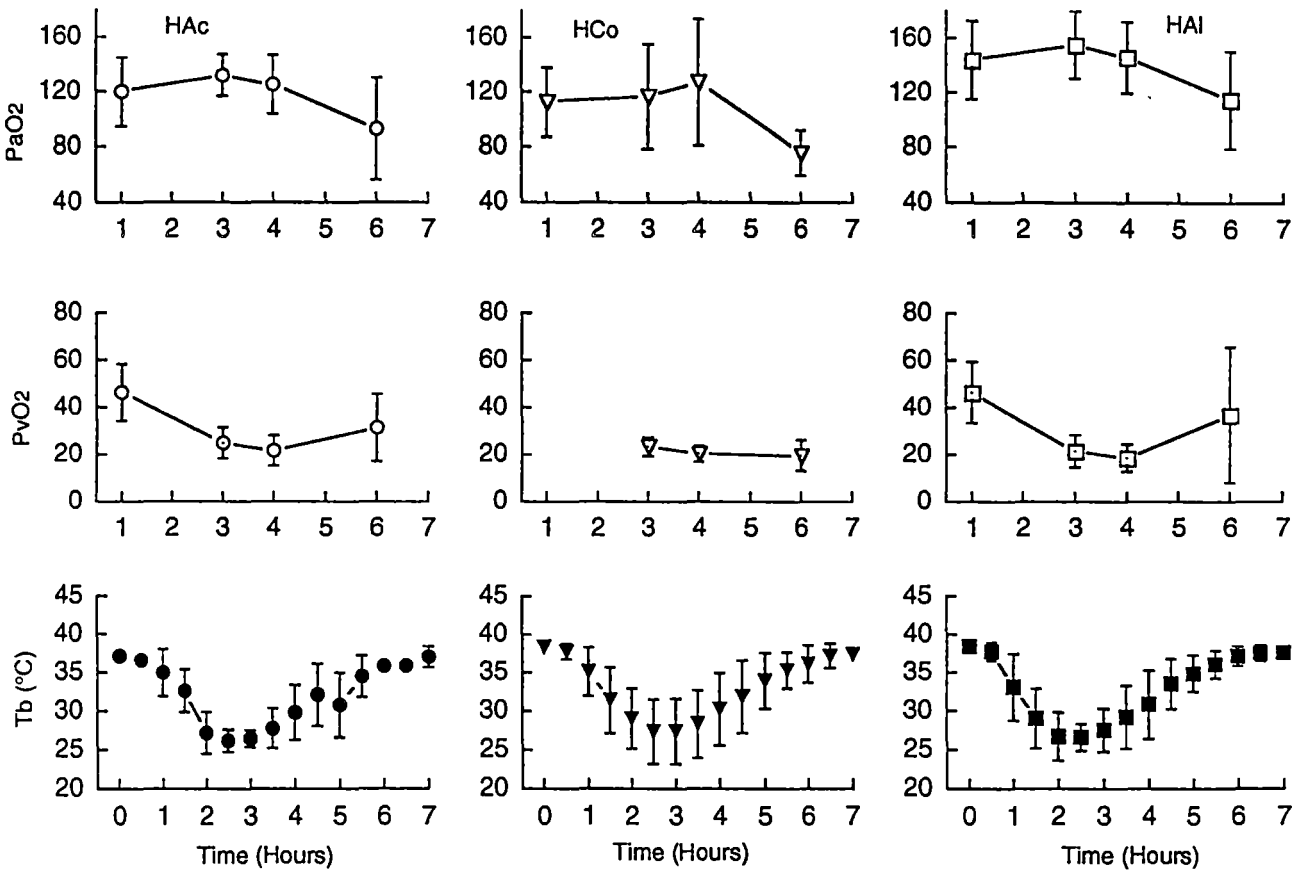


Fig 6.2.3 PO_2 changes in the three treatment groups before, during and after hypothermia (mean ± SD)

Table 6.2 Blood gases and pH (Mean ± SD) in three hypothermic groups

Time (hr) Temp (°C)	Group	pHa	pHv	PaO_2 (mmHg)	PvO_2 (mmHg)	$PaCO_2$ (mmHg)	$PvCO_2$ (mmHg)
Pre-treatment 37.5±0.5	HAl (n=12)	7.38±0.03	7.36±0.03	131.0±18.4	55.0±8.1	32.7±9.9	46.8±10.2
	HAc (n=10)	7.35±0.04	7.32±0.04	130.8±19.9	49.0±10.4	40.9±7.0	50.7±8.7
post-treatment							
1	HCo (n=9)	7.38±0.03		112.5±25.2		42.7±13.9	
37.5±0.5	HAl (n=12)	7.53±0.06 **	7.58±0.09 **	142.5±30.9 *	46.4±12.9	36.3±12.1	47.0±10.3
	HAc (n=10)	7.15±0.01 **	7.06±0.06 **	119.7±25.1	46.2±12.1	48.6±11.6	68.1±19.2 *
	HCo (n=9)	7.52±0.07	7.51±0.10	116.9±38.6	23.3±3.9	30.2±5.9	37.4±8.5
3	HAl (n=12)	7.69±0.06 **	7.66±0.10 **	155.2±24.8 **	21.5±6.9	23.1±4.1 *	27.5±5.6 *
25.5±0.5	HAc (n=10)	7.43±0.05 **	7.36±0.06 **	132.1±15.1 **	24.9±6.6	26.6±5.1	34.7±6.3
	HCo (n=9)	7.52±0.08	7.50±0.09	122.3±35.0	20.7±3.7	27.8±3.5	34.7±6.7
4	HAl (n=12)	7.67±0.06 **	7.64±0.08 **	145.5±26.3	18.4±5.2	22.5±4.1 *	27.5±5.8 **
25.5±0.5	HAc (n=10)	7.40±0.05 **	7.34±0.04 **	125.6±21.6	21.5±6.4	24.9±4.8	33.9±4.2
	HCo (n=4)	7.17±0.08 **	7.21±0.09	68.71±15.8	19.3±5.7	31.6±11.2	62.4±6.5
6	HAl (n=6)	7.37±0.09	7.33±0.10	115.0±35.8	36.9±29.0	24.9±12.0 *	43.3±14.0 *
36.5±0.5	HAc (n=3)	7.07±0.06 **	6.99±0.08 **	93.1±37.0	31.3±14.3	33.9±2.2	54.8±13.5

* $P<0.05$, ** $P<0.01$, significant differences from the control value (post-treatment) of the same group, and among three groups as well.

6.3 Discussion

6.3.1 Respiratory and metabolic alkalosis

Alkalinity can be achieved by vigorous hyperventilation or infusion of alkali (Buckberg, 1985; Prakash et al, 1978). Table 6.3 shows a brief comparison between respiratory and metabolic alkalosis.

Table 6.3 Comparison between respiratory and metabolic alkalosis

Type		Respiratory alkalosis	Metabolic alkalosis
Cause		Hyperventilation — $CO_2 \downarrow$	Excessive alkali — $HCO_3^- \uparrow$
Primary change	Blood $[HCO_3^-]$ Blood $[CO_2]$	 Decrease	Increase
Secondary /Compensatory change	Blood $[HCO_3^-]$ Blood $[CO_2]$	Decrease	 Increase
Compensatory organ		Kidneys — excrete HCO_3^-	Lungs — expire CO_2
Compensatory speed		Slower	Faster

Infusion of alkali (sodium bicarbonate is commonly used) is supposed to act faster and more efficiently than hyperventilation to buffer excessive acid and/or create an alkalosis. However, (a) an excessive Na^+ load can cause retention of water in the body; (b) infusion of sodium bicarbonate could increase the arterial PCO_2.

Hyperventilation will be easier to effect with an oxygenator during CPB procedure and satisfactory results would be achieved under a certain base deficit range otherwise infusion of alkali will still be required.

Streisand et al (1971) compared the effects of respiratory and metabolic alkalosis on myocardial contractility of seven mongrel dogs by adjusting the amount of CO_2 in the oxygenator and, applying a 5 % solution of sodium bicarbonate (pH was 7.7). The

results showed that the average percentage changes in dp/dt at a PCO_2 of 30, 20 and 10 mmHg were 18, 47 and 17, respectively. The average percentage changes in total peripheral resistance at the same PCO_2 values were 18, 13 and 20, respectively. The average percentage changes of metabolic alkalosis on dp/dt were 26, 52 and 89 and total peripheral resistances were 7, 16 and 54, respectively as pHs were 7.5, 7.6 and 7.7. However the occurrence of spontaneous ventricular fibrillation with metabolic alkalosis in this experiment was 5 out of 7 at a pH of 7.8. Baker et al (1993) investigated the myocardium protection of alkaline cardioplegia by using St. Thomas' Hospital Cardioplegic Solution II on the isolated heart of immature and mature New Zealand white rabbits. St. Thomas' Hospital Cardioplegic Solution II, which normally contains 10 mmol/L $NaHCO_2$ and has a pH of 7.8 ± 0.01, was adjusted to pH values from 4.8 to 8.8 by manual titration using HCl/NaOH. Their results showed that recovery of aortic flow from myocardial ischaemia with the modified cardioplegic solution (pH = 6.8) was 98±3 %, which was significantly greater than with standard St. Thomas' II solution (72±2 %).

I assume the failure of the alkaline solution to protect myocardium could be due to the use of $NaHCO_3$, which produces an equal amount of carbon dioxide and buffers simultaneously. For example, in Streisand's study (Streisand et al, 1971) 5% $NaHCO_3$ was used to adjust the pH. To raise pH from 7.4 to 7.7, 98 mmoles/L $[HCO_3^-]$ is required.

In the present study instead of using sodium bicarbonate, carbicarb was used to create a pre-alkalosis in rabbits. The pre-acidification was induced by ammonium chloride (Shapiro et al, 1989).

6.3.1.1 Pre-alkalinization induced by carbicarb

Del Canale et al (1988) found that at the end of CPB, a condition of extracellular metabolic acidosis was apparently sustained by enhancement of muscle cell anaerobic glycolysis with a consequent increase of both muscle and plasma lactate content. They

also detected subnormal cell phosphocreatine levels, as well as reduced bicarbonate buffer stores and decreased intracellular pH.

Sodium bicarbonate is commonly used to correct metabolic acidosis (Oliver, 1970; Narins & Cohen, 1987), but many adverse effects of sodium bicarbonate therapy have been described (Stacpoole, 1986; Graf et al, 1985a) Some of the potentially more important effects include hypercapnia and aggravation of intracellular acidosis (Von Planta et al, 1988; Graf et al, 1985b) hyperosmolality (Mattar et al, 1974) congestive cardiac failure, and ionized hypocalcaemia (Cooper & Worthley, 1987; Graf & Arieff, 1986). Hypercapnia is likely to occur during sodium bicarbonate therapy when the normally compensating respiratory reflexes are blunted, which may occur during sedation and mechanical ventilation. Hypercapnia may increase intracellular acidosis because carbon dioxide crosses cell membranes rapidly and thus may decrease myocardial cell function (Von Planta et al, 1988; Weil et al, 1988; Falk & Weil, 1988; Ng et al, 1967; Steenbergen et al, 1977). Carbicarb is a recently formulated buffer, which is a 1:1 mixture of disodium carbonate and sodium bicarbonate, which produces much lower CO_2, higher pH, and lower osmolality, compared to sodium bicarbonate (Rhee et al, 1993). It has been shown to be a more efficient alkalinizing agent than sodium bicarbonate for equal sodium loads. Moreover, carbicarb lowers PCO_2 *in vitro* and does not elevate PCO_2 *in vivo* (Sun et al, 1987). Shapiro et al (1989) studied responses of brain pH to sodium bicarbonate and carbicarb during systemic acidosis on rats. After the animals were subjected to ammonium chloride-induced metabolic acidosis and hypercapnia-caused respiratory acidosis, either sodium bicarbonate or carbicarb was given for alkalinization therapy. They found that sodium bicarbonate treatment resulted in a systemic alkalinization and an increase in arterial PCO_2 in both types of acidosis, but also caused intracellular brain acidification in rats with ammonium chloride acidosis. Carbicarb therapy led to systemic alkalinization without major changes in arterial PCO_2 and intracellular brain alkalinization in both acidosis models. Carbicarb releases less CO_2 than sodium bicarbonate.

The pK for the reaction

$HCO_3^- \Leftrightarrow H^+ + CO_3^=$ is 9.8;

so for an equimolar mixture of Na_2CO_3 and $NaHCO_3$

pH = pK = 9.8

If carbicarb is infused and pH of the infusion falls to 7.4, then:

$7.4 = 9.8 + \log (CO_3^= / HCO_3^-)$

$\log (CO_3^= / HCO_3^-) = 7.4 - 9.8$

$(CO_3^= / HCO_3^-) = 0.004 / 1$

This means 99.6 % $CO_3^=$ will be converted to HCO_3^- by taking up one H^+ per $CO_3^=$.

If pH of the infusion falls to 7.53,

$\log (CO_3^= / HCO_3^-) = 7.53 - 9.8$

$(CO_3^= / HCO_3^-) = 0.005 / 1$

1 ml carbicarb contains 0.33 mmol HCO_3^-, 0.33 mmol $CO_3^=$ and a negligible amount of CO_2 (0.004 mmol). If pHa becomes 7.53 after carbicarb is infused, we will have:

$CO_3^=$: 0.0016 mmol (negligible)

HCO_3^-: 0.556 mmol

CO_2: 0.104 mmol

which shows 1 ml carbicarb has neutralised approximately 0.43 mmol H^+ and generated 0.104 mmol CO_2.

By contrast, one mole of sodium bicarbonate contains one mole of HCO_3^-, which reacts one with one mole of H^+ producing one mole of CO_2.

In this study, carbicarb was given at a dose of 2 $ml \cdot kg^{-1}$. The average weight of the rabbits was 3 kg. The total carbicarb dose was 6 ml, which contains 1.98 mmol sodium bicarbonate and 1.98 mmol sodium carbonate. Twenty minutes after administration of Carbicarb (2 $ml \cdot kg^{-1}$) the arterial pH increased from 7.38 ± 0.03 to 7.53 ± 0.06 thus the changes in the bicarbonate buffer system were

$CO_3^=$: +0.0198 mmol (negligible)

HCO_3^-: +3.78 mmol

CO_2: +0.17 mmol

6.3.1.2 Pre-acidification induced by NH_4Cl

Acidosis was induced in HAc by administering 5 $mmol \cdot kg^{-1}$ ammonium chloride (2 M solution 2.5 $ml \cdot kg^{-1}$) (Shapiro et al, 1989) (eq 6.3.1.2).

$$2NH_4Cl + 2HCO_3^- \xrightarrow{\text{in liver}} CO(NH_2)_2 + CO_2 + 3H_2O + 2Cl^- \quad (6.3.1.2)$$

Some of the ammonium chloride is converted into HCl by passive diffusive removal of NH_3 from the body fluids via the lungs. In either case, the net effect is a reduction of HCO_3^-, and for this reason NH_4Cl has been used clinically to treat metabolic alkalosis.

In this study, twenty minutes after the administration, the mean pH value fell from an initial value of 7.31±0.08 to 7.15±0.01, which was lower than those in HAl and HCo ($P<0.01$). PCO_2 increased from a pre-treatment value of 40.9±7.0 to a post-treatment value of 48.6±11.6 ($P>0.05$).

6.3.2 Acid-base status of rabbits during and after induced hypothermia

6.3.2.1 Pre- and during cooling

As is seen in table 6.2 and Fig 6.2.1, pH showed different degrees of change in the three groups. HAc had the greatest decrease which was -0.20 ($P<0.01$) and HAl had an increase of 0.15 ($P<0.01$). During cooling from 37.5°C to 25.5°C PCO_2 in all groups decreased. The net decreases were 22, 13 and 12 mmHg in HAc, HAl and HCo respectively. Presumably, less metabolic activitiy was taking place in HAc during cooling. Therefore less CO_2 was produced. By contrast, it might be the case that HAl and HCo animals still had more enzymes working and produced more CO_2.

6.3.2.2 Hypothermia for one hour

Acidosis group (pH-stat management?)

In HAc, during the one hour period of profound hypothermia (Tb = 25±0.5°C), arterial pH was well maintained at around 7.4, which is similar to the hypothermic acid-base state by applying the pH-stat (7.43±0.05 at the beginning of hypothermia, and 7.40±0.05 at the end, which were lower than the values in the other two hypothermic groups, $P<0.05$). An interesting comparison can be made in the difference between the results of the HAc and the results of Gothgen (1988):

Gothgen et al (1988) investigated the effects of pH-stat on acid-base and oxygen status during hypothermic cardiopulmonary bypass. They concluded that the pH-stat approach to acid-base status during induced hypothermia (at 25.3 ± 1.5°C) and extracorporeal circulation is safe and that the ratio between oxygen uptake of the tissue and oxygen delivery may be more appropriate than alpha-stat. The data from the HAc showed that animals attained a pH of around 7.4 after one hour of hypothermia following administration of NH_4Cl, which is similar to the pH obtained with the pH-stat approach. However, instability of heart rate and blood pressure, arrhythmias and disturbances of conduction shown in ECG and more severe inhibition of the cerebral electric activities (see later chapters), showed that animals did not benefit from this acid-base status at a pH of around 7.4 during hypothermia.

Table 6.4 Comparison of arterial blood pH (mean ± SD) during induced hypothermia between rabbits (this study)† and humans (Gothgen et al, 1988)*

	Rabbits Tb	pHa	Humans Tb	pHa
Before cooling	37±0.5	7.31 ± 0.08	35.2±0.6	7.41 ± 0.05
Before cooling & after i.v. NH_4Cl	37±0.5	7.15 ± 0.01	—	—
Cooling	>25±0.5 <37±0.5	—	>25.3±1.5 <35.2±0.6	7.46 ± 0.04
One hour under hypothermia	25 ± 0.5	7.43 ± 0.05 to 7.40 ± 0.05	25.3 ± 1.5	7.39 ± 0.06
After rewarming	36.5 ± 0.5	7.07 ± 0.06	34 ±3.8	7.30 ± 0.05

* In their study pH was maintained at around 7.4 by ventilating with O_2 and CO_2 during whole experimental period.

† Rabbits were ventilated with a gas mixture of 35% O_2 and 65% N_2.

Alkalinity management

When the animals in HAl were cooled to 25 ± 0.5°C both pHa and pHv increased to 7.69 ± 0.06 and 7.67 ± 0.10 respectively, and were higher during the one hour period of profound hypothermia than the other two groups. At the end of this period pHa and pHv were still higher in all hypothermic groups at 7.67 ± 0.06 and 7.64 ± 0.08 respectively ($P<0.01$). Because of the use of carbicarb for the pre-alkalinization, HAl did not have high $PaCO_2$ and $PvCO_2$. The average values were actually lowest throughout the experiment among the three groups ($P<0.05$) .

6.3.2.3 During and after rewarming

After rewarming pH values for HAl were 7.37 ± 0.09 (pHa) and at 7.33 ± 0.10 (pHv). These results were not significantly different from those of the Nom group. The other

two hypothermic groups gave lower pH values, 7.17 ± 0.08 (pHa) and 7.21 ± 0.09 (pHv) for the HCo and 7.07 ± 0.06 (pHa) and 6.99 ± 0.08 (pHv) for the HAc (Table 6.2). The blood pH values indicated that a hypothermic acidosis seemed inevitable after rewarming and the pre-acidification treatment exacerbated such an inevitable acidosis. However, pre-alkalinization treatment, by providing extra buffering, has achieved a near normal pH.

After rewarming, $PaCO_2$ was 24.9 ± 12.0 in HAl, which was lower than the level before cooling and lower than those in the other two groups. After rewarming $PvCO_2$ in HAl did not show any significant difference from the pre-cooling value. $PvCO_2$ were higher in HAc, 54.8 ± 13.5 and HCo, 62.4 ± 6.5 respectively (Venous blood gases and pH were not measured in the first HCo samples), which suggests that the supply and demand of oxygen was out of balance. By comparison, $PvCO_2$ in HAl was 43.3 ± 14.0 ($P<0.05$) (Table 6.2). Compared to the PCO_2 changes during cooling (see above discussion), it seems HAl animals had a better balance of oxygen demand and supply. All these results indicate that pre-alkalinization, by producing a more alkaline pH at low temperature, enables the animal to achieve a more normal acid-base status after rewarming.

6.4 Summary

We have compared the effects of different acid-base approaches on acid-base status during and after induced hypothermia on rabbits. The data showed that pH increased as the body temperature dropped. Animals pre-alkalinized with carbicarb showed a significantly higher pH during hypothermia and rewarming. After rewarming, normal pHa and pHv were retained. HAc animals showed a "normal" pH (pH = 7.4) during hypothermia and a serious acidosis after rewarming. $PaCO_2$ decreased during hypothermia and was lower in HAl than the other two hypothermic groups during and after hypothermia, and after rewarming. In HAc and HCo, $PvCO_2$ was around 40 mmHg during hypothermia and became significantly higher than the precooling values when the body temperature was returned to normal. $PvCO_2$ was 43.3±14.0 in HAl and not significantly different from the Nom group. By a brief comparison of the effects of different pH status (Table 6.5), it is concluded that pre-alkalinization created an optimum acid-base state (more physiological) after rewarming and seemed to cause no harm during hypothermia and rewarming at a relatively higher pH. Pre-acidification led to a worse acidosis after rewarming although animals in this group had a pH at around 7.40 during hypothermia.

Chapter 7

Effects of acid-base status on haemodynamics during and after induced hypothermia

7.1 Introduction

Hypothermia in experimental animals results in complex haemodynamic changes that are not yet well understood (Sinet et al, 1985; Simon & Bers, 1990) although much work has been published on the correlation between hypothermia per se and haemodynamics (Robert, 1959). Hypothermia may affect haemodynamic state changes through changes in vascular tension (Barcroft, 1943; Morimoto et al, 1984), blood viscosity (Bigelow et al, 1950a; Lofstrom, 1959; Schönbein et al, 1973; Chen & Chien, 1978), flow rate (Nose, 1982; Mills 1990; Wolf et al, 1992) and myocardial function (Covino & Hegnauer, 1955b; Lloyd & Mitchell, 1974; Landymore et al, 1992). Based on the results of experimental application of alpha-stat, Becker et al (1981) and Buckberg (1985) postulated that a more alkaline acid-base state would be even more effective in preserving hypothermic circulation although it has not been observed in any naturally occurring physiological systems. Alkalinity could be achieved by vigorous hyperventilation or infusion of alkali.

It is known that there will be a metabolic acidosis during and after rewarming; and that delays in changes in ventilation, cardiac output and local blood flow may cause oxygen debt and early lactate accumulation in hypothermic procedure, and after rewarming (Di Prampero, 1981). Therefore in the present study the effects of pre-alkalinization and pre-acidification on the hypothermic haemodynamics were investigated.

7.2 Results

7.2.1 Normothermic group

Mean aortic blood pressure (MAP), heart rate (HR), central venous pressure (CVP) values did not vary significantly during the 7 hours of recording ($P > 0.05$). Carotid artery blood flow (CrdBF) showed a slow decrease during the last few hours of the experiment and a difference of 11.6 ml min^{-1} between the first and last hours ($P < 0.05$) (Fig 7.2.1).

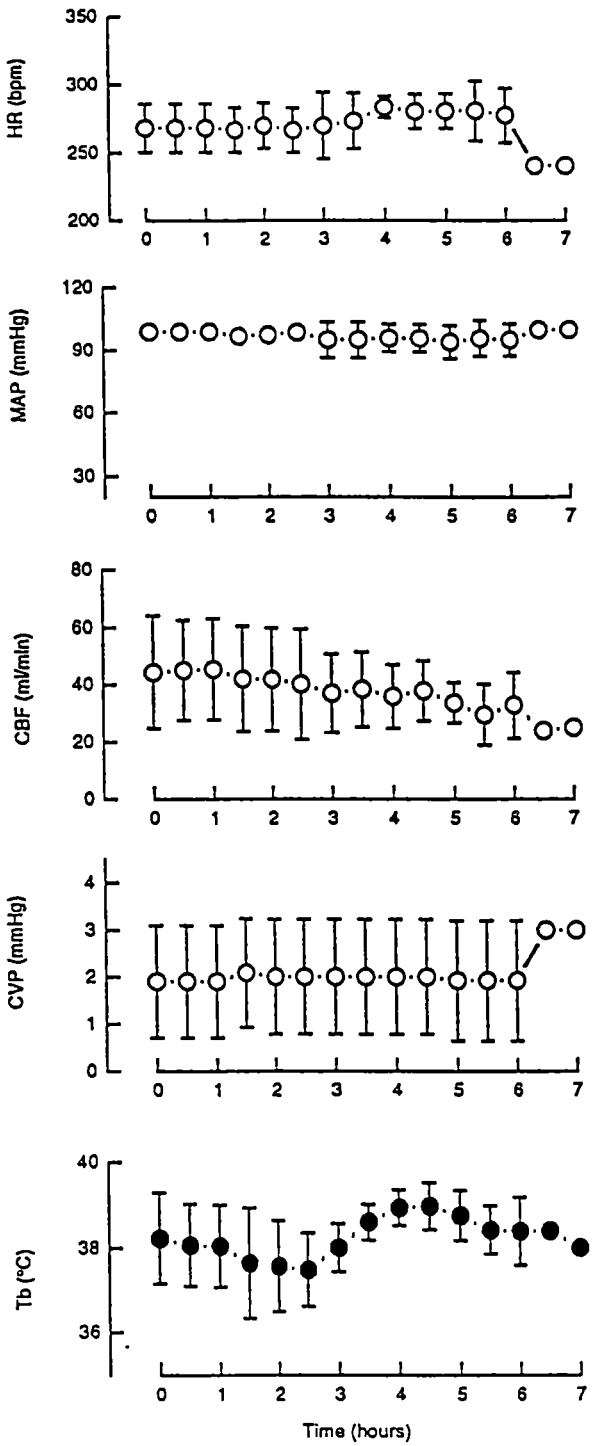


Fig 7.2.1 Changes of haemodynamics and body temperature in normothermic group (Mean ± SD)

7.2.2 Hypothermic group

7.2.2.1 Heart rate (HR)

HR fell significantly in all rabbits ($P<0.05$) as their body temperature (Tb) was lowered. During rewarming HR in both HCo and HAl increased rapidly. By contrast, the recovery of HR in HAc was slower and unstable (Fig 7.2.2).

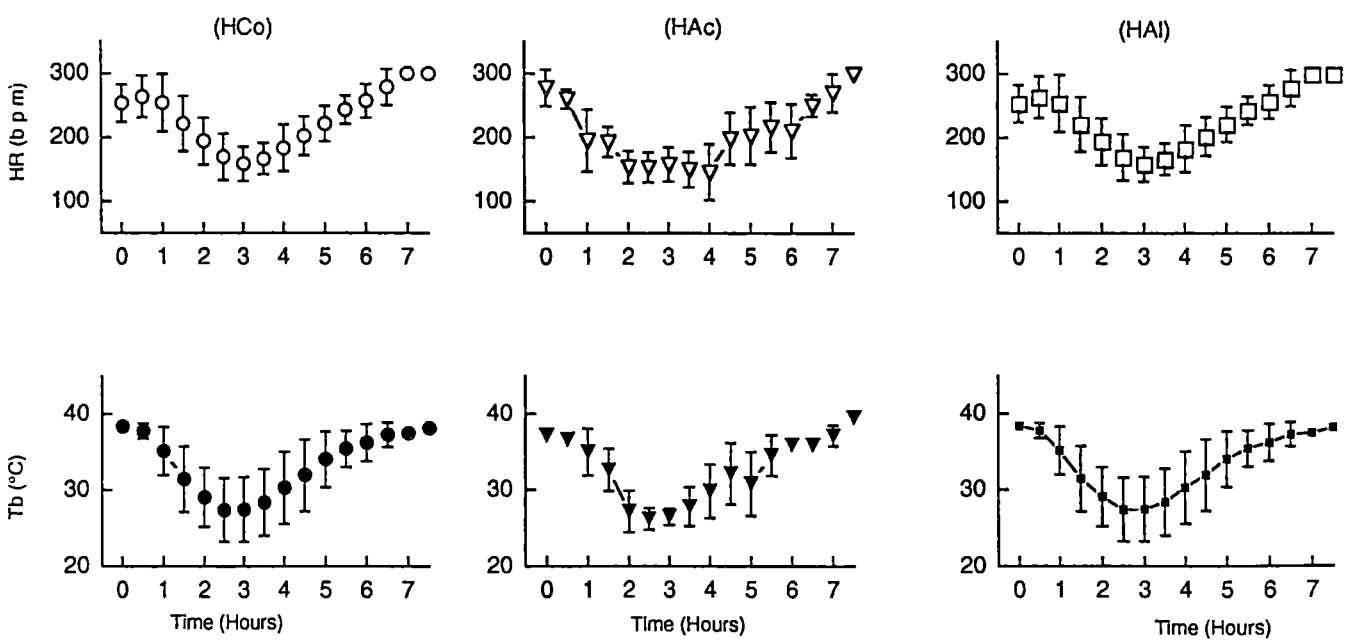


Fig 7.2.2 Changes of HR at different body temperatures in three hypothermic groups (Mean ± SD)

7.2.2.2 Carotid artery blood flow (CrdBF)

CrdBF declined immediately in all animals when hypothermia was induced. During rewarming, it increased gradually but never reached group control values. CrdBF in HAl was higher than that in HAc at the late rewarming stage (8 ml min^{-1} ± 5, n = 4 versus. 3.5 ml min^{-1} ± 1, n = 9 at the 5th hour and 10 ml min^{-1} ± 5, n = 10 versus. 4.8 ml min^{-1} ± 1.8, n = 5 at half hour later, $P < 0.05$) and showed an upward trend after rewarming. There was no significant difference between HCo and HAl (Fig 7.2.3).

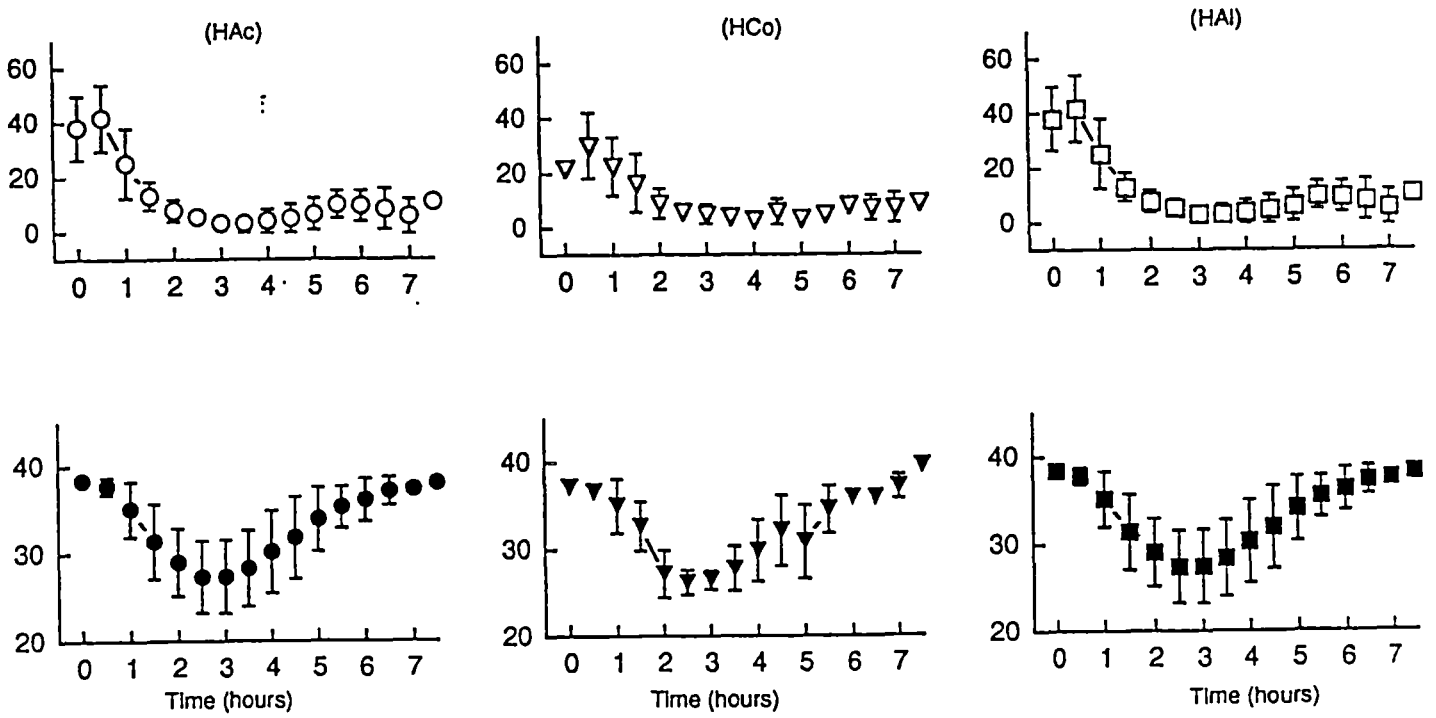


Fig 7.2.3 Changes of CrdBF at different body temperatures in three hypothermic groups (Mean ± SD)

7.2.2.3 Central Venous Pressure (CVP)

CVP decreased sharply as Tb was reduced in HAc, and fell progressively throughout the experimental period. HAl showed a slight decrease of CVP when Tb was reduced then remained relatively constant during the rest of the experimental period. (Fig 7.2.4).

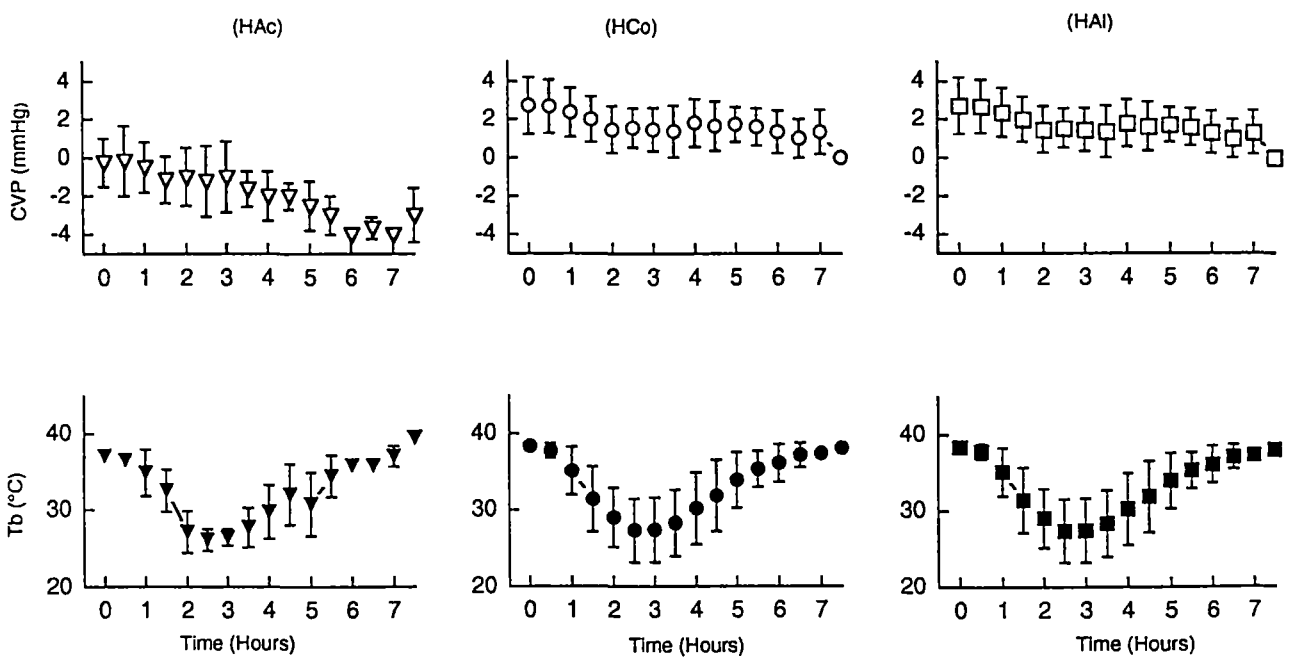


Fig 7.2.4 Changes of CVP at different body temperatures in three hypothermic groups (Mean ± SD)

7.2.2.4 Mean Aortic blood pressure (MAP)

The changes in MAP with hypothermia were different in each group (Fig 7.2.5).

In HCo, MAP did not change until the Tb was lowered to 25°C ± 0.5. Thereafter it decreased continuously during the rest of the hypothermic period and most of the rewarming phase. MAP did not return to normal until one hour after Tb recovered.

In HAl, MAP increased progressively while Tb was reduced, then decreased while animals were rewarmed (from 103 ± 6 mmHg to the lowest value 86 mmHg ± 5). After Tb reached 33.5°C (4.5 hours after the beginning of the experiment) MAP increased and reached the mean value of 107 mmHg ± 12 at the 6th hour.

In HAc, MAP was unstable and tending to decrease through the entire monitoring period and never returned to the control value.

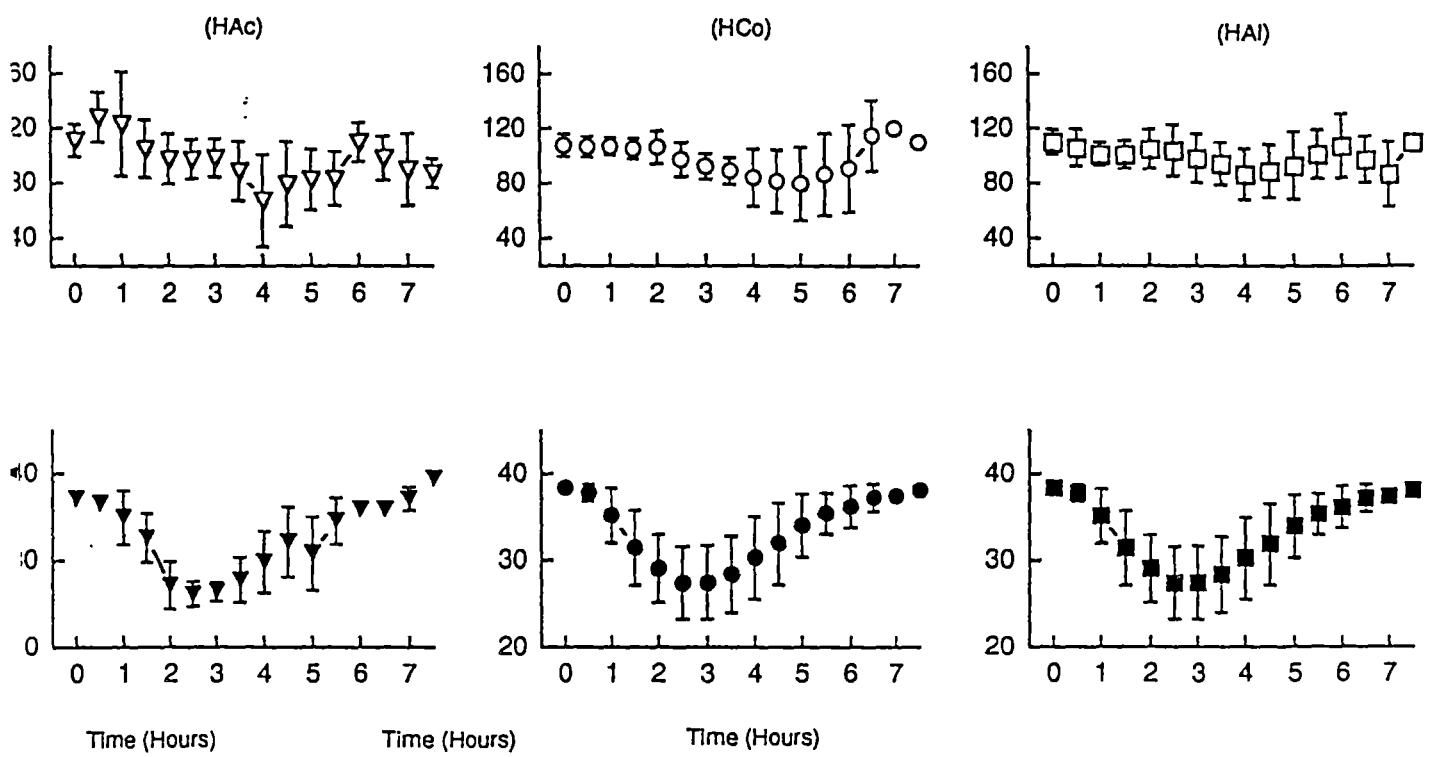


Fig 7.2.5 Changes of MAP at different body temperatures in three hypothermic groups (Mean ± SD)

7.3 Discussion

7.3.1 HR and Cardiac Contractility

In all hypothermic groups, HR decreased when body temperature was reduced and recovered when the temperature returned to normal. An overall correlation between HR and Tb for all data was highly significant ($n = 495$, $r = 0.9$, $P<0.01$). By comparison, HR was relatively constant in normothermic group. Goldman (1986c) demonstrated that hypothermia decreases the slope of phase 4 (the phase of depolarization of the SA action potential), which causes a reduced speed of depolarization and thereby slows the sinus rate. All intervals are prolonged.

When rewarming was commenced HCo and HAl animals showed an immediate increase in heart rate, which returned to the control rate by the third hour of rewarming. By contrast, HAc animals showed a delayed response to rewarming, with HR remaining low for the first 2 hours. Animals in this group did not retain a normal heart rate until the 4th hour of rewarming.

During the entire experimental period arterial pH value in HAl was always greater than that in HCo which in turn was greater than that in HAc. Acidification in HAc produces more H^+_o, which leads the movement of H^+ into the intracellular fluid from the extracellular fluid and is reflected by an increase of the extracellular $[K^+]$ (Masoro & Siegel, 1971a). The spontaneous rate of the sinus node is influenced by $[K^+]_o$. An increased extracellular $[K^+]$ will inhibit the outward flow of potassium (I_K: delayed rectifier potassium current, one of the major voltage-dependent potassium currents. Heidbuchel et al, 1990), and reduce the membrane potential. If this less negative membrane potential fails to be at a threshold potential of about -60 to -50 mv, the channel for the slow inward calcium current (I_{si} or I_{Ca}. Bean, 1985) will not be able to open. The rate of spontaneous diastolic depolarization in phase 4 of the action potential will fall, and sinus bradycardia will occur.

Harrison and Bers (1989) found that decrease in myofilament Ca^{2+} sensitivity occurred with decreased temperature in cardiac ventricular muscles of the frog, rat, rabbit and guinea pig. This could result in a decrease of myocardial contractility and occurrences of arrhythmias (Opie, 1991). An increase in resting tension and after-contractions in isolated ventricular muscles of rats and rabbits were observed when temperature was lowered from 37°C to about 15°C (Liu et al, 1990). Furthermore direct measurement of cytosolic free Ca^{2+} ($[Ca^{2+}]i$) with a fluorescent dye, Indo-1 has shown that $[Ca^{2+}]i$ in isolated rat ventricular myocytes increased significantly when temperature was decreased from 37C° to 15°C and 5°C (Liu et al, 1990). It is believed that a disturbance in regulation of intracellular Ca^{2+} is responsible for the cardiac arrhythmia and / or dysfunction in the cold (Liu & Belke, 1991). Several groups (Farber, 1981; Tani, 1990; Cranefield & Wit, 1979) have found that an abnormal increase of $[Ca^{2+}]$ can lead to energy depletion and membrane damage due to activation of energy-dependent processes and cellular phospholipases. Also a transient calcium inward current induced by excessive $[Ca^{2+}]i$ rise will trigger after-depolarizations and after-contractions leading to severe cardiac arrhythmias.

Katz and Hecht (1969) proposed that an intracellular acidosis decreased contractility because protons displaced calcium from binding sites on the thin contractile filaments. A very similar proposal stresses the retention of carbon dioxide during ischaemia, also acting by the production of intracellular acidosis (Cobbe & Poole-Wilson, 1980)

There is strong experimental evidence for favourable effects of both respiratory and metabolic alkalosis on cardiac contractility and more importantly, on the recovery of contractility after a period of prolonged ischaemia. At 37°C, the canine heart on cardiac pulmonary bypass increased contractility (dp/dt) incrementally with increases in pH, achieving a maximum at pH 7.7 (Streisand et al, 1971). Ebert (1962) found that in dogs cooled to 12°C, which were subject to 30 minutes of aortic occlusion, if the perfusate was made alkaline (pH 7.6) by the addition of $NaHCO_3$, post-occlusion myocardial contractility was markedly improved compared with dogs in the control group or dogs

given diluted hydrochloric acid. Wang and Katz (1965) reported that a myocardial acidosis induced by coronary infusions of DMO (5,5-dimethy1-2, 4-oxazolidinedione) and decreased myocardial contractile force. Experiments in skinned cardiac fibres have demonstrated that a decrease in pH to 6.2 results in a fivefold increase in the amount of free calcium required to produce half-maximal tension (Fabiato & Fabiato, 1978). In close agreement, ATPase measurements at pH 6.4 indicated a consequent fivefold increase in the free calcium concentration required to achieve maximal ATPase activation (Kentish & Nayler, 1979).

By comparison to the calcium desensitising effect of acidification, alkalinization may help to sensitise the myofilament (myocardium) to calcium and minimise the effect of hypothermia on calcium desensitisation. As a result, myocardial contractility could be maintained better under alkalinization. It is believed that a disturbance in regulation of intracellular Ca^{2+} is responsible for the cardiac arrhythmia and/or dysfunction in the cold. The results of this study showed that cardiac haemodynamics and ECG recovered better and faster after rewarming in pre-alkalinized animals of HAl than those of the other two groups, which suggested myocardium was functioning better under alkalinization.

Fleckenstein (1971) first emphasised the role of calcium overload in cardiac pathology. He proposed that calcium overload could damage myocardial cells by excessive splitting of ATP as a result of the increased activity of the contractile mechanism in response to calcium. Second, excess calcium may stimulate the phospholipase enzymes that break down cell membranes. Third, calcium overload may cause the development of contracture, which is a state of sustained excess contraction. Fourth, excess calcium cycling in and out of the sarcoplasmic reticulum may explain certain arrhythmias. Recently, Aoki et al (1994) indicated that hypothermia causes calcium to accumulate in the myocyte and an increase in intracellular calcium during ischaemia may worsen the effect of ischaemia and impair the postischaemic recovery of function. I assume that alkalinity may alleviate this hypothermic calcium overload by increasing myofilament calcium sensitivity and bonding, reducing the number of intracellular free calcium ions.

Perhaps this is the reason that HAl rabbits showed a better recovery in cardiac function (HR, MAP, CVP and CrdBF) than HAc and HCo animals.

7.3.2 Blood pressure, Blood Perfusion and Peripheral Resistance

Any changes in HR and cardiac performance will affect the cardiac output (CO), which in turn affects the blood pressure and organ blood perfusion. Another factor which will affect cardiac output is the peripheral resistance, attributable to the blood vessels and the blood itself.

Poiseuille's law (Guyton, 1991c) states:

$$Q = \pi \Delta P r^4 / 8\eta l \qquad (1)$$

Where Q is the rate of blood flow in millilitres per second, ΔP is the pressure gradient in dynes per square centimetre, r is the radius of the vessel in centimetres, η is the viscosity in poise, and l is the length of the vessel in centimetres.

In another expression of this law we have:

$$\Delta P = Q 8\eta l / \pi r^4 \qquad (2)$$

According to equation (2) if Q or η increase and r falls, ΔP will increase (usually l, the length of vessel, does not change).

An increased blood viscosity during hypothermia may be caused by:

a. The direct effect of low temperature on blood cell aggregation;

b. Haemoconcentration as a result of plasma loss;

c. The low-flow (low shear) state induced by hypothermia.

Previous studies showed that low temperature increased intravascular aggregation which became severe between 20-25°C (Schönbein et al, 1973; Bigelow et al, 1950b; Lofstrom, 1959) Chen and Chien (1978) studied haemodynamic functions and blood viscosity

changes at a core temperature of 25°C in 14 pentobarbital-anaesthetised dogs subjected to surface cooling. They found the viscosity of blood increased progressively to 173% of that at 37°C. During body cooling, plasma volume decreased progressively in parallel with the fall of body temperature and reached a level about 93% of the control value when deep body temperature reached 31°C (Morimoto et al, 1984). Wolf et al (1992) investigated the effects of cold temperatures on microvascular protein permeability in the isolated constant-flow perfused cat's hind limb. Their results indicated a significant increase in permeability under hypothermia in whole blood perfused cats. Zhang and Wolf (1991) observed that postcapillary resistance increased as the temperature was lowered. This led to an increase in microvascular hydrostatic pressure above the isogravimetric level, and fluid filtration ensued. Nose (1982) reported that the temporary rise in arterial pressure due to cold-induced sympathetic action causes a shift of a fraction of water from the active circulatory area. After the initial increase in arterial pressure, the colloid osmotic pressure was maintained while plasma volume declined further.

The low flow (low shear) rate induced by hypothermia could be another factor which changes the blood viscosity. The effects of shear rate were tested in normothermic and hypothermic situations by Chen and Chien (1978). When the measurement temperature was lowered to 25°C , they observed that if the shear rate was kept at 200 sec^{-1} (the control value used in normothermic experiment), the blood viscosity still increased by 12%. From the ratio of cardiac output during hypothermia and normothermia, the mean shear rate in the body at 25°C would be only one fourth of that at 37°C, i.e., approximately 50 sec^{-1}. At a temperature of 25°C, this reduction in shear rate causes the viscosity of the hypothermic blood samples to further increase by 39%. It is clear that a large portion of the increase in blood viscosity is attributable to the low-flow rate induced by hypothermia.

The present study showed that when the core temperature of the rabbit was lowered the heart rate and carotid blood flow fell linearly (Fig 7.2.2 and 7.2.3) in all hypothermic animals. However, the blood pressure showed different patterns in the three hypothermic

groups (Fig 7.2.5). During cooling, blood pressure in HAc showed an immediate decrease while heart rate dropped also. During rewarming the increase in heart rate was parallel with the increase in temperature whereas the arterial pressure declined again from the 6th hour of the experiment. The blood gases and pH showed that there was a severe acidosis in HAc during rewarming. This acidosis had a negative inotropic effect. Although there was a compensatory increase of heart rate, it still failed to bring the cardiac output back to a normal level. During rewarming, particularly in HAc, a lowered CVP (which in HAc was significantly different from all other groups (Fig 7.2.3)) indicated there may be a "pooling of blood" in the peripheral circulation, which could have resulted from acidosis-induced peripheral vasodilatation (Miki et al, 1983).

The results of HCo showed that hypothermia per se did maintain the MAP before the body temperature fell below 25°C (Fig 7.3) then MAP declined gradually. Rittenhouse et al (1971) used 11 adult mongrel dogs to investigate circulatory dynamics during surface-induced deep hypothermia and after cardiac arrest for one hour. Their results showed mean aortic pressure remained essentially unchanged in the range 38 — 30°C. At temperatures below this, it gradually declined, dropping to 112 mmHg at 25°C from an average of 123 mmHg at 38°C. Peripheral resistance gradually increased from 67 PRU {peripheral resistance units, mean aortic pressure (mmHg)/ cardiac output (L· per minute)} to 79 PRU as body temperature declined from 38°C to 30°C. Thereafter, resistance increased at a faster rate. At 20°C, it averaged 212 PRU, which was 310 per cent above the precooling control.

Compared to HCo, MAP in HAl rose although HR and CrdBF both fell during the cooling and one hour of steady hypothermia phase (Fig 7.4 and 7.5). These changes were similar to those of HR and CrdBF in HCo. This demonstrates that there was a great increase in the peripheral resistance, which is contributed to by the changes of blood viscosity and/or the radius of the vessel. That is, h increased and/or r decreased. It is assumed that if there were a similar decrease on blood viscosity in both HCo and HAl groups when Tb was low, then, the change of MAP in HAl must have been caused by a

longer and stronger effect of alkalinization in increasing vascular resistance. If there were different changes in blood viscosity, then at least, the alkalinization had stronger and longer influences on both blood viscosity and vascular tone than hypothermia per se. Despite these doubts the results strongly suggest that alkalinization increased peripheral resistance and maintained a higher blood pressure throughout the hypothermic period.

During rewarming, MAP fell slightly although HR and CrdBF both increased in both HCo and HAl. This implies that rewarming reduced blood viscosity and probably reduced vascular constriction. After rewarming MAP in both HAl and HCo returned to their control values while HAc declined rapidly. This demonstrates that pre-cooling acidification produced a more acidotic state after rewarming. This post-rewarming acidosis had deleterious effects on both peripheral circulation and cardiac function.

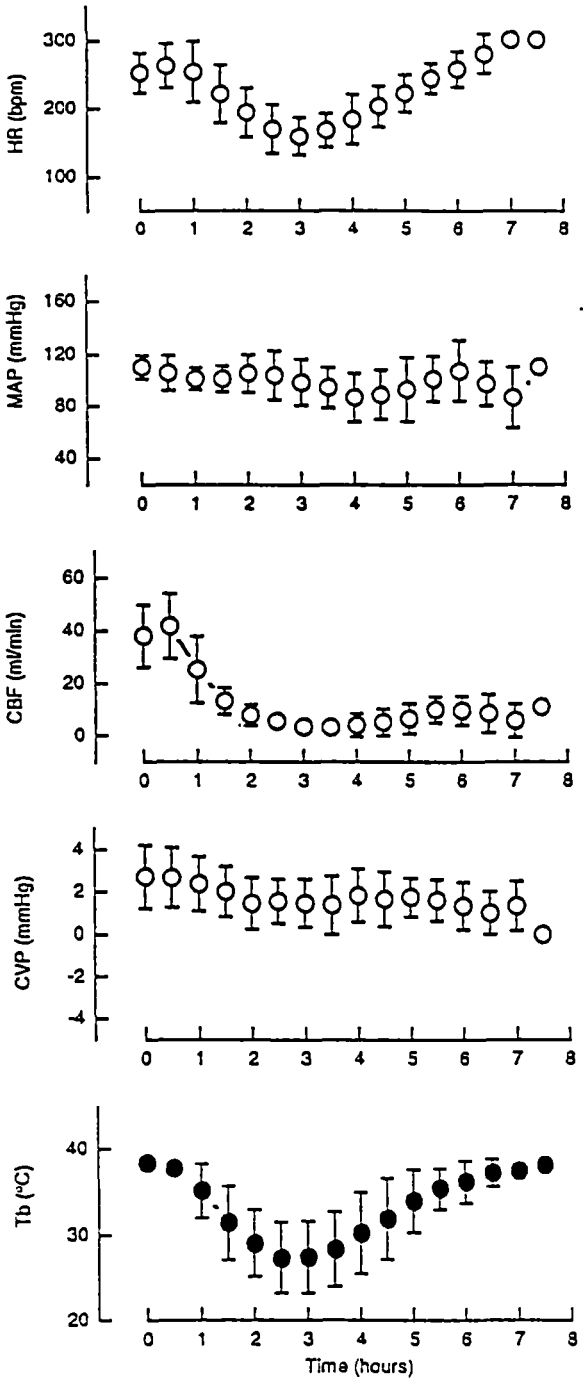


Fig 7.3 Changes of haemodynamics and body temperature in HCo (Mean = ± SD)

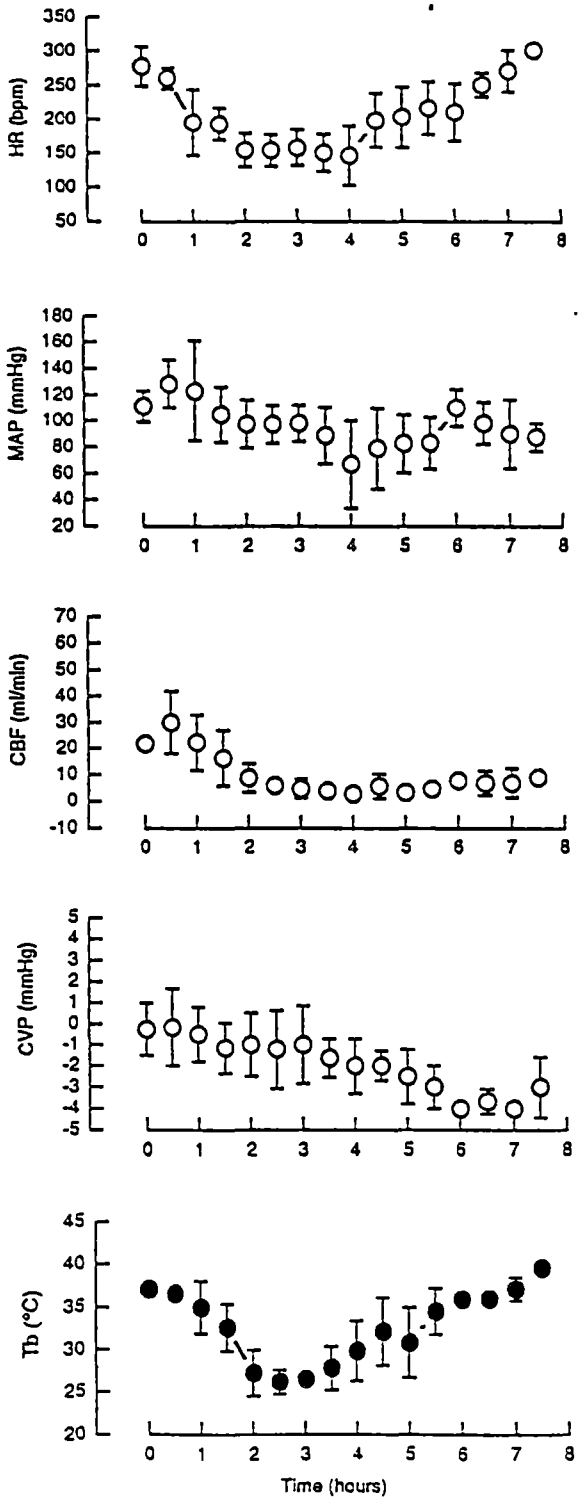


Fig 7.4 Changes of haemodynamics and body temperature in HAc (Mean = ± SD)

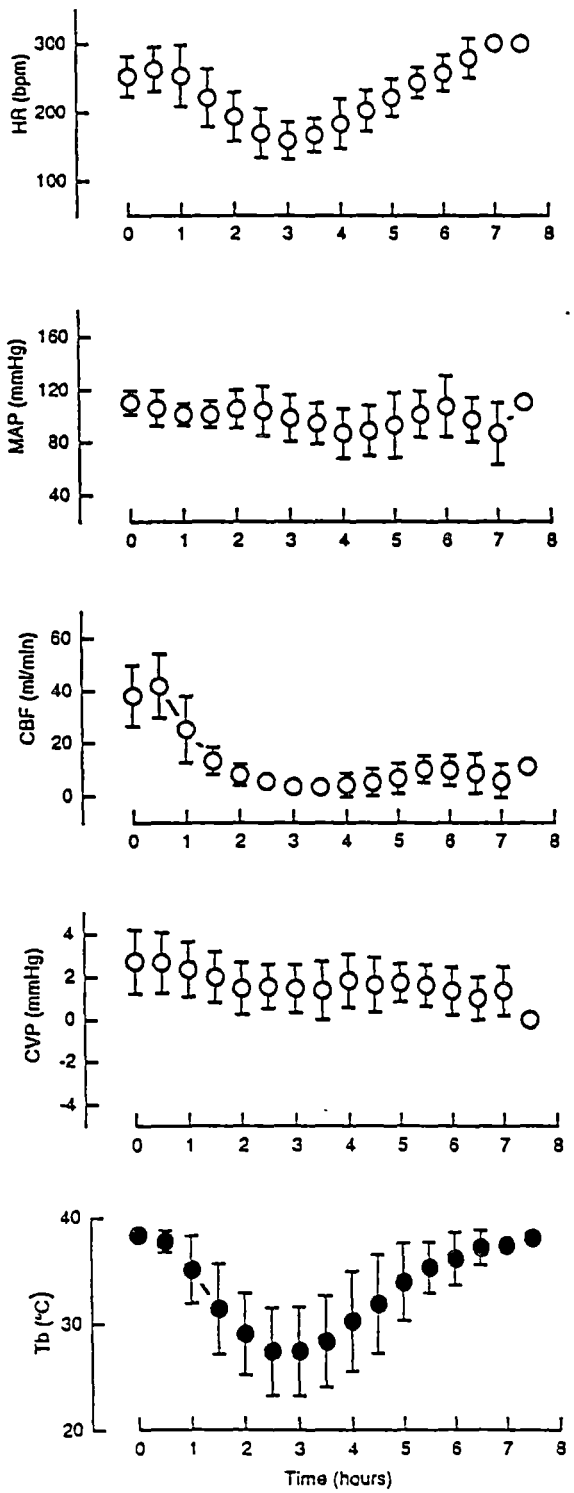


Fig 7.5 Changes of haemodynamics and body temperature in HAI (Mean = ± SD)

In conclusion, the results of this study showed that in HAl animals, HR recovered after rewarming; MAP was higher and stayed at a relatively constant level during hypothermia and returned to normal after rewarming; CrdBF dropped during hypothermia but showed an upward trend after rewarming; CVP showed a slight decrease when cooling started then gradually increased during rewarming. By comparison, in HAc animals CVP dropped sharply during cooling and stayed at a very low level after rewarming; MAP was unstable during hypothermia and declined linearly after rewarming; HR slowly returned to normal and CrdBF declined after rewarming. These results demonstrate that animals in HAl achieved a better cardiac haemodynamic status during one hour of hypothermia and a better recovery after rewarming. This indicated that a pre-alkalinization has a beneficial effect upon circulatory function during and after an induced hypothermia. It is probable that alkalinity preserves cardiac function and optimum blood distribution by (1) resisting a decrease in myofilament Ca^{2+} sensitivity with decreased temperature in cardiac ventricular muscles to protect cardiac contractility; (2) maintaining tone of peripheral vessels particularly under low temperature; (3) reinforcing the effect of hypothermia on the blood viscosity.

Chapter 8

Observations of the electrocardiogram in acidotic and alkalotic rabbits under low temperatures

8.1 Introduction

The electrocardiogram (ECG) is a graphic recording of the electrical potentials produced in association with the heartbeat. The ECG is of particular value in the following clinical conditions (Goldman, 1986a):

(a) Myocardial ischaemia and infarction.

(b) Arrhythmias.

(c) Disturbances in electrolyte metabolism, especially potassium abnormalities.

(d) Atrial and ventricular hypertrophy

(e) Pericarditis.

(f) Systemic diseases that affect the heart.

With the clinical application of total body hypothermia or cold cardioplegia it is essential that one is aware of untoward effects as evidenced in the ECG because cardiac function is depressed by low temperatures. Similar abnormalities occur in individuals accidentally exposed to cold (Dexter, 1990; Solomon et al, 1989). Sinus bradycardia supervenes at 32°C. Bradycardia and cold diuresis all reduce the circulating volume. As a result, oxygen delivery will be depressed. Although the body's oxygen demands are less during hypothermia, hypoxia coupled with metabolic acidosis and the irritability of cold myocardium may lead to various arrhythmias. These abnormalities are ST depression, flat to inverted T waves, marked prolongation of the Q-T interval, and prominent U waves. In the normal ECG, U waves may or may not be present (Seelig, 1992). The exact cause of this wave is unknown. It is currently thought to be the result of the slow repolarization of the intraventricular (Purkinje) conduction system (Goldman, 1986b)., but progressive slowing of the sinus nodal rate with cooling is masked early on by a sympathetically mediated tachycardia. Among these abnormalities, sinus bradycardia, AV junctional rhythm, and prolongation of the Q-T interval are common findings. With more

extreme degrees of hypothermia, an intraventricular conduction delay develops that is characterised by prominent notching of the terminal portion of the QRS complex (Osborn wave or J wave), which is also considered as a pathognomonic wave of hypothermia. When human rectal temperature falls below 28°C, ventricular fibrillation may occur. (Bashour et al, 1989; Clements, 1972).

Previous observations have shown that hypothermia is associated with decreased myocardial electrical stability (Lloyd & Mitchell, 1974; Covino & Hegnauer, 1955b). The propensity for ventricular fibrillation (VF) is measured by the VF threshold, which is defined as the smallest electrical stimulus applied directly to the heart that will initiate VF. Hypothermia leads to a decreased VF threshold and an increase in arrhythmia and fatal VF (Osborn, 1953; Covino & Hegnauer, 1955c). Rhythm abnormalities are probably due to the existence of transmyocardial temperature gradients (Mounitzen et al, 1965). At approximately 30°C, atrial irritability may provoke atrial flutter or fibrillation. Ventricular irritability increases with further cooling and ventricular ectopics are followed by ventricular fibrillation. Conduction disturbances accompany rhythm abnormalities (Solomon et al, 1989). His bundle electrocardiography has demonstrated that there may be a conduction defect at the level of AV node, which is fully reversible on rewarming (Bashour et al, 1989). Abnormal ECGs may be seen in association with cerebral disease, especially cerebral and subarachnoid haemorrhage (Abildskov et al, 1970).

Although there are many reports regarding the use of ECG with human beings, information on the ECG with hypothermic rabbits is quite limited. Such information would be beneficial in studies of cardiac function and some cerebral disorders.

For a better understanding of abnormal ECG it is important to review some essential concepts of electrophysiology and the normal ECG pattern

8.1.1 The electrophysiological basis of the ECG

8.1.1.1 Ventricular action potential

The characteristic appearance of the ventricular action potential can now be interpreted in terms of opening and closing of sodium, calcium, and potassium channels, with the resultant flow of the corresponding currents (Fig 8.1.1).

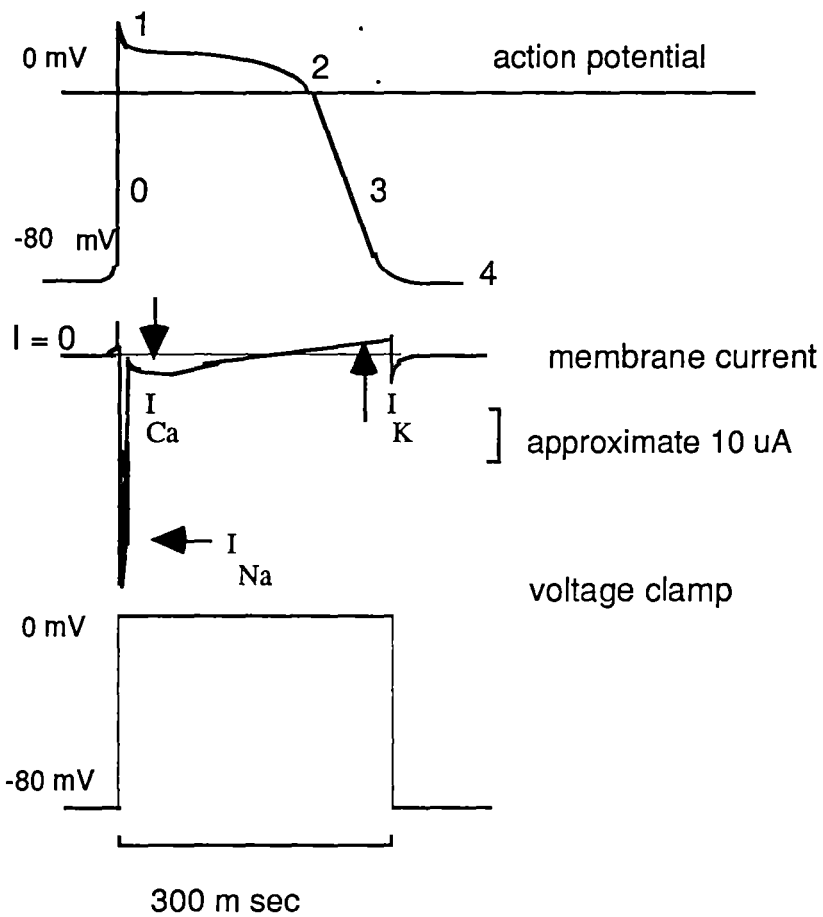


Fig. 8.1.1 Action potential of a ventricular muscle cell.

This diagram shows how the voltage-clamp technique can be used to explain the currents flowing during the cardiac action potential. When the voltage across the heart membrane is artificially fixed by the voltage-clamp technique, the change in current required to keep the voltage constant reflects the change in membrane conductance for various ions. If the voltage is changed in a steplike fashion (voltage-step), the patterns of the current required

to keep the voltage fixed allow a diagram of the currents to be constructed. The sodium current is triggered by a depolarizing clamp to a level more positive than -60 mV. When the voltage-step rises to above -30 mV, the L-calcium (slow-calcium) calcium current (I_{Ca}) is activated. Later, the outward potassium current is activated (Reuter, 1984).

When the cell membrane is penetrated by a capillary electrode, a negative potential of about 70 - 90 millivolts (mV) will be recorded (depending on the type of heart cell involved). This is known as the membrane resting potential (MRP). The major factor that determines the MRP is the gradient of the potassium ions (K^+) across the cell membrane. The intracellular concentration of K^+ is approximately 140 $mEqL^{-1}$, and the extracellular concentration is approximately 4 $mEqL^{-1}$. This K^+ gradient is 35:1. On the other hand, an opposite gradient exists for the sodium ions (Na^+). There is a relatively high extracellular Na^+ concentration in relation to intracellular Na^+ concentration. This Na^+ gradient, opposite in polarity to that of the K^+ gradient, does not appreciably alter the MRP because the cell membrane is considerably less permeable to Na^+ than to K^+. A group of special channel proteins in the cell membrane, called a "potassium-sodium 'leak' channel" is far more permeable to potassium than to sodium, normally about 100 times as permeable, which produces a resting membrane potential of approximately -70 — -90 mV (Guyton, 1991a; Opie, 1991)

Onset of depolarisation of a cardiac muscle cell (eg, a ventricular muscle cell) makes the membrane potential less negative, which opens the sodium channel activation gates at -70 to -60 mV and almost immediately initiate the events leading to delayed closing of the inactivation gates. Thus, sodium conductance first increases very rapidly, as does the flow of the inward current (I_{Na}), peaking within 1 msec and then falling off equally rapidly. This rush of inward sodium movement, carrying positive charges, fully depolarises the cell, causing the rapid upstroke, which is designated as phase 0 and represents the fast inward current typical of normal myocardial cells and Purkinje fibres. Pacemaker cells of the SA node and cells in the proximal region of the AV node are depolarised by a slow inward current of calcium. Under abnormal conditions, cells

whose fast inward current via sodium channels is inhibited can be depolarised by the slow inward current via calcium channels.

Following depolarisation, there is a relatively slow and gradual return of intracellular potential to the MRP (phase 4). This is repolarisation and is divided into 3 phases:

Phase 1: An initial rapid return of intracellular potential to 0 mV. This is largely the result of abrupt closing of the sodium channels. It has been suggested that chloride ions entering the cell may contribute to phase 1.

Phase 2: A plateau phase of repolarisation owing to a slow inward current of calcium (I_{Ca} or I_{si}) into the cell by L-calcium channel and T-channels while the sodium current fades away.

These two channels are the major subpopulations of calcium channels relevant to the cardiovascular system (Bean, 1985). The T-(transient) channels open at a more negative voltage (-60 to -50 mV), have short bursts of opening, and do not interact with calcium antagonists. The T-channels presumably account for the earlier phase of the opening of the calcium channel, because they open at a more depolarized voltage, which may also give them a special role in the early electrical depolarization of the sinoatrial node, and hence of initiation of the heart beat. In contrast, T-channels have much less activity in the ventricles. The L-(long-lasting) channels open at a less negative voltage, thus accounting for the later phases of calcium channel opening. The L-channels have two patterns in which their gates work (modes of gating). Mode 1 has short periods of opening, and mode 2 has longer periods of opening. Calcium antagonist drugs change the mode of opening of L-channels to a preponderance of short-acting channels, so that the amount of calcium entering through the channel is reduced. Thus, such drugs can have a negative inotropic effect on the heart or cause arteriolar dilation, or some of them can inhibit the sinus or atrioventricular nodes (Bean, 1985).

Phase 3: This represents the slow, gradual return of the intracellular potential to MRP. It results from flow of potassium ions out of the cell, which re-establishes the normal

negative resting potential. However, the cell is left with an excess of sodium ions and a deficit of potassium ions.

The factors leading to repolarization (phase 3) are not fully understood. One of the major proposals is that as a result of the initial depolarization (phase 0) and potassium currents are activated after a delay (I_{k1} and I_K), thereby terminating the action potential. Second, the calcium gate shuts possibly by buildup of intracellular calcium ions (Tseng, 1988) or by a delayed voltage-operated mechanism, hence stopping inflow of positive ions and promoting repolarization. In arterial tissue, a third and less favoured possibility is that the calcium current while it flows switches on the rectifying potassium current (Giles & Imaizumi, 1988). Once the action potential is over, the resting membrane potential is restored and maintained (phase 4). During this diastolic phase of electrical rest, the activity of the sodium-potassium pump and the various exchange systems restore ionic balance across the sarcolemma.

Phase 4: To restore the original ion concentration, a cell membrane sodium-potassium pump mechanism becomes effective. The energy required for this pump is derived from conversion of ATP to ADP. This pump removes sodium from the cell and permits potassium influx.

8.1.1.2 Normal ECG

As shown in Fig 8.1.2, waves, complex, and intervals express the cardioelectric activities in rabbit.

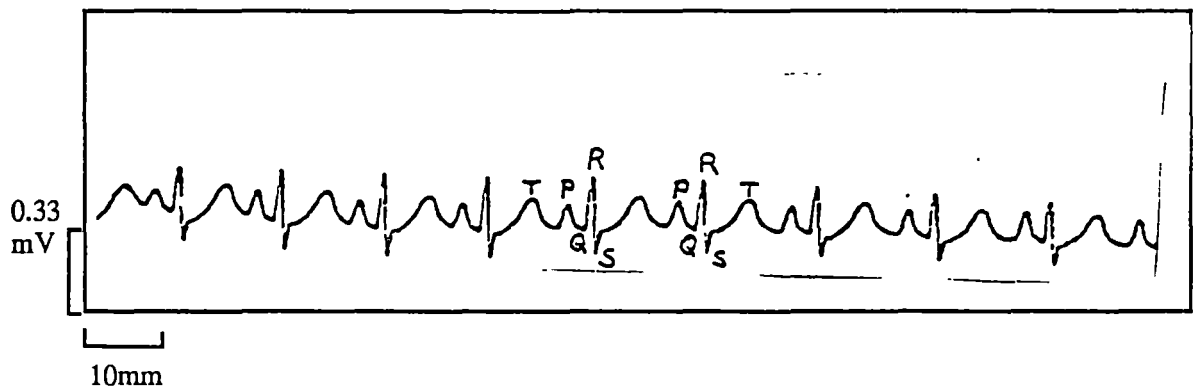


Fig 8.1.2 A normal ECG pattern of rabbit

8.1.1.3 Intracellular potential of heart muscle and ECG

Because of the mass of the heart muscle, the changes in membrane potential can be detected on the body surface as the ECG. The summation of all phase 0 potentials of atrial myocardial cells results in the P wave of the ECG. All phase 0 potentials of ventricular muscle cells produce the QRS complex. Phase 2 correlates with the ST segment and phase 3 with the T wave of the ECG (Fig 8.1.3). The duration of this action potential varies from an atrial muscle cell to a Purkinje fibre (Fig 8.1.4). The duration of action potential is longer in a Purkinje fibre than in any other site. This is due to prolongation of phase 2 and 3 and results in the U wave of the ECG .

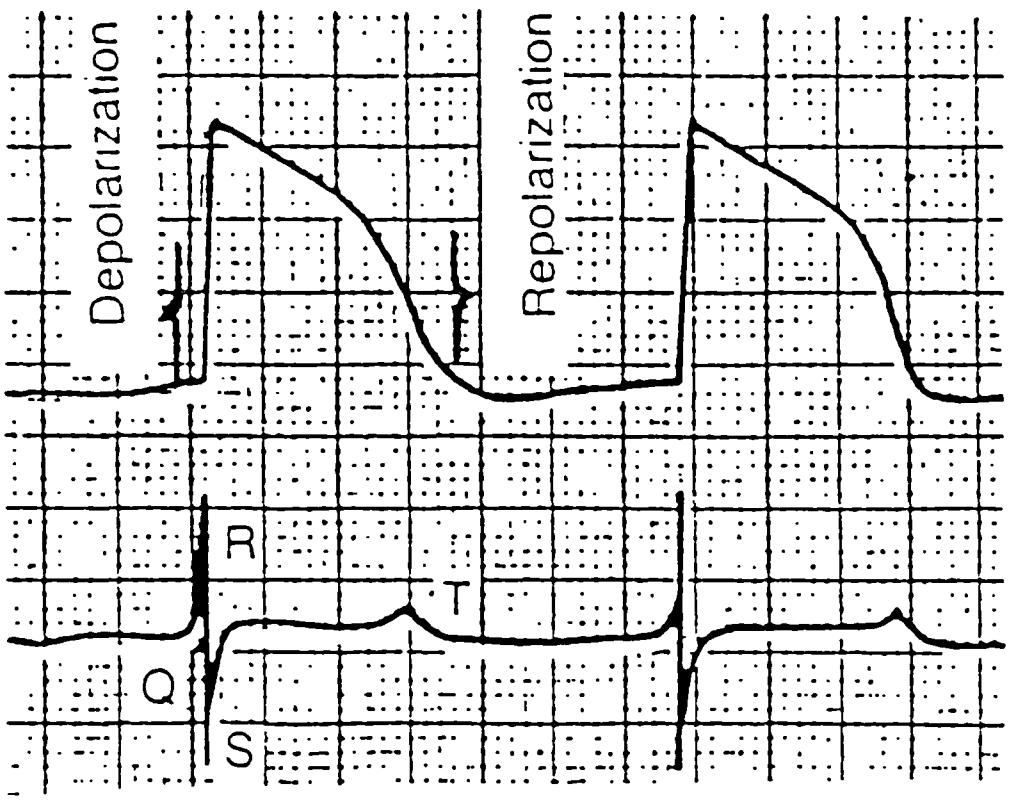


Fig 8.1.3. Monophasic action potential from a ventricular muscle fibre during cardiac function, showing depolarization and then repolarization. Recording paper speed is 25 mm /second (Goldman, 1986b).

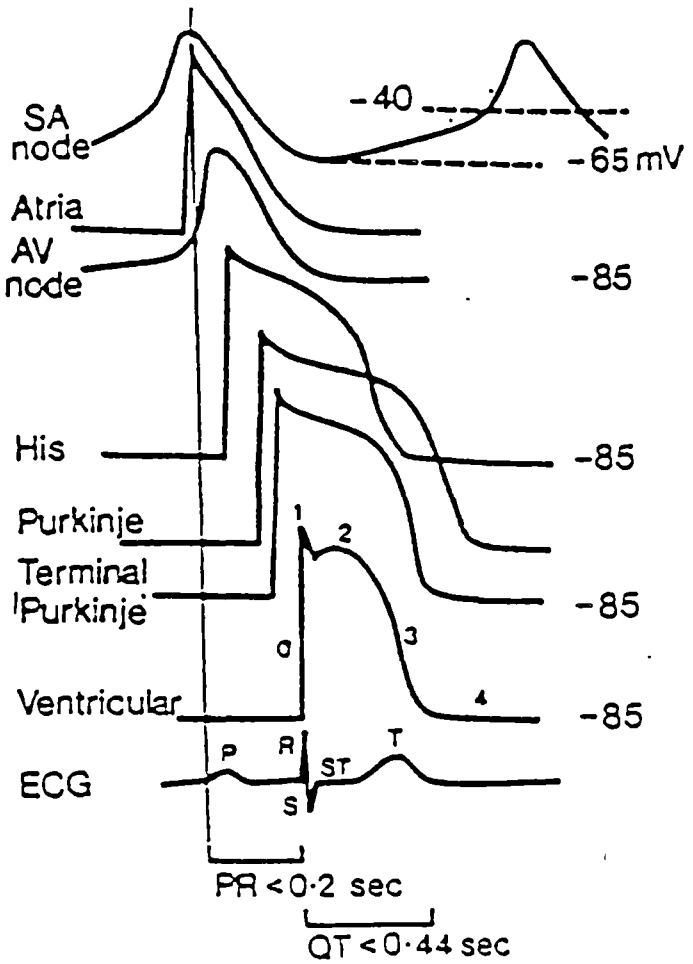


Fig 8.1.4 The patterns of the cardiac action potential in different sites. Electrocardiogram recorded simultaneously (Opie, 1991).

8.2 Results

8.2.1 Normal rabbit ECG

Two measurements were taken for each rabbit during control monitoring period. Normal ECG values of anaesthetised, ventilated rabbits are as follows (Mean ± SD, n = 22 and total 44 measurements. :

P-P or R-R interval	0.27 s ± 0.03
P-R interval	0.09 s ± 0.01
QRS	0.08 s ± 0.01
Q-T	0.20 s ± 0.03

P wave is always positive in lead II.

8.2.2 Hypothermic rabbit ECG

Two groups—one hypothermic acidotic, the other alkalotic, were investigated in this study through five distinct phases:

* phase 1. Control: the normothermic period before cooling (Temperature = 37±1.0°C);
* phase 2. Cooling: during the period of decreasing body temperature;
* phase 3. Steady hypothermia: body temperature was maintained at 25 ± 0.5°C for one hour;
* phase 4. Rewarming: during the period of increasing body temperature;
* phase 5. After rewarming: when the body temperature had returned to 37± 1.0°C.

The following results are copies of some original records from each group. The recording paper speed was 25 mm·second^{-1} (Goldman, 1986b) and a calibration signal of 0.33 mv gave a 10 mm deflection.

8.2.2.1 Hypothermic Acidotic group (HAc)

(a). Phase 1 — Normothermia

The HR was 216 ± 37 bpm (beats per minute) at body temperature of 37 ± 1.0°C with a regular sinus rhythm; PP was 0.28 ± 0.05s. PR was within 0.1 s (0.085 ± 0.009); QRS was from 0.06 to 0.1s (0.077 ± 0.013). S-T interval and T wave were normal. Q-T interval was 0.2 ± 0.03s. Fig 8.2.1 shows an ECG record during the control period.

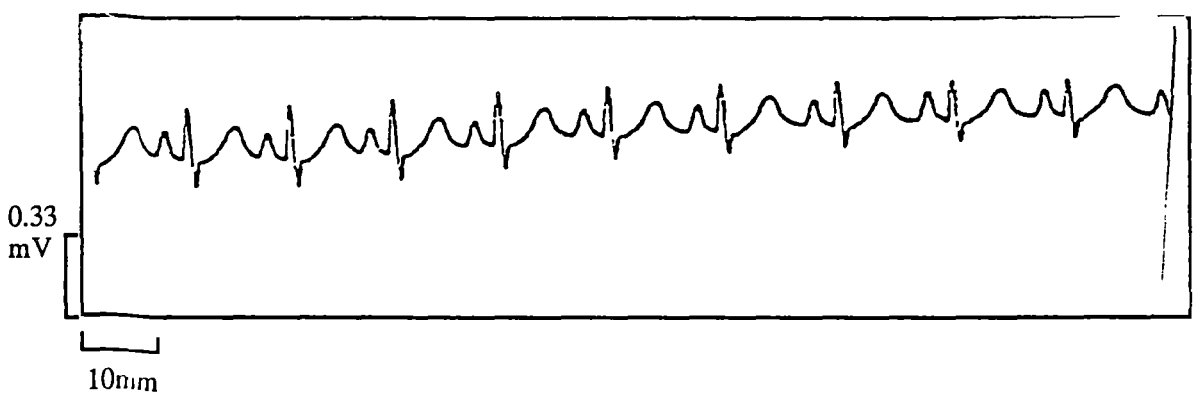


Fig 8.2.1 Normal ECG in acidosis group
Regular sinus rhythm; HR = 200 bpm; P-P = 0.3 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.2 s

(b). Phase 2 — Cooling

The heart rate decreased with body temperature. Q-T interval was prolonged from 0.2s ± 0.03 to 0.23s ± 0.03. T waves became tall and peaked when the temperature reached 30°C approximately. ST segments were depressed among 5 of 10 animals.

One case presented AV junctional escape beats. Sinus arrhythmias were seen in approximately half of the animals. Figs 8.2.2-4 show ECG records during cooling.

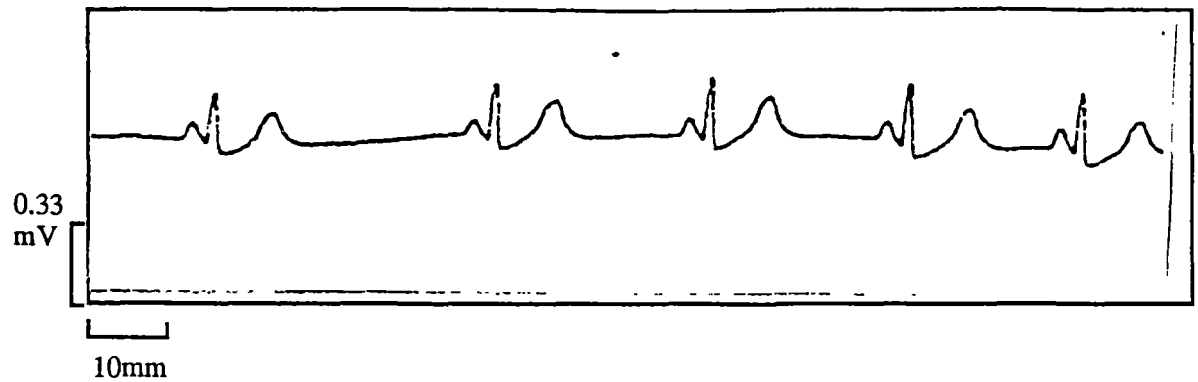


Fig 8.2.2 Sinus arrhythmia

P-Ps are irregular; P-R = 0.08 s; QRS = 0.06 s; Q-T = 0.24 s

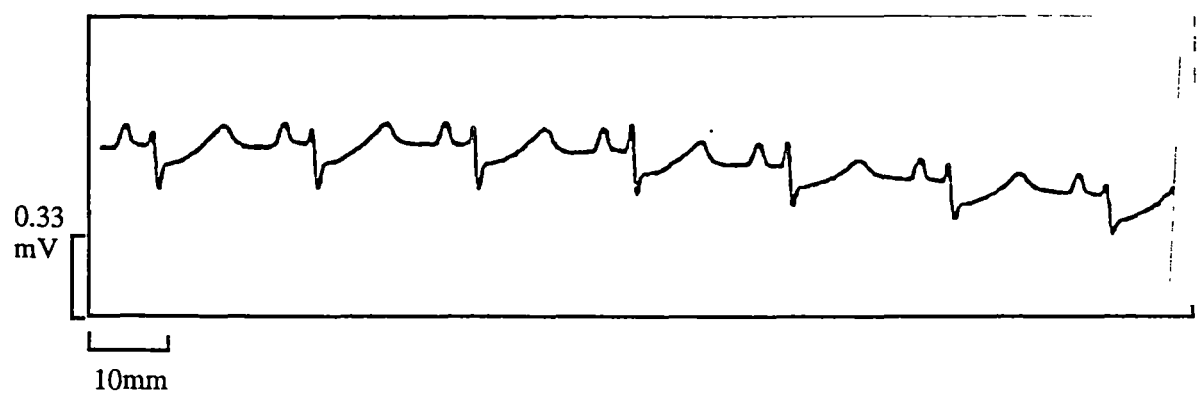


Fig 8.2.3 Sinus bradycardia

Regular sinus rhythm; P-P = 0.44 s; P-R = 0.08 s; QRS = 0.08 s; Q-T = 0.28 s. S-T segments are depressed obliquely.

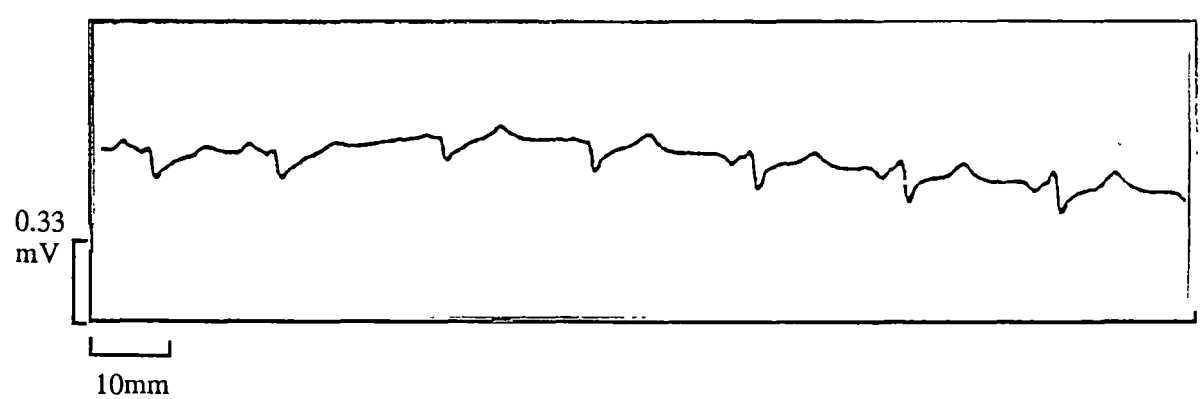


Fig 8.2.4 AV junctional rhythm

P wave is inverted; P-P = 0.32 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.24 s.

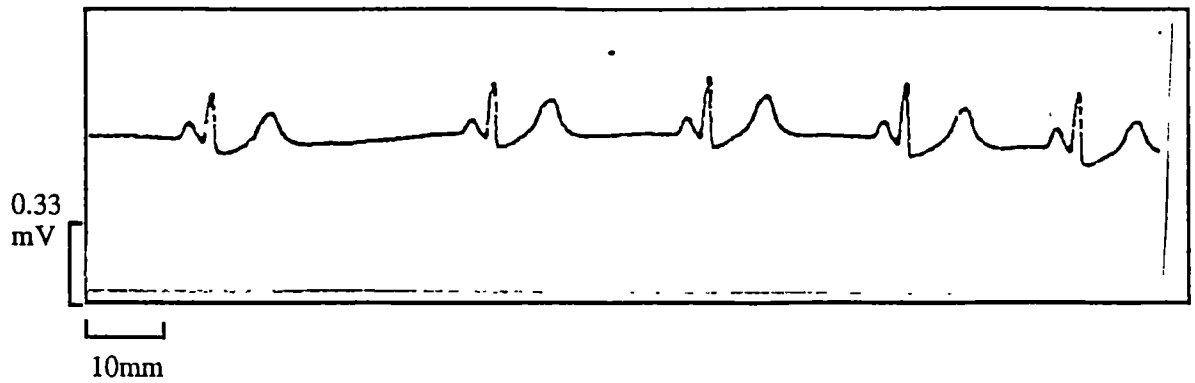


Fig 8.2.2 Sinus arrhythmia

P-Ps are irregular; P-R = 0.08 s; QRS = 0.06 s; Q-T = 0.24 s

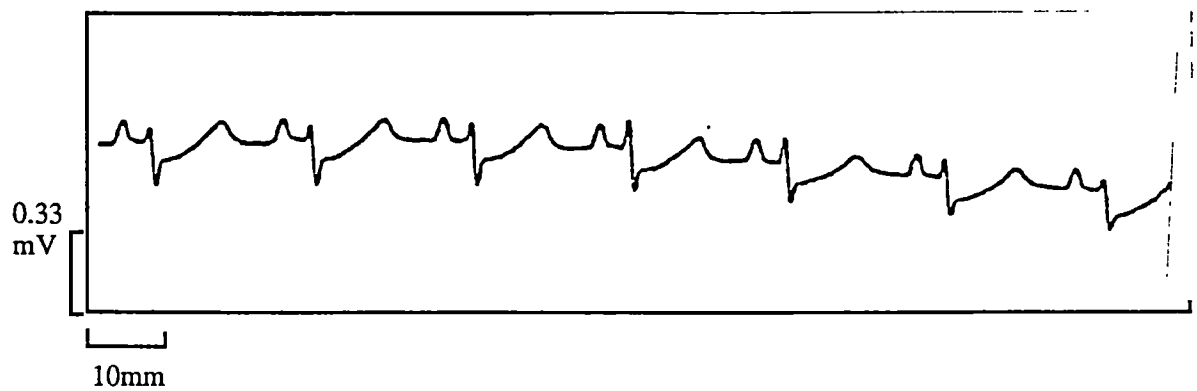


Fig 8.2.3 Sinus bradycardia

Regular sinus rhythm; P-P = 0.44 s; P-R = 0.08 s; QRS = 0.08 s; Q-T = 0.28 s. S-T segments are depressed obliquely.

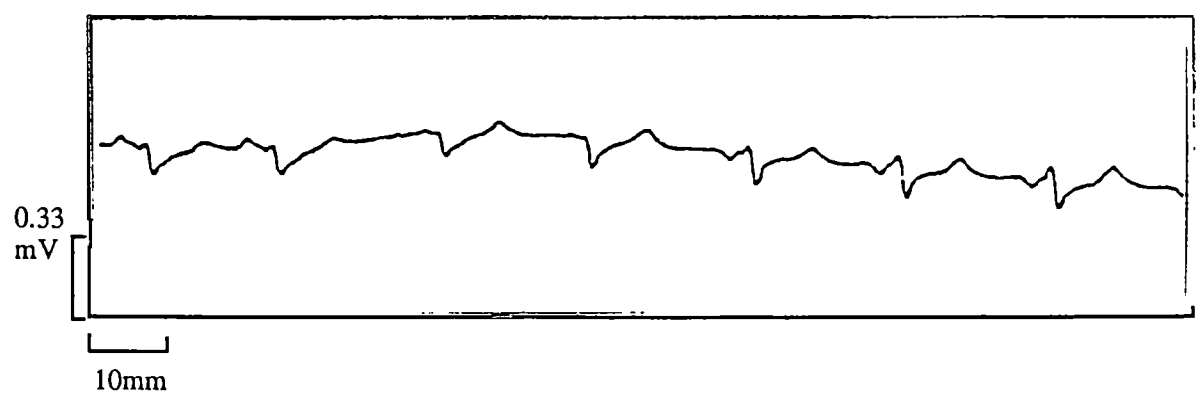


Fig 8.2.4 AV junctional rhythm

P wave is inverted; P-P = 0.32 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.24 s.

(c) Phase 3 — Hypothermia

Heart rates decreased markedly from the control value of 216 ± 37 bpm. The average value was 128 ± 15 bpm ($p<0.01$). QRS values ranged from 0.06 to 0.1s. Q-T intervals were prolonged considerably. The average was at 0.31 ± 0.01s ($p<0.01$). T waves were abnormally tall, peaked and slender. ST segments displayed a variety of depressions which were oblique, horizontal and scooped, from -0.03 mV (1 mm) to -0.06 mV (2 mm). Arrhythmias were seen in two of ten cases. Fig 8.2.5-7 show ECG records of two cases during one hour hypothermia.

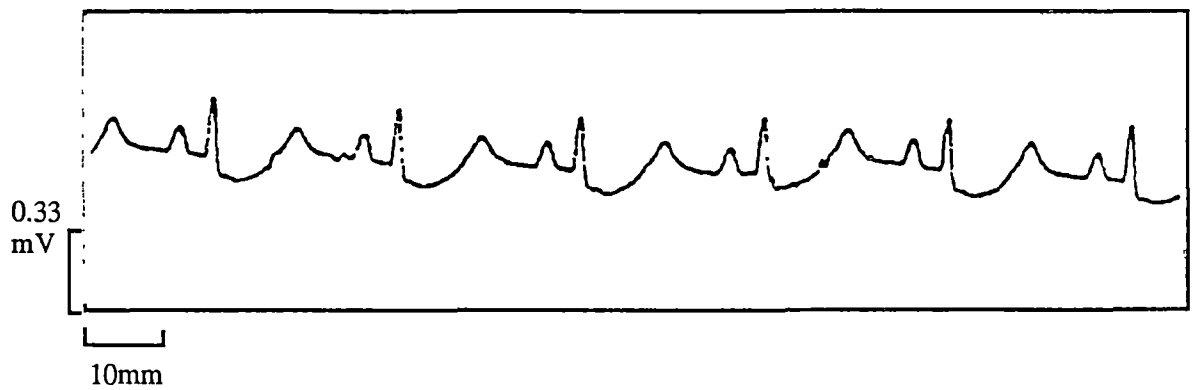


Fig 8.2.5 Sinus bradycardia

Regular sinus rhythm; P-P = 0.5 s; P-R = 0.08 s; QRS = 0.08 s; Q-T = 0.36 s; S-T segments are depressed as scooped pattern.

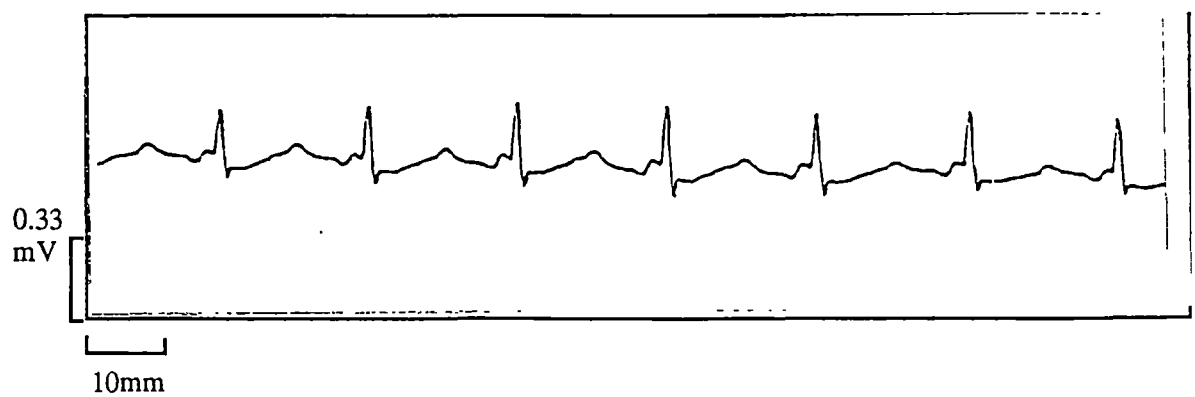


Fig 8.2.6 AV junctional rhythm

P wave is inverted; P-P = 0.32 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.24 s. S-T segments are depressed horizontally.

Fig 8.2.7 AVB with IVB

P-P = 0.36 s; R-R is greatly prolonged and irregular; P-R is irregular; QRS and Q-T are prolonged and irregular.

(d) Phase 4 — Rewarming

Heart rates gradually increased. The average value was 0.27 ± 0.04s. ST segments depressed to -0.034 mV (1.1 mm). Abnormal T waves were prominent, displayed as a tall, slender, peaked configuration. Atrioventricular block (AVB), intraventricular block (IVB) were observed. Three animals in this group showed an atrioventricular junction rhythm (AV junction rhythm) and sinus arrhythmia. Fig 8.2.8-10 show ECG records of three cases during rewarming.

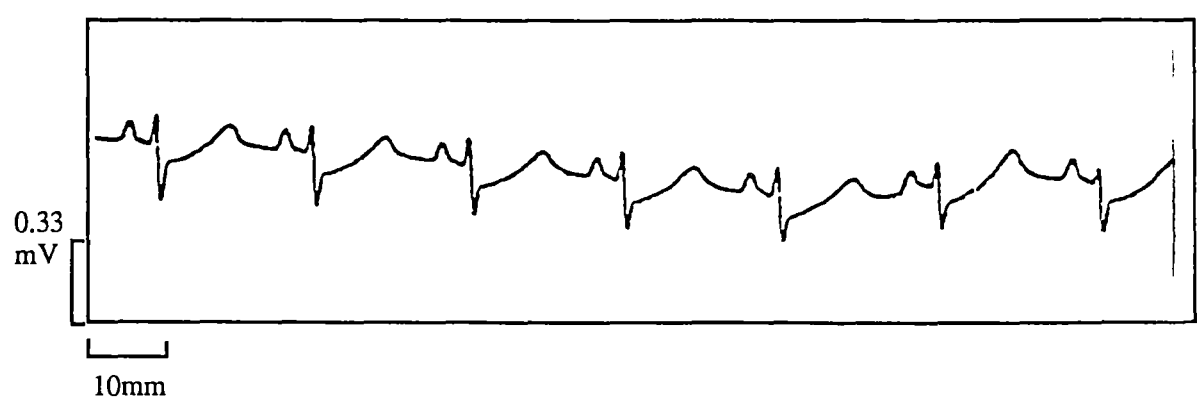


Fig 8.2.8 Sinus bradycardia

Regular sinus rhythm; P-P = 0.4 s; P-R = 0.08 s; QRS = 0.08 s; Q-T = 0.24 s. ST segment is depressed.

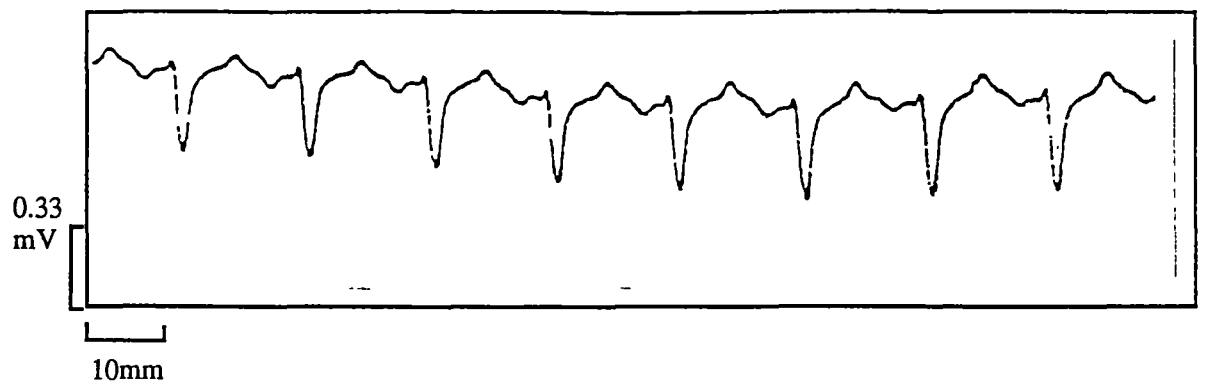


Fig 8.2.9 AV junctional rhythm

P wave is inverted; P-P = 0.34 s; P-R = 0.12 s; QRS = 0.08 s; Q-T = 0.24 s.

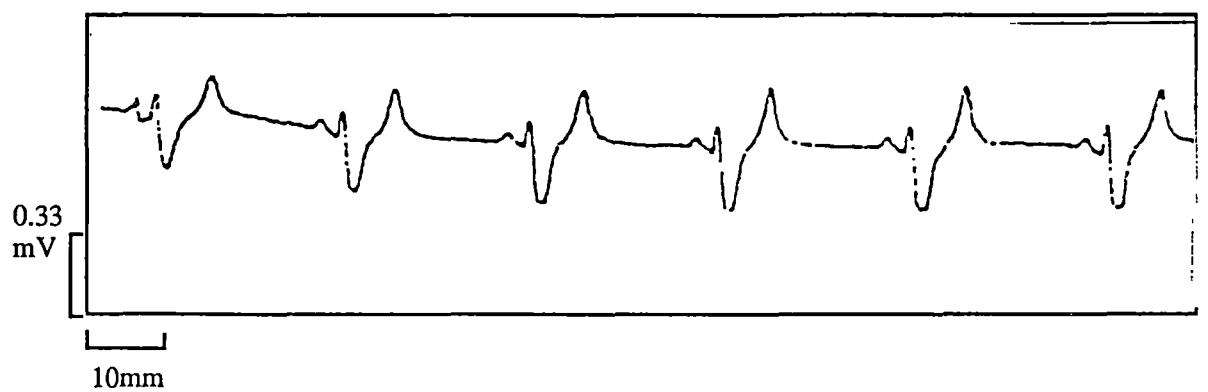


Fig 8.2.10 Intraventricular block (IVB)

Regular sinus rhythm; P-P = 0.5 s; P-R = 0.08 s; QRS = 0.12 s; Q-T = 0.24 s.

T wave is tall, peaked and "tent"-like.

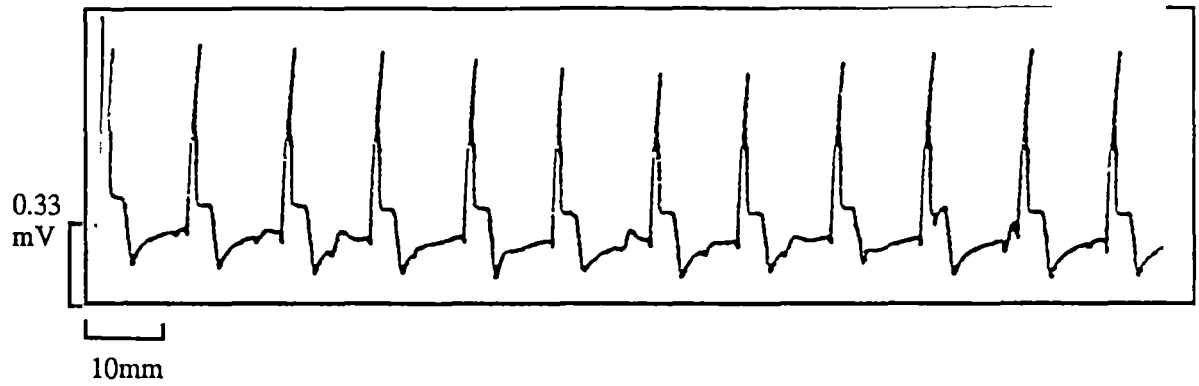


Fig 8.2.11 "Osborn" waves or "J waves", which occur at the end of QRS complex and just overlapping the beginning of the S-T segment (Goldman, 1986c; Leatham et al, 1991). The animal died soon after this recording was made.

(e) Phase 5 — After rewarming

After the body temperature had returned to normal, only three rabbits survived to this stage. One of the survivors had a normal ECG with HR of 215 bpm, PP interval of 0.28s, PR interval of 0.1s, QRS of 0.08s and Q-T interval of 0.2s. The other had different degrees of supraventricular tachycardia and atrial fibrillation with IVB, respectively. ST segment and T wave recovered well. Figs 8.2.12-14 show ECG records from each of these three rabbits after rewarming.

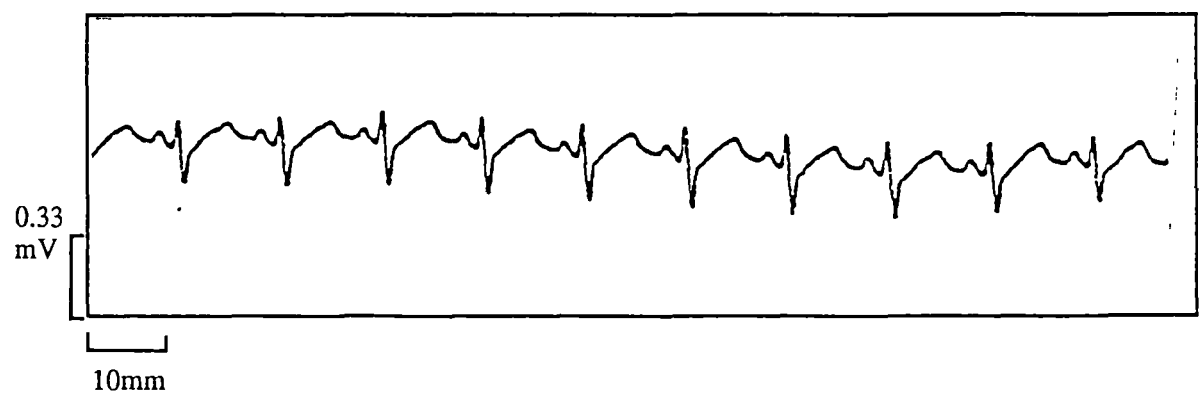


Fig 8.2.12 Recovered ECG

Regular sinus rhythm; HR = 215 bpm; P-P = 0.28 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.2 s.

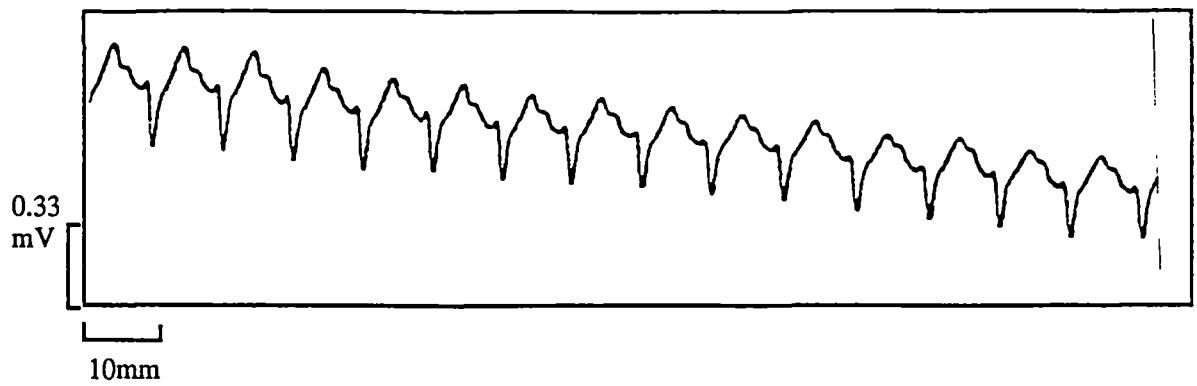


Fig 8.2.13 Supraventricular tachycardia

HR = 300; P-P = 0.2 s; P-R = 0.08 s; QRS = 0.08 s; Q-T = 0.16 s.

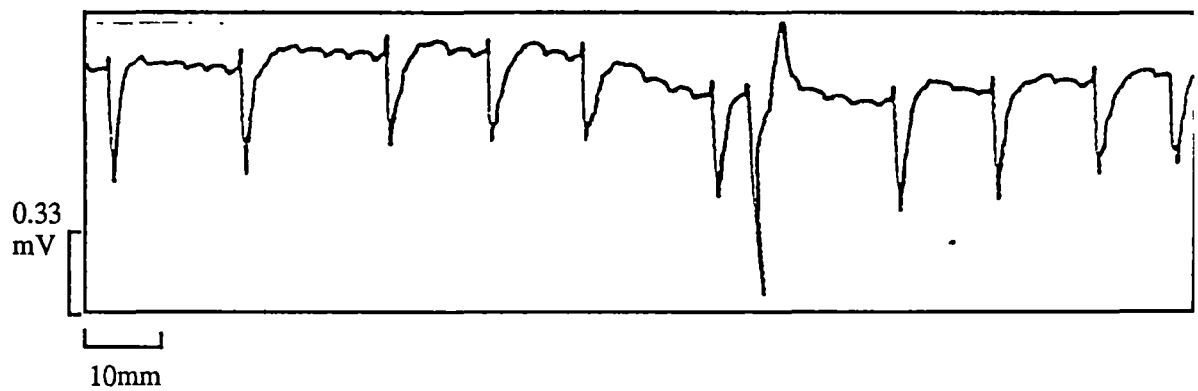


Fig 8.2.14 Atrial fibrillation with IVB. The animal died soon after this recording was made.

8.2.2.2 Hypothermic Alkalotic group (HAl)

(a) Phase 1 — Normothermia

HR was 235 ± 29 bpm during the control phase at a body temperature of 37 ± 1.0°C with a regular sinus rhythm; PR was within 0.1 s, from 0.08 to 0.1; QRS was 0.08 ± 0.009s. Q-T interval was 0.2 ± 0.03s. S-T interval and T wave were normal. Fig 8.2.15 shows an ECG record during control period.

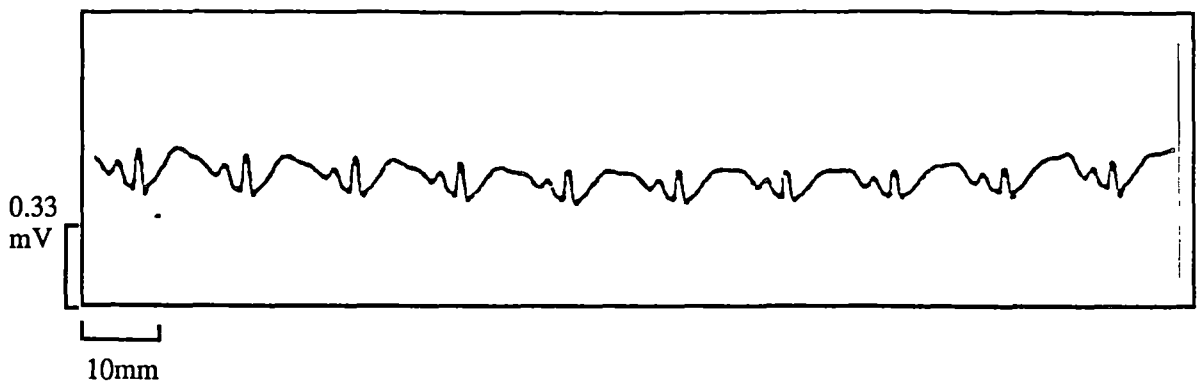


Fig 8.2.15. Normal ECGs in alkalosis group

Regular sinus rhythm; HR = 215 bpm; P-P = 0.28 s; P-R = 0.1 s; QRS = 0.06 s; Q-T = 0.2 s.

(b) Phase 2 — Cooling

Heart rate decreased (from 235 ± 29 to 191 ± 15 bpm) as body temperature was reduced. Q-T interval was prolonged from 0.2s ± 0.03 to 0.26s ± 0.03. ST segment showed a depression of -0.049 mV (1.5 mm). Regular sinus rhythm and normal conduction were seen. Fig 8.2.16 shows an ECG record during cooling.

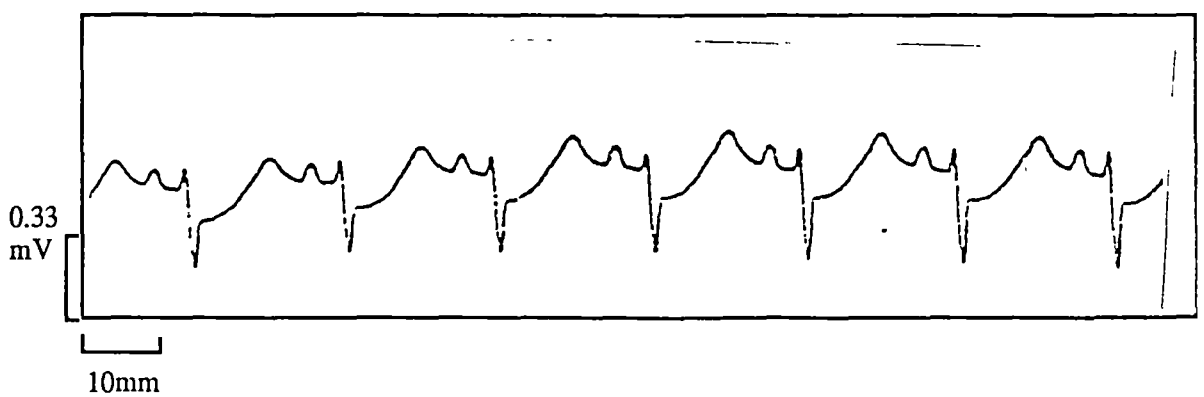


Fig 8.2.16. Sinus bradycardia

Regular sinus rhythm; P-P = 0.4 s; P-R = 0.1 s; QRS = 0.1 s; Q-T = 0.36 s. S-T segments are depressed obliquely.

(c) Phase 3 — Hypothermia

Heart rates decreased markedly from 235 ± 29 to 136 ± 12 bpm ($p<0.01$). QRS ranged from 0.08 to 0.12s. ECGs from two animals showed an increase in QRS magnitude

during this period. Q-T intervals were prolonged greatly from 0.2s ± 0.03 to 0.33s ± 0.04 ($p<0.01$). T waves were abnormally biphasic, tall, peaked and slender. ST segments displayed a variety of depressions that were oblique, horizontal and scooped, from -0.03 mV (1 mm) to -0.1 mV (3 mm). In one case, a U wave was observed. Arrhythmias occurred in one case only. Most of the animals retained sinus rhythms. Fig 8.2.17-18 show ECG records of two cases during the one hour of profound hypothermia.

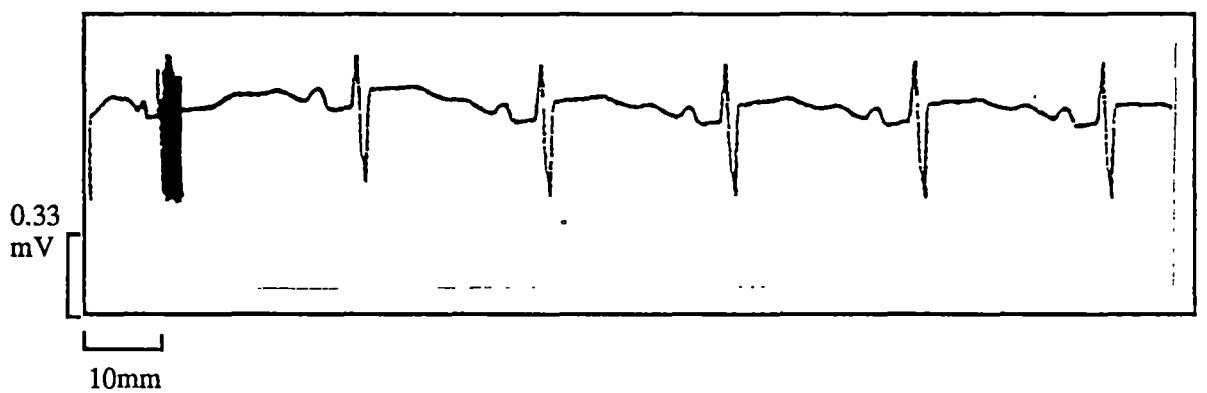


Fig 8.2.17. Sinus bradycardia

Regular sinus rhythm; P-P = 0.5 s; P-R = 0.12 s; QRS = 0.1 s; Q-T = 0.28 s. Note the U wave (A deflection, usually positive, seen following the T wave and preceding the next P wave). The exact cause of this wave is unknown. It is currently thought to be the result of the slow repolarization of the intraventricular conduction system (Goldman, 1986b; Leatham et al, 1991).

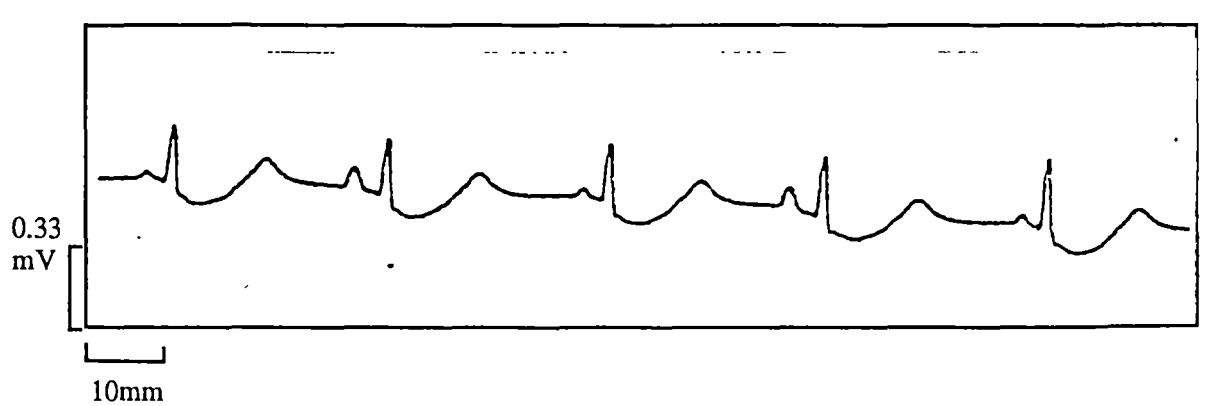


Fig 8.2.18 Sinus arrhythmia

P-Ps and P-Rs are irregular; QRS = 0.08s; Q-T = 0.38s. S-T segments are depressed as a scooped pattern.

(d) Phase 4 — Rewarming

Heart rates gradually increased before the body temperatures reached normal. ST segment depressed to -0.046 mV (1.5 mm). Abnormal T waves were prominent, and displayed mainly as a tall pattern. Atrioventricular block (AVB) was observed. Two of this group showed an atrioventricular junction rhythm (AV junction rhythm). Atrial tachycardia and ventricular bigeminy were seen in the other two cases. Figs 8.2.19-21 show ECG records of three cases during rewarming.

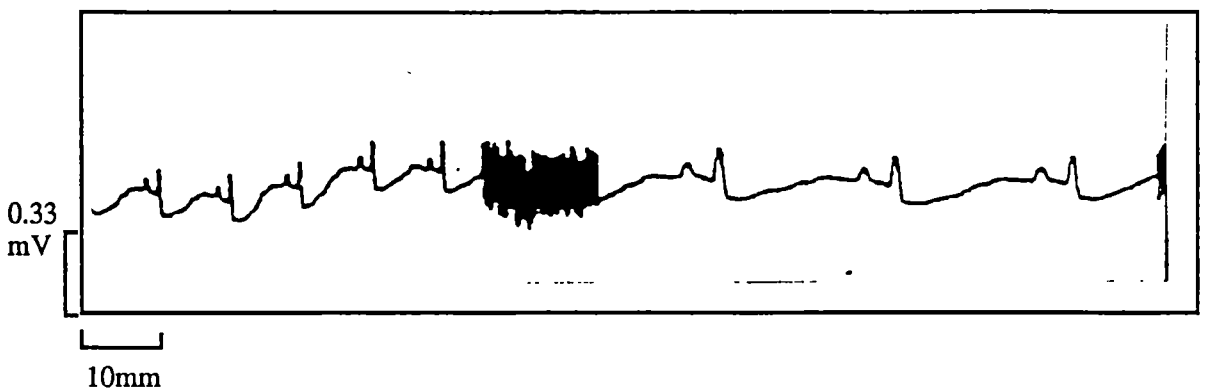


Fig 8.2.19 Sinus bradycardia

Regular sinus rhythm; P-P = 0.46 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.3 s. ST segment is depressed obliquely.

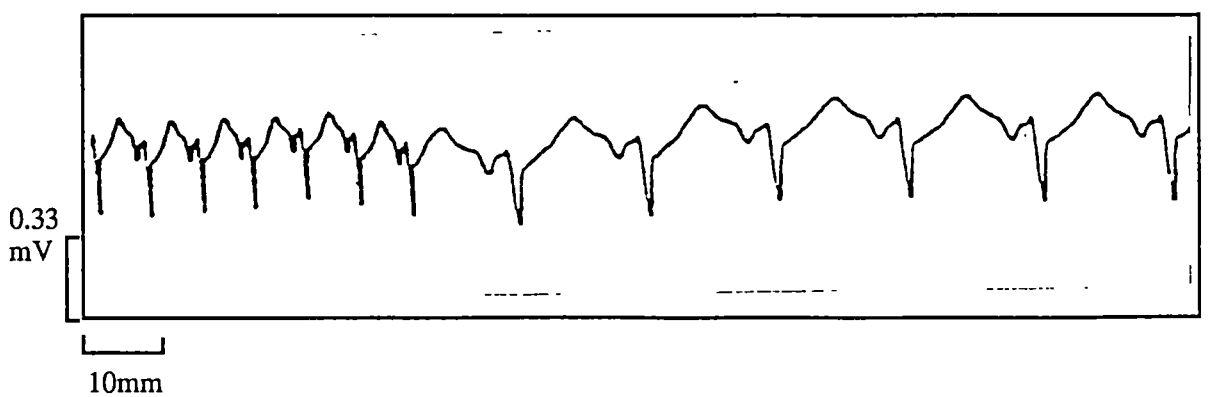


Fig 8.2.20 AV junctional rhythm

P wave is inverted; P-P = 0.34 s; P-R = 0.1 s; QRS = 0.08 s; Q-T = 0.26s.

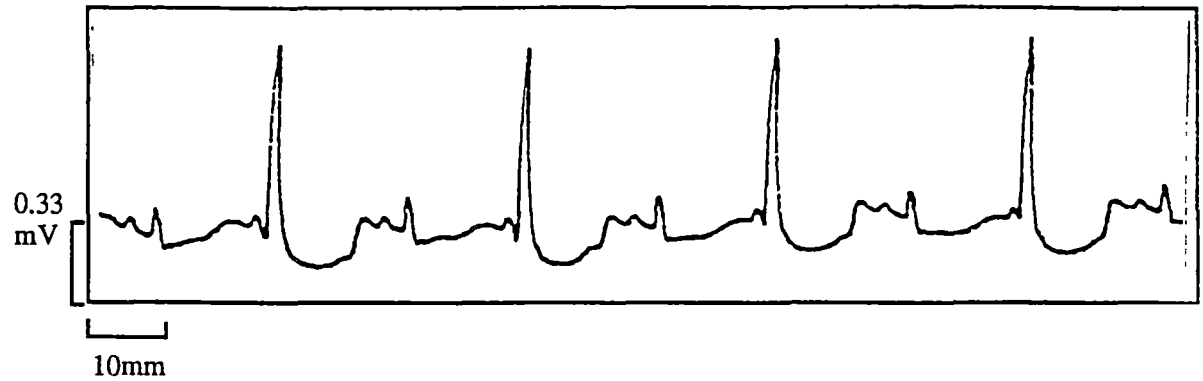


Fig 8.2.21 Ventricular bigeminy

The rhythm alternates between a regular sinus beat and a ventricular premature beat. There is a constant interval between the sinus beat and the ventricular premature beat. P-P = 0.34 s; R-R = 0.68 s; QRS = 0.08 s; Q-T = 0.3 s.

(e) Phase 5

Compared to the control value at the beginning of the observation, rabbits regained a normal ECG with a sinus rhythm that showed HR of 265 ± 27 bpm (P>0.05), PR interval of 0.08s ± 0.17 (P>0.05), QRS of 0.08s ± 0.013 (P>0.05) and Q-T interval of 0.2s ± 0.008 (P>0.05). ST segments and T waves recovered well. Fig 8.2.22 shows an ECG record after rewarming.

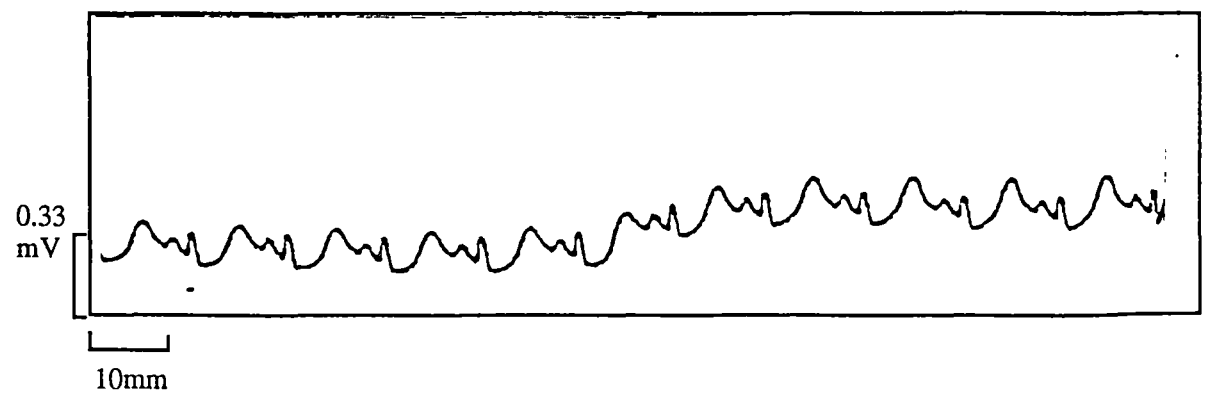


Fig 8.2.22 Recovered ECG

Regular sinus rhythm; HR = 230 bpm; P-P = 0.26 s; P-R = 0.1 s; QRS = 0.06 s; Q-T = 0.2 s.

Table 8.2. ECG intervals and ST voltages in two groups (Mean ± SD)

Group	Treatment	HR (bpm)	PP/RR (s)	PR (s)	QRS (s)	Q-T (s)	ST (mV)
Alkalosis	Control (n=12)	247±30	0.24±0.02	0.08±0.01	0.08±0.01	0.18±0.02	
	Cooling (n=12)	191±15 a**	0.31±0.05 a**	0.1±0.01	0.09±0.01 b**	0.26±0.03 a**, b*	– 0.05±0.01
	25.5°C (n=12)	136±12 a**	0.45±0.04 a**	0.12±0.01	0.09±0.01	0.32±0.04 a**	– 0.03±0.01
	Rewarming (n=8)	193±40 a**, b*	0.32±0.07 a**, b*	0.1±0.01	0.08±0.01	0.26±0.06 a**	– 0.05±0.01
	After rewarming (n=6)	265±27	0.22±0.02	0.08±0.01	0.08±0.01	0.18±0.02 a**	
Acidosis	Control (n=10)	216±37	0.28±0.05	0.09±0.01	0.08±0.01	0.2±0.03	
	Cooling (n=10)	189±28 a**	0.33±0.06 a**	0.09±0.01	0.07±0.01 b**	0.23±0.03 a**, b*	– 0.04±0.01
	25.5°C (n=10)	128±15 a**	0.48±0.05 a**	0.1±0.01	0.09±0.01	0.3±0.01 a**	– 0.04±0.01
	Rewarming (n=4)	158±32 a**, b*	0.38±0.07 a**, b*	0.1±0.02	0.08±0.01	0.28±0.05 a**	– 0.03±0.01
	After rewarming§ (n=3)	215	0.28	0.1	0.08	0.16	

"a" — comparison within group; "b" — comparison between groups.

* P<0.05, ** P< 0.01

§ The data shown are from the only rabbit in this group that retained a normal ECG. The other two had supraventricular tachycardia and atrial fibrillation with IVB, respectively. Their intervals and ST changes are irregular and not shown in this table.

8.3 Discussion

8.3.1 PP, PR, QT intervals and duration of QRS complex

Hypothermia causes fairly characteristic ECG changes. During the period of cooling and profound hypothermia, my results show that PP, PR and Q-T intervals were prolonged as body temperatures were reduced. Hypothermia decreases the slope of phase 4 (the phase of depolarization of the myocardial action potential), which causes a reduced speed of depolarization and thereby slows the sinus rate. All intervals are prolonged (Goldman, 1986c; Lin et al, 1994). The duration of QRS complex was prolonged during cooling and steady hypothermia in this study. The QRS complex corresponds to the spread of depolarization through the ventricles. The cause of a prolonged QRS complex is a slowing of spread of the impulse through the ventricles. Guyton (1991b) suggests hypothermia decreases permeability of the muscle membrane to the ions, thus slowing conduction. Statistical analysis showed that there were no significant differences in PP and PR intervals between the alkalosis and acidosis groups in the present study. The QRS complex and QT interval were significantly longer in HAl than those in HAc, which suggested that alkalinization reinforced the effect of hypothermia on the QRS complex and QT interval. A slow spreading of the ventricular electric activity was particularly observed in this study with pre-alkalinization. By contrast, it seems that A-V node was not affected by either pre-acidification or pre-alkalinization because there was no significant prolongation of PR interval. During rewarming, the prolongations of all intervals were gradually reduced and completely recovered in the HAl when the body temperatures returned to normal. Most rabbits in HAc did not survive long enough to recover completely. Three survived until the end of the experiment. Only one rabbit retained a normal ECG. The other two had supratachycardia and atrial fibrillation with IVB, respectively compared to HAl group. HAc rabbits had much lower pHa and pHv, with values of 7.07±0.06 and 6.99±0.08. This acidosis might decrease calcium sensitivity of the myofilaments and cause a rise of cytosolic calcium (Marban et al, 1990;

Steenbergen et al, 1990). Consequences of the increased calcium level could increase depolarization which could evoke supratachycardia (Owen et al, 1990).

8.3.2 ST segment

In both alkalosis and acidosis groups the ST segment was depressed obliquely, horizontally or as a "scooped" pattern during hypothermia. The depression of ST segment was -0.04 mV ± 0.01(-1 to -1.5 mm) in the acidosis group and -0.05 mV ± 0.01 (-1 to -2 mm) in the alkalosis group. When the body temperatures were reduced to 25.5°C ± 0.5, the depression of the ST segment was less in the alkalosis group than that in the acidosis group (-0.03 mV ± 0.01 and -0.04 mV ± 0.01, $P < 0.07$). This depression lasted through the rewarming period within the acidosis group. Six of the alkalosis group returned to normal baseline during the late period of rewarming.

It has been suggested that the ST depression could be produced by currents of injury from the subendocardium, which is believed more sensitive to changes of oxygen tension, temperature and pH (Klocke, 1976). Under physiological conditions, in conscious dogs, the ratio of endocardial to epicardial blood flow averaged throughout the cardiac cycle is approximately 1.25:1 as a consequence of preferential dilatation of the subendocardium because calculated wall stress and oxygen consumption are greater in this region than in the subepicardium. There could be mal-distribution of transmural blood flow and metabolic impairment of subendocardial tissue, even though net transmural blood flow may remain near normal (Brazier et al, 1974).

As myocardial ischaemia occurs, hypoxia coupled with metabolic acidosis could firstly affect the subendocardium. The injured area is electrically negative in relation to normal resting muscle. The overlying electrode will record a depressed baseline which shows a depression of ST segment with the standard ECG. McConnell et al (1975) reported that at a temperature of 28°C there was significant increase in coronary blood flow, left ventricular O_2 consumption and lactate utilisation in the canine heart at pH 7.7 by comparison with pH 7.4. The results of the alkalosis group show that the changes of S-T

segments are less serious than those in the acidosis group. At the end of the experiment most animals in the alkalosis group survived normally with a well-recovered ST segment. This implies that, to a certain extent, alkalosis could protect the myocardium from injury by retaining the intracellular K^+ with reducing K^+-H^+ exchange, or by increasing the lactate utilisation. The occurrence of U wave (Fig 8.2.17) in HAl group also gives another clue to this assumption (see 3.4. U wave for more details).

8.3.3 T wave.

The T wave was always tall, slender and peaked in the acidosis group after temperature was reduced. By contrast, only one animal in the alkalosis group had a tall T wave during the period of cooling. When the temperature was reduced to 25.5°C ± 0.5 the majority of both groups displayed tall, slender and peaked T waves. This T wave change suggests that there might be an elevated extracellular potassium level.

Data in Chapter 7. showed that a reduction in carotid blood flow during cooling was caused by reduced myocardial contractility and heart rate, resulting in a low cardiac output, which caused an associated fall in coronary blood flow as well. This may then have led to an acute myocardial ischaemia. Such an ischaemia can activate and open the potassium channels that normally are inhibited by ATP (ATP-sensitive K^+ channel, Kubota et al, 1993) then increase the membrane permeability to potassium. Additionally the membrane undergoes a phase transition from a more liquid to a less liquid state as the temperature is reduced (McMurchie et al, 1973; Charnock et al, 1980), which could influence a wide variety of membrane-associated functions including the activation of sodium-potassium ATP pump (Na^+-K^+-ATPase) during hypothermia. Na^+-K^+-ATPase extrudes sodium out of the cell and potassium into the cell against the electrochemical gradients to restore the resting heart cell membrane after membrane depolarization (Opie, 1991). When the activity of this pump decreases during hypothermia, excess K^+ in the extracellular space could accumulate gradually after membrane depolarization. Acidosis can promote this accumulation. An increase of extracellular potassium may then prolong the repolarization and show a high-voltage T wave. This might be one of the reasons that

most of the acidosis group showed a tall, slender and peaked "tent"-like T wave pattern but only two within the alkalosis group presented a tall pattern during rewarming.

Metabolism increased when Tb increased. Metabolic products would have accumulated because of a poor washing-out when the local perfusion has not yet been improved soon after rewarming started. This mismatching of metabolism and temperature during rewarming could easily create severe tissue acidosis particularly in HAc, leading to hyperkalemia and producing a "tent"-like pattern of T wave.

8.3.4 U wave

U waves were observed in a few cases of the alkalosis group. As mentioned before, the exact cause of this wave is unknown. Currently it is thought to be the result of the slow repolarization of the Purkinje fibre. Presumably, the extracellular alkalinization during hypothermia in the HAl animals decreased the potassium conductance and reduces the speed of repolarization, resulting in U waves. Furthermore, whether the U wave could be used to estimate acid-base buffering activity during acid-base treatment is worth further investigation.

8.3.5 Conduction disturbances and arrhythmias

There were no conduction disturbances observed during hypothermia. Two cases from both groups showed atrioventricular block (AVB) over the period of the rewarming. When the body temperatures had returned to normal all animals of the alkalosis group showed a normal ECG with normal conduction and sinus rhythm.

Arrhythmias were detected during hypothermia mainly within the acidosis group. The types included sinus arrhythmia, AV junctional rhythm and escape beats. Only one animal within the alkalosis group had sinus arrhythmia. Previous observations have shown that hypothermia is associated with decreased myocardial electrical stability (Lloyd & Mitchell, 1974; Covino & Charlson, 1955a; Covino & Hegnauer, 1955b). This leads to a decrease of ventricular fibrillation threshold (when this threshold decreases,

ventricular fibrillation is easily evoked) and an increase in arrhythmia and fatal ventricular fibrillation (Osborn, 1953; Covino & Hegnauer, 1955c). Swain and his group (1984) showed the values of the ventricular fibrillation thresholds (VFT) between alpha-stat and pH-stat groups were significantly different. In the alpha-stat group VFT did not show a significant difference between normothermia and hypothermia (23.7±3.2 mA versus 22.8±2.8 mA, P>0.05). Five of their eight dogs in the pH-stat group had spontaneous VF (ventricular fibrillation) while being cooled , while only one of the seven in alpha-stat group had spontaneous VF. This implies that there is less chance of spontaneous VF during cooling under more alkaline conditions. The existence of transmyocardial temperature gradients could contribute to rhythm abnormalities as well (Mouritzen et al, 1965). At approximately 30°C, atrial irritability may provoke atrial flutter or fibrillation. Ventricular irritability increases with further cooling and ventricular ectopics are followed by ventricular fibrillation. It has been suggested that hypothermia may impair coronary autoregulation and affect oxygen delivery (McConnell et al, 1977). Lack of oxygen coupled with metabolic acidosis and the intrinsic irritability of a cold myocardium may lead to various arrhythmias, including several types and degrees of heart block, atrial fibrillation, ventricular fibrillation, and asystole. Fuquay et al (1962) and Best (1961) suggested that the following factors might cause conduction disturbances and arrhythmias during hypothermia:

(a) During cooling there would be a shift to the left in the oxygen-haemoglobin dissociation curve that would make less oxygen available to the tissues.

(b) Differential cooling may be an important factor contributing to the metabolic acidosis.

During rapid total-body cooling, there is a temperature lag in certain regions. This will produce acidosis in those tissues with the greater oxygen demand. Liu and Belke (1991) indicated that the cardiac arrthymia typically observed in hypothermia may be due to an increased cytosolic $[Ca^{2+}]$. Excessive $[Ca^{2+}]i$ rise induces a transient inward current that will trigger after-depolarizations and after-contractions leading to severe cardiac arrhythmias (Farber, 1981; Tani, 1990; Cranefield & Wit, 1979). There were fewer cases

of arrythymias and abnormal conduction during cooling and hypothermia in HAl than in HAc. This implies that alkalinization may protect the myocardium from excessive $[Ca^{2+}]i$ and stabilise the cellular membrane, thereby reducing the occurrences of hypothermic conduction abnormalities and arrhythmias.

During rewarming, animals in acidosis group displayed a variety of arrhythmias including sinus arrhythmia, atrial tachycardia, and AV junctional rhythm. AVB and IVB (intraventricular block) were found. Two rabbits showed a large, slowly inscribed terminal portion of the QRS complex or "Osborn" wave that appeared before the animals died. In the alkalosis group, AVB, AV junctional rhythm and ventricular bigeminy were seen. After Tb returned to normal six animals were still surviving in the alkalosis group and retained a normal ECG. In acidosis group three animals survived after Tb returned to normal. However, only one of them retained a normal ECG. The other two had atrial fibrillation with IVB and supraventricular tachycardia respectively. They died soon after rewarming. Blood gases showed animals in HAc had severe acidosis that could cause a consistent rise in cytosolic calcium, which in turn will lead to activation of phospholipases, increased depolarization, and mitochondrial damage (Steenbergen et al, 1990). Finally this acidotic condition is harmful to the survival of the ischaemic myocardium (Bing et al, 1973). By contrast, animals in HAl, which regained normal pH, showed a better recovery of cardiac electric activities after rewarming.

8.4 Summary

8.4.1 Normal ECG values from this investigation are as follows:

P-P or R-R interval	0.27 s ± 0.03
P-R interval	0.09 s ± 0.01
QRS	0.08 s ± 0.01
Q-T	0.20 s ± 0.03

P wave is always positive in lead II.

8.4.2 All intervals were prolonged in both groups during cooling and rewarming periods. The alkalosis group retained normal intervals when the body temperature returned to normal.

8.4.3 Depression of ST segments and "tent" T waves were common during hypothermia and rewarming. As the temperature increased the depression of ST segments became less and T waves tended to a normal pattern. Animals in the alkalosis group achieved a normal configuration of ST segment and T wave.

8.4.4 Conduction disturbances and rhythm abnormalities occurred more frequently and were more severe in the acidosis group than in the alkalosis group. Pre-alkalinization reinforced the slow-down effect of hypothermia on intraventricular conduction but seemed to have no effect on A-V node and junction area. When the temperature returned to normal, animals of the alkalosis group showed no sign of conduction disturbances and arrhythmia. The presence of J, or Osborn, waves were occasionally seen in the acidosis group.

8.4.5 The sinus node seems to be strongly affected by hypothermia.

8.4.6 The depression of S-T segment, prolongation of Q-T interval and peaked T wave are obvious during hypothermia. This suggests that repolarisation is greatly affected by low temperature.

8.4.7 Proper pre-alkalinization could be beneficial to myocardium preservation.

Chapter 9

Cerebral function during and after induced hypothermia

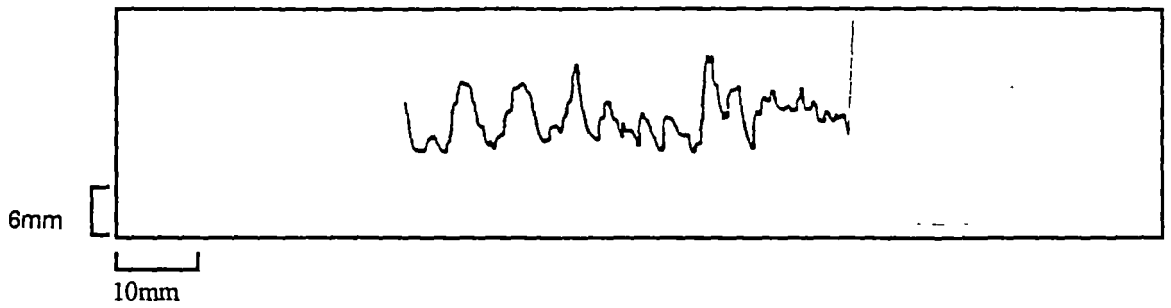
9.1 Introduction

In an awake subject about 60% of the oxygen utilisation supports electrophysiological activity while the remainder is used for maintenance of membrane/organelle homeostasis (Michenfelder, 1988). There are no oxygen reserves in the brain, and, when oxygen consumption exceeds energy production for a prolonged period, cellular dysfunction and death can occur. During cardiopulmonary bypass, cerebral blood flow may vary significantly, and clinically unsuspected cerebral ischaemia may occur. Electroencephalogram (EEG) monitoring may ultimately be required to detect such ischaemia reliably. Stockard et al (1974) recorded EEGs in 75 patients undergoing CPB for various cardiac procedures. Fifteen of them experienced significant hypotension during CPB; all had associated bilateral EEG changes with slowed or decreased activity. Eight patients who developed postoperative neurological deficits had EEG disturbances that began at the time of the hypotensive episodes during CPB, persisted postoperatively, and corresponded to the nature and evolution of the lasting deficits. In the present study, EEG was monitored as evidence of the cerebral effects of pre-alkaliniization and pre-acidification during and after the induced hypothermia.

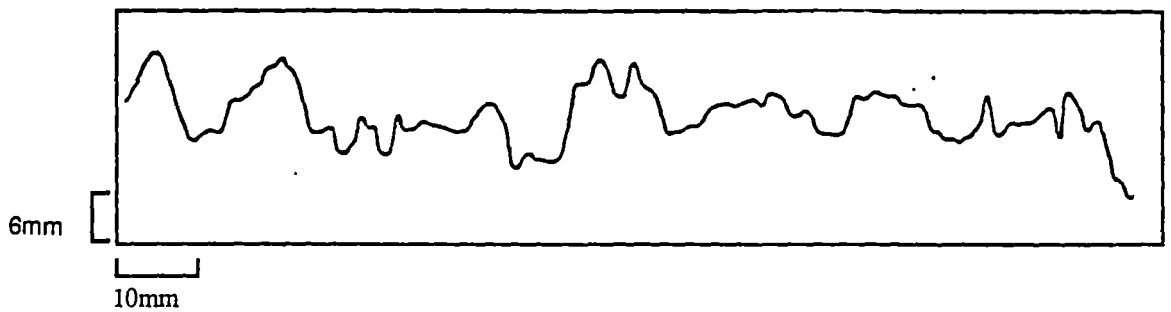
9.2 Results

In hypothermic groups, EEG records during control, cooling, profound hypothermia, rewarming and after rewarming were monitored and are shown in appendix records. Recording paper speed was 25 mm·second^{-1} and the sensitivity of the recorder set so that a calibration signal of 50 μv produced a 6 mm deflection (Sadove et al, 1967). Representitive records, showing the various types of suppression, are shown in the following graphs.

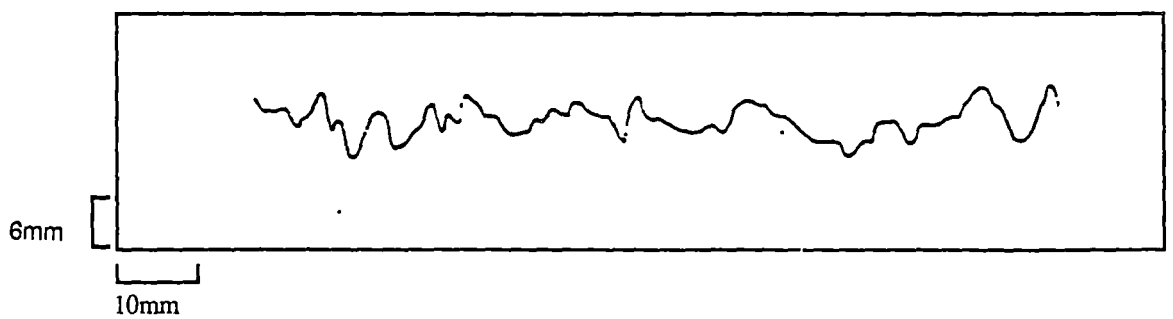
9.2.1 High voltage irregular activity (HIA).



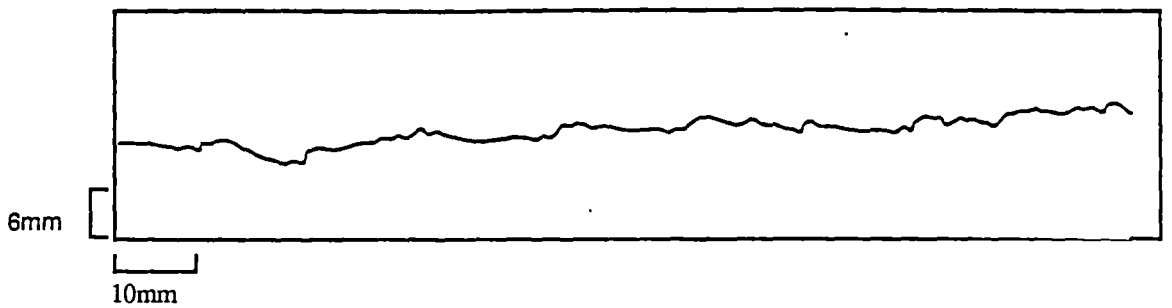
9.2.2 Slight suppression (SSH): High voltage, slow waves interrupted by flattening.



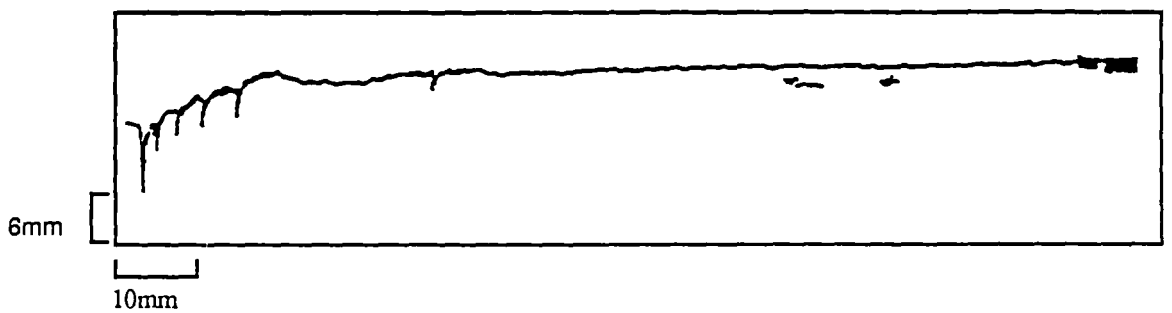
9.2.3 Moderate suppression (MOSL): Low voltage, slow waves interrupted by flattening.



9.2.4 Marked suppression (M_ASL): Low voltage, slow waves interrupted by flattening.



9.2.5 Complete suppression (CSI): Isoelectric state.



9.2.6 The time courses of the appearance and disppearance of different suppression on EEG records from all recorded animals (Table 9.1-2).

Table 9.1 Time (min) before cerebral electrical suppression appeared

Group	M_OSL	M_ASL	CSI
HCo (n=3)			
1	90	100	—
2	50	90	110
3	0	100	—
HAl (n=3)			
1	—	120	240
2	90	140	—
3	70	90	—
HAc (n=6)			
1	—	20	70
2	10	20	—
3	—	30	—
4	20	100	195
5	40	160	—
6	170	230	—

Table 9.2 Recovery time (min) of cerebral electrical suppression

Group	M_ASL	M_OSL	SSH	HIA
HCo (n=3)				
1	—	260	290	—
2	370	260	—	—
3		310	—	—
HAl (n=3)				
1	260	270	350	—
2	—	220	250	—
3	—	190	240	265
HAc (n=6)				
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	270	—	—	—
5	—	300	—	—
6	—	280	360	410

9.2.7 Body temperatures at which the various levels of suppression of the EEG occurred during hypothermia in three hypothermic groups (Table 9.3).

Table 9.3 The relationship between the body temperature range (°C) and the various EEG suppression degrees

Group	M_OSL	M_ASL	CSI	M_OSL	SSH
HAc (n=3)	35.0-36.5	34.0-35.5	25.0-25.5	35.0-37.5	NC§
HCo (n=3)	NC	34.0-34.6	25.0-29.5	27.0-30.0	NC
HAl (n=6)	NC	27.5-29.0	NC	31.0-33.0	37.0-38.5

§. NC — This type of suppression did not occur in this group.

9.3 Discussion and conclusion

In the present study, the EEG was recorded over dura mater which has a strongly attenuating effect on original signals (Cooper, 1965). The results showed that the EEG became suppressed (in both amplitude and frequency) as the body temperature dropped (Table 8.3). According to the degree of suppression, the EEG showed different patterns, from high voltage irregular activity (HIA, 5—10 per second), through slight suppression (high voltage, slow waves interrupted by flattening. SSH), through moderate suppression (low voltage, slow waves interrupted by flattening. $M_{O}SL$), through marked suppression (low voltage, slow waves interrupted by flattening. $M_{A}SL$) to a complete suppression (an isoelectric state. CSI). After rewarming, the EEG recovered gradually in the opposite direction from CSI to HIA. An isoelectric state occurred at about 25°C in both HAc and HCo group but not shown in HAl group. During rewarming, EEG suppression did not recover from an isoelectric state to a moderate suppression until the Tb reached at least 35 °C After rewarming, only HAl showed a slight suppression.

Gaenshirt (1954) studied the effects of both high and low temperatures on the electrical activity of the brain in the isolated cat head. Slowing occurred when the temperature rose above 38°C or fell below 32°C. The relationships between temperature and mean frequency was exponential except for two breaks, one at 38°C and the other at 33°C to 31°C. At these temperatures, specific enzyme systems apparently became blocked. Callaghan (1954) using monkeys as test animals, found a reduction in the voltage of fast frequencies below 33°C. At 25°C, fast activity was hardly discernible, the record consisting almost entirely of 1 to 2/sec. waves. At 20°C, EEG was relatively flat.

My results showed that all degrees of suppression started earlier in HAc group than in HCo group, which started even earlier than that in HAl. After rewarming, HAl recovered faster than the other two groups (Table 9.1-2). All three EEG records in HAl reached SSH stage after rewarming but only one of three in HCo group and one of six in HAc

group reached the same stage. Three of six in HAc failed to recover after CSI or MASL (Table 9.2).

Because the brain of awake subject uses about 60% of its oxygen supply for support of electrophysiological activity (Michenfelder, 1988) the EEG change is very closely related to the cerebral oxygen delivery and utilization. Arfel (1961) stated that the EEG has proved to be a sensitive indicator of cerebral perfusion problems during open heart surgery. Interruption of the cerebral circulation leads to disappearance of the anaesthesia-induced fast activity followed by emergence of very slow (0.5-3 /sec) activity. Thereafter, output of the cerebral electrical activity declines. With global perfusion failure of long duration, massive cortical necrosis will occur, while a more selective type of necrosis is prompted by shorter duration of ischaemia (Prior, 1982). The lower pH in HAc and HCo groups severely inhibited cerebral electrical activity during and after profound hypothermia, while this effect was not seen in HAl which had a higher pH. HAl group animals had higher blood oxygen content, lower oxygen extraction ratio and higher pH values during the experiment (for more details see chapter 6 and 10). This indicated that pre-alkalinization with a rather high pH before cooling buffered an inevitable rewarming acidosis and led to a lower oxygen debt metabolism during and after rewarming. This alkalinity may allow cerebral autoregulation to function adequately and promote an appropriate ratio of cerebral metabolic needs and blood perfusion as the body temperature changes. Earlier recovery with less suppression of the EEG implied that the brain had less insult after exposure to the induced hypothermia. By contrast, acidosis in HAc caused deterioration of the cerebral function with a suppression developing after rewarming.

Chapter 10. Cerebral oxygen consumption during and after induced hypothermia

10.1 Introduction

Normal brain function is dependent on an adequate supply of energy derived from oxygen and glucose and stored in the form of high-energy phosphates. Chiang et al (1968) reported microscopic and macroscopic cerebral morphologic alterations occur after brief periods of circulatory arrest at normothermia. Following 30 minutes of arrest, swelling of perivascular glial cells and collapse of capillary lumen can be observed electronmicroscopically. If a biological suspension of colloidal carbon is infused into the cerebral circulation after a 15-minute period of normothermic arrest, white areas of variable size representing regions with no microcirculatory perfusion are visible against a uniformly grey background on the brain surface and on cut sections. This obstructive lesion of the microcirculation following reperfusion after circulatory arrest has been called "no-reflow lesion" (Ames et al. 1968). Such evidence led to the conclusion that recovery of cerebral function depends on the restoration of the blood circulation (Hossmann & Sato, 1970a and 1970b).

Dysfunction of central nervous system following cardiopulmonary bypass has been reported to occur in most patients in the early postoperative period. Two-thirds of them demonstrate new neurological signs (Shaw et al., 1985; Smith et al. 1986). Although most of such abnormalities are transient and non-disabling, they are often present at the time of discharge from hospital (Shaw et al, 1985), when clinically obvious stroke is found in 5% and severe disability in 1 - 2% of patients. It is generally assumed that neurological injury is caused by inadequate cerebral blood flow or is embolic in origin (Reeves & Greeley. 1989). Because the brain is so dependent on O_2 supply that even a brief period of hypoxia can lead to serious disruption of neural function (Wilson et al, 1991). Greeley and his colleagues (1989) studied the effects of deep hypothermic cardiopulmonary bypass and total circulatory arrest on cerebral blood flow in infants and children. They found a highly significant correlation of cerebral blood flow with temperature during cardiopulmonary bypass and a significant association of cerebral blood flow with mean arterial pressure during deep hypothermic bypass. Operations with

profound hypothermia, with or without an arrest period, can be followed by cerebral dysfunction (Van der Linden et al, 1991a). Such dysfunction is not always obvious on simple clinical examination but is detected by cerebral injury markers (Rossi, 1989) and long-term neuropsychometric follow-ups (Wells et al, 1983). These may result from inadequate cerebral perfusion and oxygenation. Cerebral blood flow decreases with hypothermia and cerebral ischaemia leads to biochemical, functional, and structural alternations. However, little information is available regarding cerebral blood flow or how metabolic requirements are affected by applying different pH managements (Van der Linden et al, 1991b; Reeves & Greeley, 1989; Swain et al, 1991; Aoki et al, 1994), and there is no information available regarding how cerebral oxygen utilization is effected by pre-alkalinization during profound hypothermic procedures. For this reason, this part of my study focused on the investigation of the effects of pre-alkalinization and pre-acidification on cerebral oxygen consumption.

In this study carotid artery blood flow has been used as an index to estimate the cerebral perfusion. Three arterial stems ascend to supply the brain: right and left internal carotids and the basilar artery which results from the union of the two vertebral arteries. These three stems form an arterial circle (the circle of Willis) at the base of the brain through the linkages provided by the anterior communicating artery and two posterior communicating arteries. The cerebellum is mainly supplied by branches from the vertebral or basilar arteries. The cerebrum is shared by the anterior and middle cerebral arteries from the internal carotids, and the posterior cerebral arteries from the basilar (Anderson, 1983; Barr & Kiernan, 1988). On the basis of this unique anatomical feature, the variations of the carotid arterial blood flow (CrdBF) have been used to evaluate the changes of the cerebral blood flow in many previous studies (Arbeille et al 1991; Bailliart et al, 1993; Jalili et al, 1994). Unlike a number of mammalian species which have carotid retia, the rabbit has a very similar carotid anatomy to humans (Hayward & Baker, 1969).

10.2 Results

10.2.1 Normothermic group

Arterial and venous blood oxygen content ($CtaO_2$ and $CtvO_2$) showed no significant changes. Cerebral oxygen delivery ($DO_2 = CrdBF \times CtaO_2$) and oxygen uptake [$\dot{V}\ O_2 = CrdBF \times (CtaO_2\text{-}CtvO_2)$] showed no significant changes during the experiment. The oxygen extraction ratio ($ER = \dot{V}\ O_2/DO_2$) was stable.

10.2.2 Hypothermic groups

10.2.2.1 Carotid artery blood flow (CrdBF)

CrdBF declined immediately in all animals when hypothermia was induced. During rewarming, it increased gradually but never reached group control values. CrdBF in HAl was higher than that in HAc at the late rewarming stage (8 ml $min^{-1} \pm 5$, n = 4 versus. 3.5 ml $min^{-1} \pm 1$, n = 9 at the 5th hour and 10 ml $min^{-1} \pm 5$, n = 10 versus. 4.8 ml $min^{-1} \pm 1.8$, n = 5 at half hour later, $P < 0.05$) and showed an upward trend after rewarming. There was no significant difference between HCo and HAl (Fig 10.2.1).

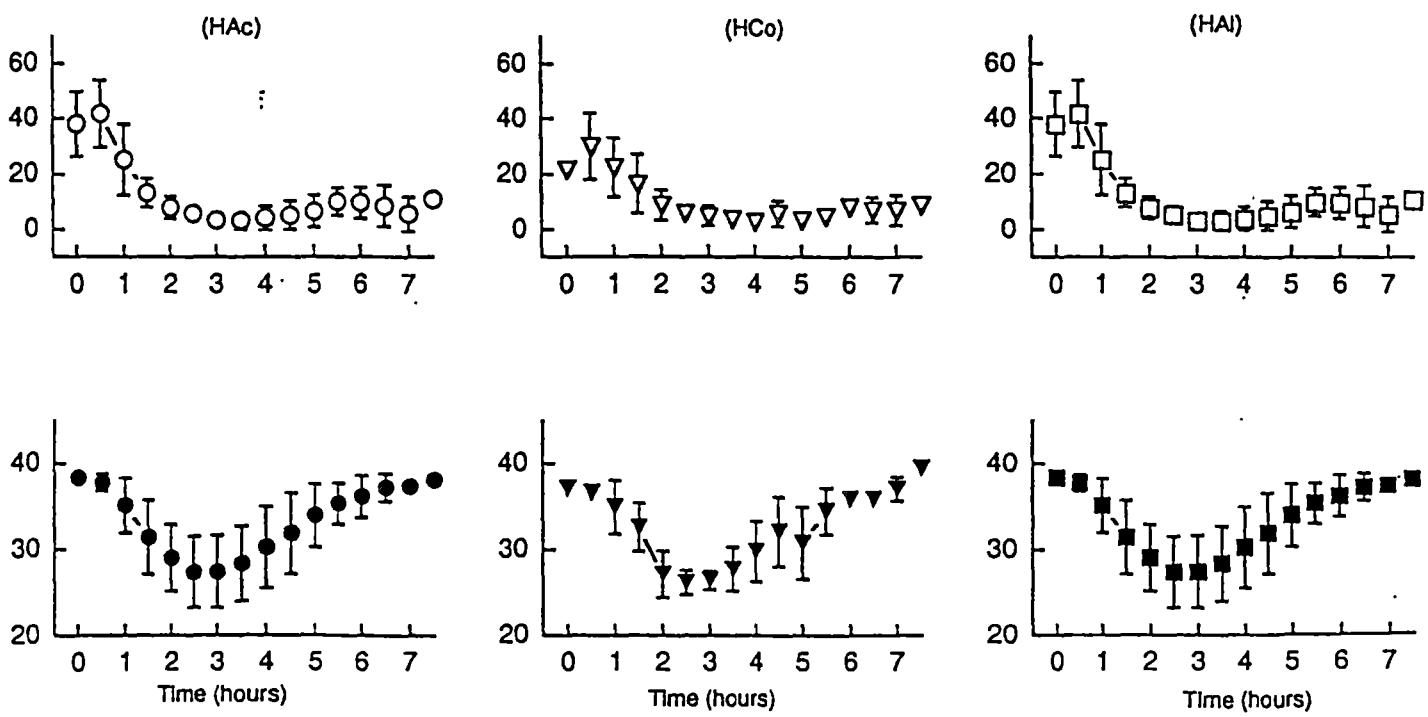


Fig 10.2.1 Changes of CrdBF at different body temperatures in three hypothermic groups (Mean ± SD)

10.2.2.2 Arterial and venous oxygen content ($CtaO_2$ and $CtvO_2$)

$CtaO_2$ increased slightly in all three groups when the body temperature was reduced. $CtvO_2$ was stable. During rewarming, $CtaO_2$ and $CtvO_2$ in both HAc and HCo declined, particularly in HAc ($P<0.05$ in HCo and $P<0.01$ in HAc). By contrast, during the same period, $CtaO_2$ increased further and $CtvO_2$ was nearly constant in HAl. $CtaO_2$ increased and reached a higher level at the end of the experiment in HAl. $CtvO_2$ of HAl decreased at a lesser degree than that in HAc and HCo, but the changes of HAl during the whole experimental period were not significant ($P>0.05$) (Fig 10.2.2).

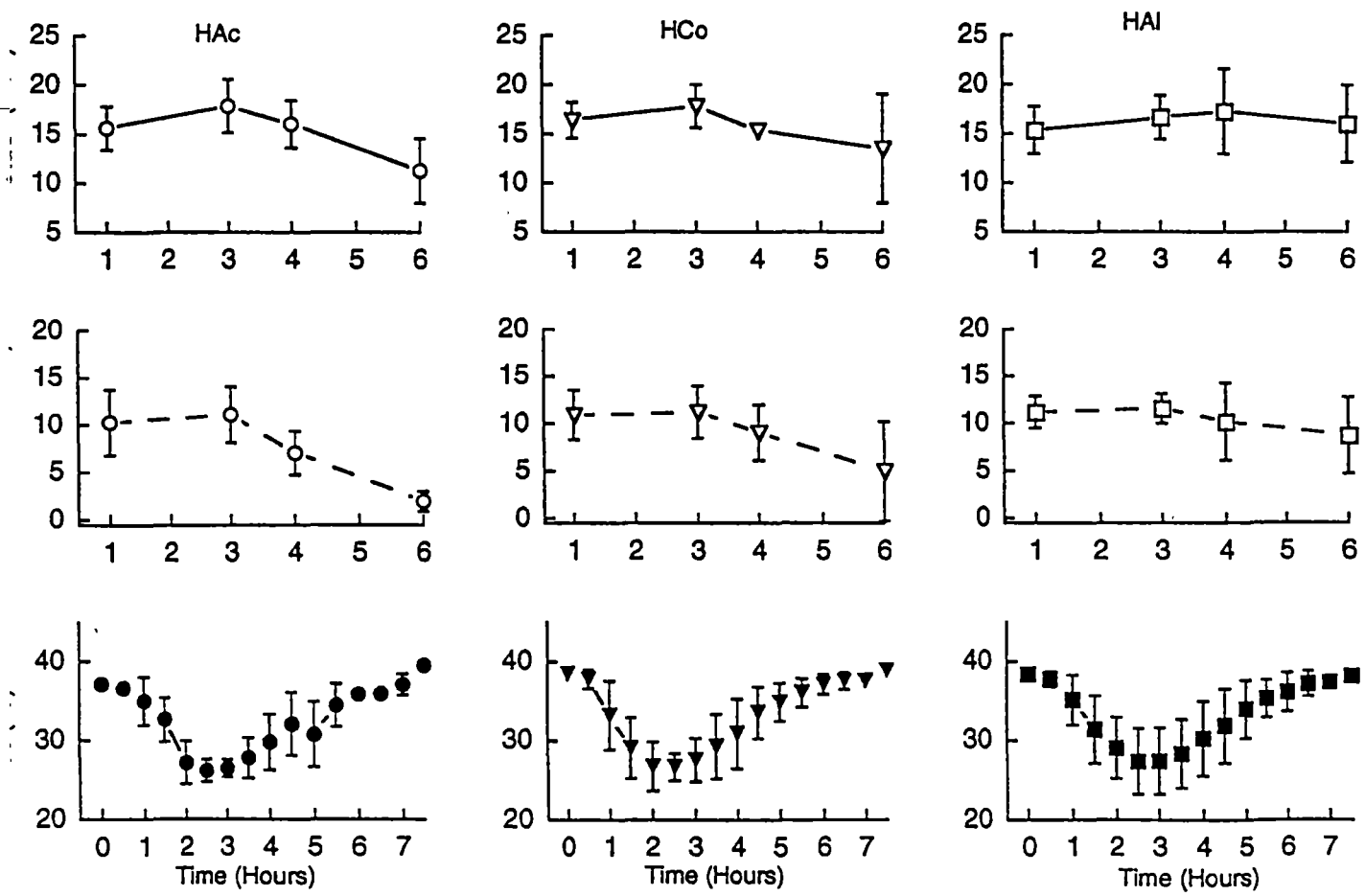


Fig 10.2.2 Changes of oxygen content at different body temperatures in three hypothermic groups (Mean ± SD)

10.2.2.3 Arteriovenous O_2 content difference ($AVDO_2$)

$AVDO_2$ increased gradually in all groups while the body temperature was reduced and maitained the same trend in HAc and HAl after the body temperature was increased. It declined rapidly in HCo during rewarming (Fig 10.2.3). There was no significant difference between groups.

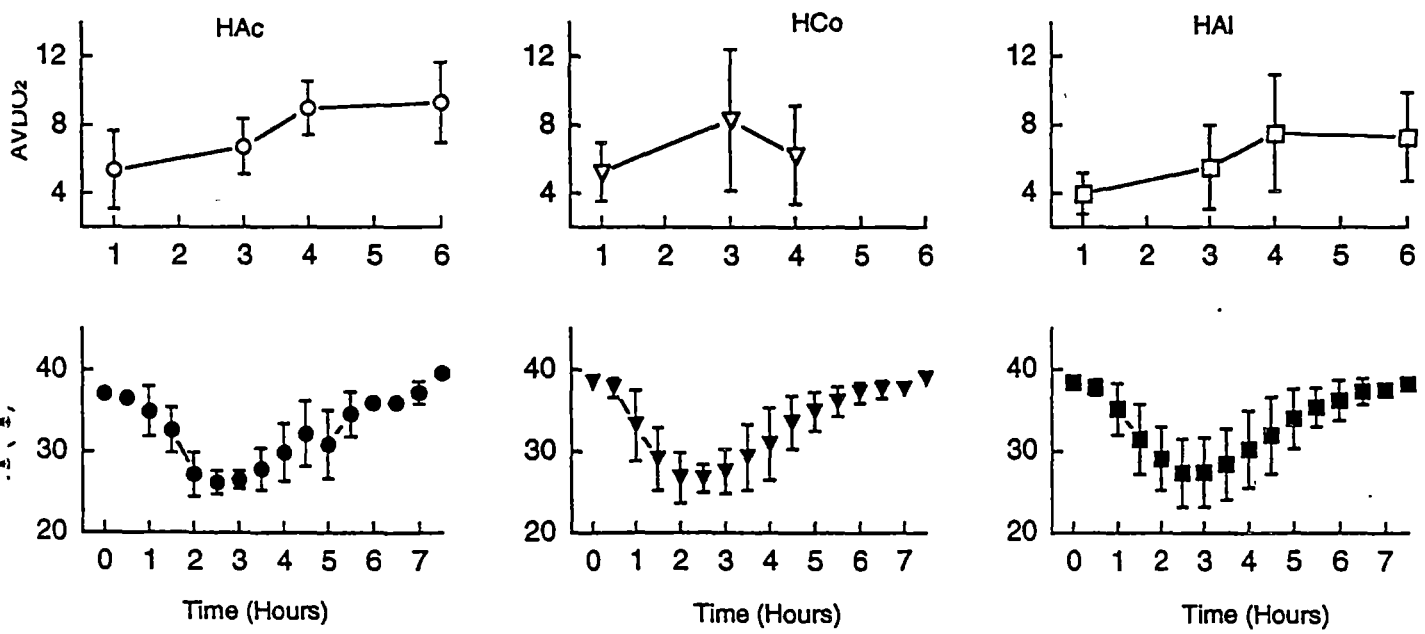


Fig 10.2.3 Changes of $AVDO_2$ at different body temperatures in three hypothermic groups (Mean ± SD)

10.2.2.4 Oxygen delivery (DO_2)

DO_2 decreased in all groups when animals were cooled ($P<0.05$ in HAc, $P<0.01$ in HCo and in HAl) thereafter stayed relatively stable (Fig 10.2.4).

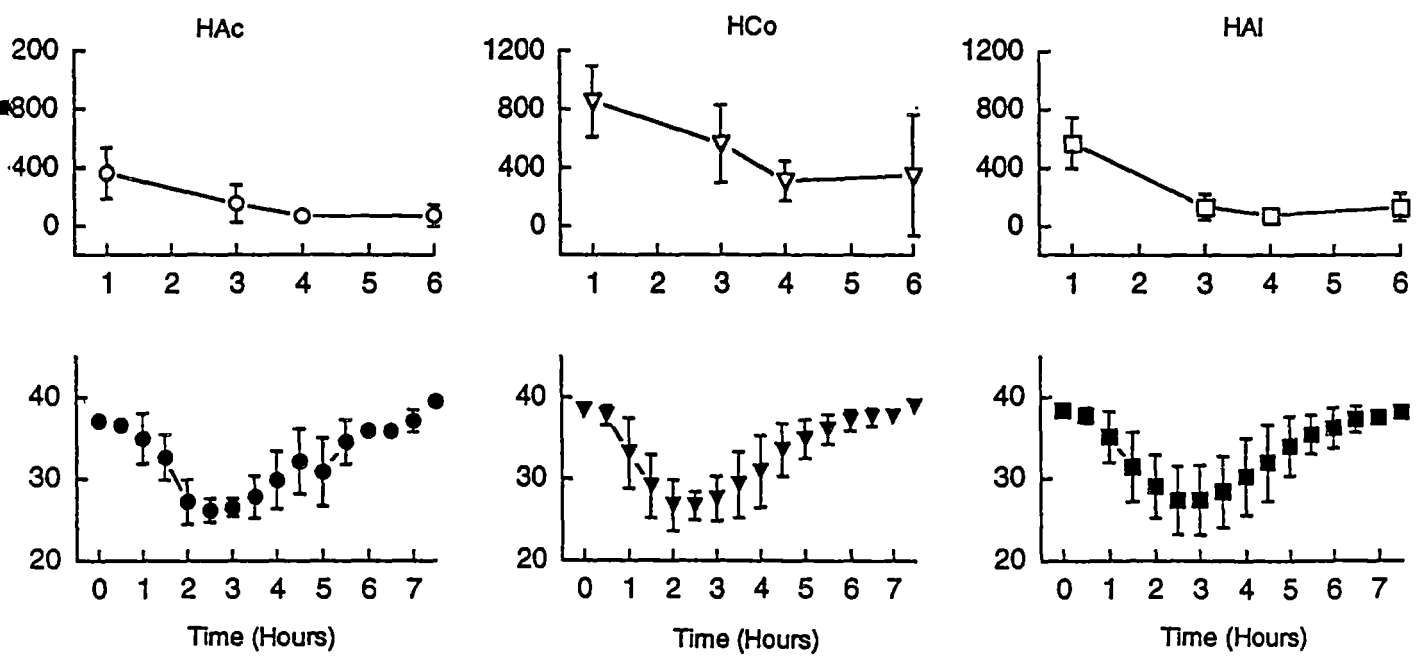


Fig 10.2.4 Changes of DO_2 at different body temperatures in three hypothermic groups (Mean ± SD)

10.2.2.5 Oxygen uptake ($\dot{V}O_2$)

In the HAl and HAc groups $\dot{V}O_2$ decreased gradually as body temperature fell during cooling from 111.26±34.02 ml·min^{-1} to 58.93±57.36 ml·min^{-1} in the HAc group ($P<0.05$), from 169.27±65.59 to 45.74±41.05 ml·min^{-1} in the HAl group ($P<0.001$) and then stayed at a relative stable level during one hour hypothermia and after rewarming. There was no significant difference between these two groups. In the HCo group, $\dot{V}O_2$ did not change during cooling but declined sharply during one hour hypothermia and was stable after rewarming (Fig 10.2.5).

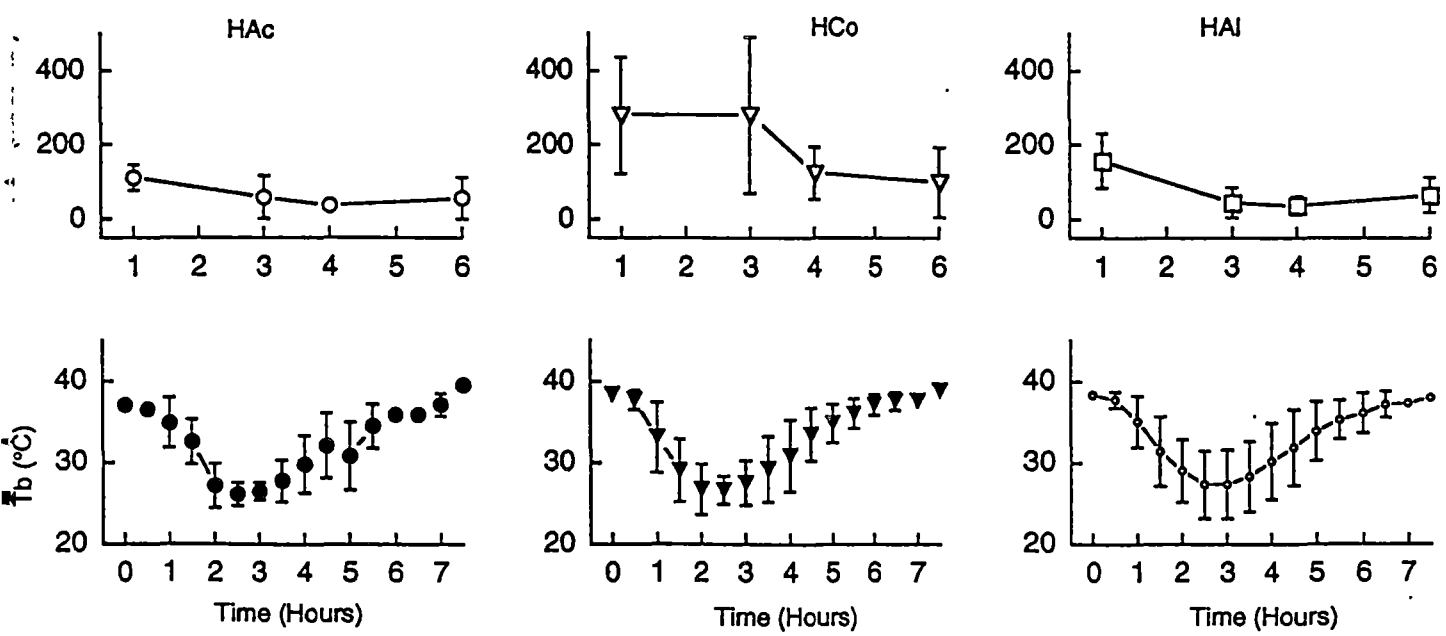


Fig 10.2.5 Changes of $\dot{V}O_2$ at different body temperatures in three hypothermic groups (Mean ± SD)

10.2.2.6. Oxygen extraction ratio (ER)

ER did not change significantly in any group when the animals were cooled. During rewarming, the HAc group showed a rapid increase until the end of the experiment. By comparison, ER in HAl group increased slightly at the beginning of rewarming then stabilized. The value of ER in HAl group was lower than those in HAc and HCo groups at the end of the rewarming (0.47 < 0.83 and 0.70 respectively, P<0.05) (Fig 10.2.6).

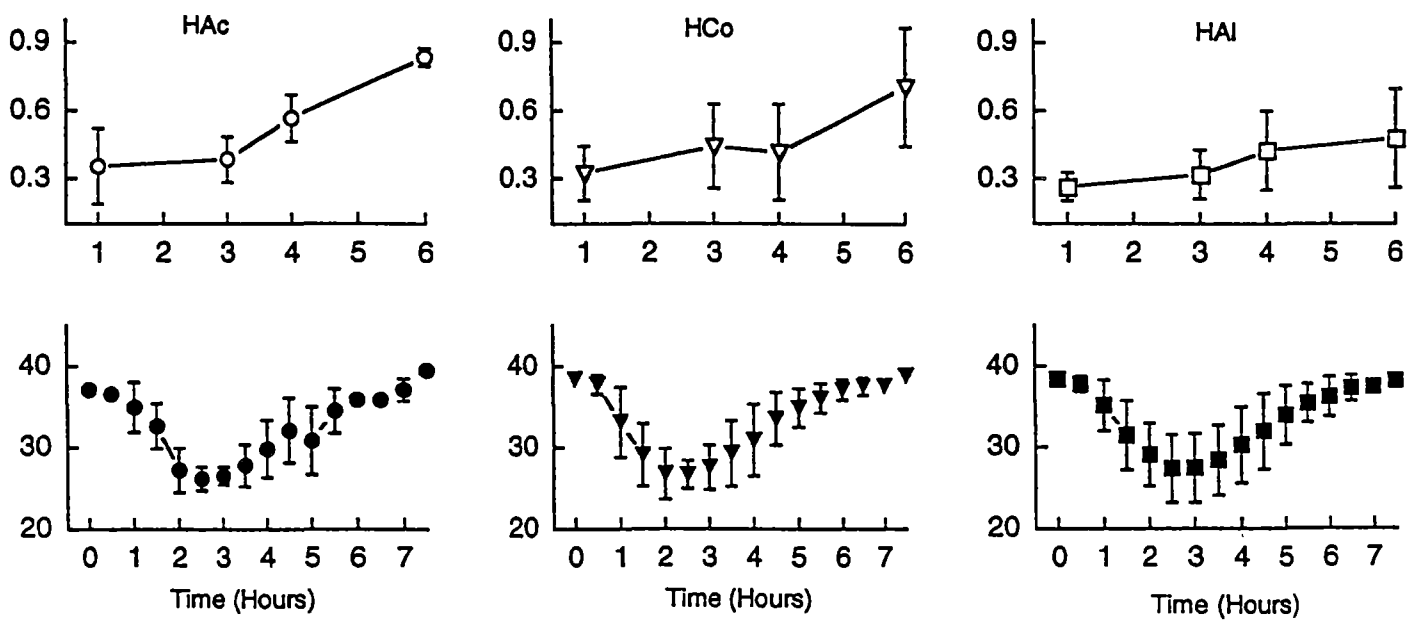


Fig 10.2.6 Changes of ER at different body temperatures in three hypothermic groups (Mean ± SD)

10.3 Discussion

The most important reason for applying hypothermia in clinical practice is that low temperature reduces O_2 requirements of the body (Schumacker et al, 1987; Alston et al, 1989). Two major factors influence the oxygen metabolism: oxygen supply or oxygen delivery and oxygen consumption. Hypothermia reduces the oxygen requirements by changing oxygen supply and consumption. The relationship between temperature and oxygen consumption has been studied extensively in animals. A decrease in cerebral temperature from 37°C to 20°C results in a five-fold decrease in metabolic rate and oxygen consumption ($Q_{10}O_2 = 2.2$) (Perna et al. 1973). An approximate 50% reduction of total body oxygen consumption is observed when temperature is reduced by 10°C and a similar relationship exists between temperature and cerebral metabolic rate (Tanaka et al. 1988).

10.3.1 Oxygen delivery

For a subject with no respiratory disorders, oxygen supply is determined mainly by cardiac output or organ blood perfusion and the amount and function of erythrocytes. Under most situations, the numbers of erythrocytes are stable and are not changed by a short period of hypothermia (Bigelow et al, 1950a). Any changes that affect these two factors could in turn affect the oxygen delivery. Hypothermia increases the affinity of haemoglobin for O_2 and adversely affects the release of O_2 to the tissues (Honig, 1988). DO_2 in the present study was calculated from the arterial oxygen content ($CtaO_2$, vol%) and the carotid blood flow (CrdBF, ml·min^{-1}). That is: $DO_2 = CtaO_2$ ¥ CrdBF (Gutierrez et al, 1986).

The results from this study showed that alkalinized rabbits had the highest arterial blood oxygen content, particularly during one hour hypothermia, rewarming and after rewarming. Although there was no significant difference in the venous oxygen content among the three groups, the HAl group had less decrease of $CtvO_2$ and HAc animals had the lowest level of $CtvO_2$ during one hour hypothermia, rewarming and after. The

oxygen content of blood is the sum of physically dissolved O_2 plus chemically bound O_2. With a normal PO_2, a normal oxygen content only occurs if the Hb concentration is in the normal range and the Hb is adequately saturated with oxygen (Zander, 1991). When PCO_2 increases and pH falls the position of the O_2 dissociation curve is shifted to the right. This is the Bohr effect (West, 1979) which allows more unloading of O_2 at a given PO_2 in a tissue capillary. In other words, the PO_2 for 50% O_2 saturation (P_{50}) increases. Opposite changes, like an increase in pH, a fall in PCO_2 or a fall in temperature shift the oxyhaemoglobin dissociation curve (ODC) to the left. An increased arterial oxygen content and more physically dissolved oxygen in the plasma of hypothermic subjects occurred (Carlsson et al, 1976; Keykhah et al, 1982 and Michenfelder et al, 1977). Baer (1989) investigated the effect of left-shifting of the ODC by hypothermia and concluded that an increase in haemoglobin-O_2 affinity is capable of limiting myocardial O_2 delivery and that increases in convective O_2 transport play a minor role at best in the coronary adaptation to small decreases in P_{50}. Because alkalinity increases haemoglobin-O_2 affinity it could have a synergistic effect with hypothermia on myocardial preservation. In the present study, when PaO_2 declined gradually a slight increase of $CtaO_2$ indicated a reduced oxygen uptake and an increased solubility of oxygen (Shapiro et al, 1989). Willford and his colleague (1986) reported that use of constant relative alkalinity (pH = 7.58 at 25°C) further reduces the P50 from 13.2 to 10.8 mmHg (In this case, on cooling from 37°C to 25°C at pH 7.4, the P50 decreases from a normal 26.8 mmHg to 13.2 mmHg).

In this experiment oxygen delivery dropped in all groups during cooling and was maintained at a low but stable level during rewarming. Fig 10.3.1-2 display the changes of DO_2, CrdBF and $CtaO_2$ in three hypothermic groups as Tb varies. Because the changes of oxygen content and oxygen delivery were directionally opposed in HAl the level of DO_2 during rewarming was slightly higher than that in HAc group although the CrdBF trends of both HAl and HAc groups were at a similar level. The decrease of DO_2 in HAc, however, could be caused by the reduction of both $CtaO_2$ and blood flow. Despite HAl and HAc showing a decrease of CrdBF there could be different

mechanisms. As discussed earlier in this chapter and previous chapters, alkalinity with hypothermia seemed to reduce oxygen delivery to match reduced cerebral activities and to protect the brain from an ischaemic insult. In HAc, acidosis caused myocardial damage and produced a low cardiac output, which could worsen the oxygen delivery to the brain and cause hypoxic acidosis. Becker et al (1981) measured brain blood flow by injecting puppies with 15±3 μ microspheres labeled with ^{125}I. They found due to the higher levels of blood pressure and cardiac index in the pH-adjusted group (in which pH changes following the changes of the temperature) total brain blood flow at 27°C and 22°C were better maintained despite the production of respiratory alkalosis with PCO_2 values of 14 and 11 mmHg, respectively. They also found at PCO_2 40 mmHg and pH 7.4, total brain flow fell 75% below controlled values, whereas cerebral flow fell only 25% in dogs in which PCO_2 was reduced and pH adjusted (increased). The regional distribution of brain blood flow showed similar disparities during hypothermia; at 22°C brain stem flow was reduced 56% in the pH 7.4 group and only 30% in pH adjusted group, while cerebellar flow was reduced to 60% versus 40% in these groups, respectively ($p< 0.05$).

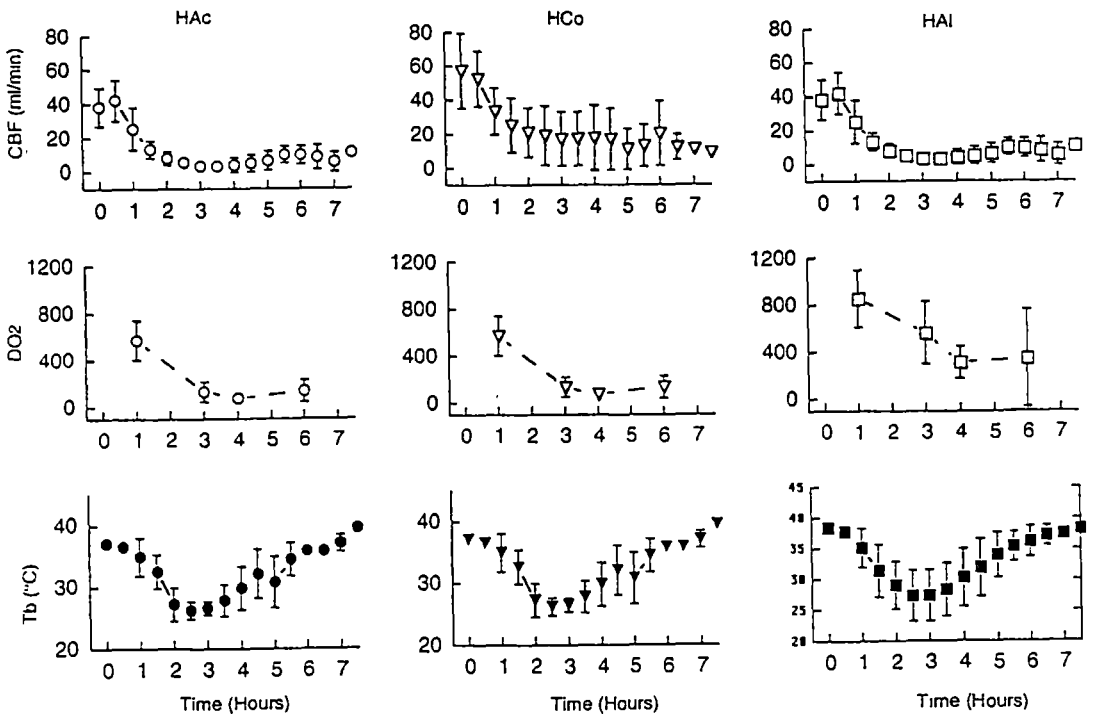


Fig 10.3.1 Changes of CrdBF and DO_2 in three hypothermic groups at different body temperatures (Mean ± SD)

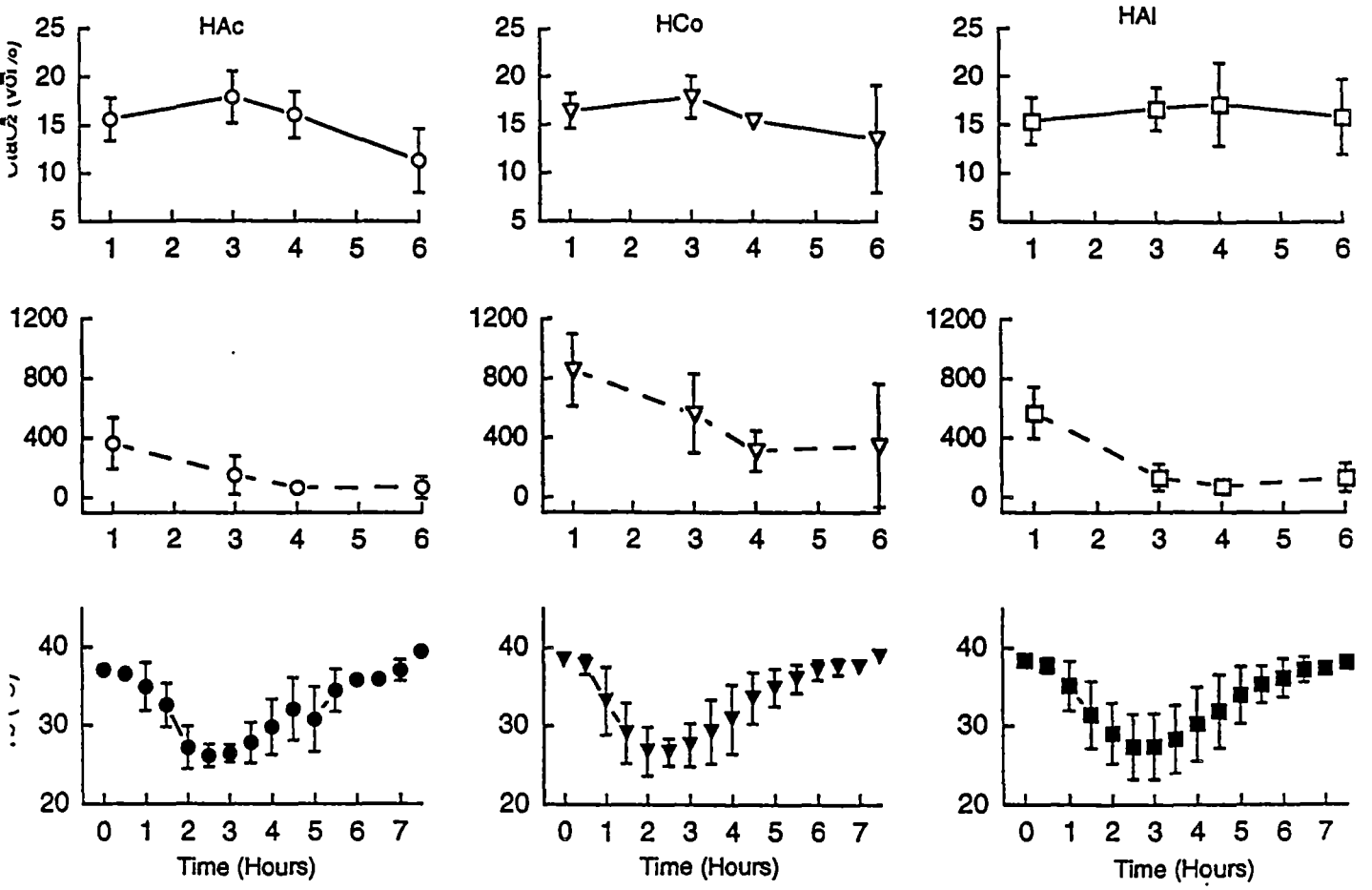


Fig 10.3.2 Changes of $CtaO_2$ and DO_2 in three hypothermic group at different body temperatures (Mean ± SD)

10.3.2 Oxygen consumption

Oxygen consumption is normally determined by the metabolic needs (Willford et al, 1986. Sumimoto et al, 1989). In the present study $\dot{V}O_2$ was estimated from product of blood flow and $AVDO_2$ (Miki et al, 1983). Prakash et al (1978) found in human body, during surface cooling, O_2 consumption, CO_2 production, and $PaCO_2$ decreased proportionally and linearly with body temperature. Another report showed hypothermia reduced whole-body $\dot{V}O_2$ by 31% on ventilated dogs (Schumacker et al, 1987).

The results of this study showed that $\dot{V}O_2$ decreased gradually as body temperature fell in all hypothermic groups during cooling and then remained constant during one hour hypothermia. The reduction in $\dot{V}O_2$ is a reflection of Van Hoff's law, where the logarithm of the reaction rate is directly related to the temperature (Hegnauer et al, 1954). After rewarming, $\dot{V}O_2$ showed an uprising trend in both HAc and HAl (Fig 10.2.5). In the HCo, $\dot{V}O_2$ had no changes during cooling but declined sharply during profound hypothermia and was stable after rewarming.

As shown in Fig 10.2.6, oxygen extraction ratio (ER) dramatically increased in HAc during rewarming although the differences of oxygen consumption among three groups failed to reach significant level. By comparison, oxygen extraction was lower in the HAl after rewarming than in HCo and HAc (0.4787 ± 0.2181 for HAl; 0.7045 ± 0.2610 for HCo group and 0.8329 ± 0.0395 for HAc group, $P<0.05$). At a given DO_2, a higher extraction ratio reflects a higher O_2 demand (Schumacker et al, 1987). An increase of ER could compensate for an increased oxygen consumption. A higher ER in the HAc indicated that this group of animals required more oxygen than those in the HAl. This could result from the lowered pH reducing haemoglobin affinity for O_2 (see the above discussion for more details). The different effects on vascular tone of acidosis and alkalinity may also affect the distribution of blood flow in the cerebral microcirculation. Chapter 7 showed that blood pressure in HAl increased progressively while Tb was reduced, and decreased while animals were rewarmed (from 103 ± 6 mmHg to the lowest

value 86 mmHg ± 5) (Fig 7.2.4). After Tb reached 33.5°C (four and a half hours after the experiment started) MAP started to increase and reached the mean value of 107 ± 12 mmHg at the 6th hour. In HAc, MAP was unstable, decreasing through the entire monitoring period, and never reaching the control value again. These results imply that, in HAl, animals may have a higher peripheral vascular tone, which probably contribute to the blood supply of the important organs after rewarming. As a result, there would be an adequate blood perfusion to the brain and central nervous system thus dysfunction following CPB may rarely happen. Fig. 10.3.3 show the changes of MAP, CVP and ER in three hypothermic groups as Tb changes.

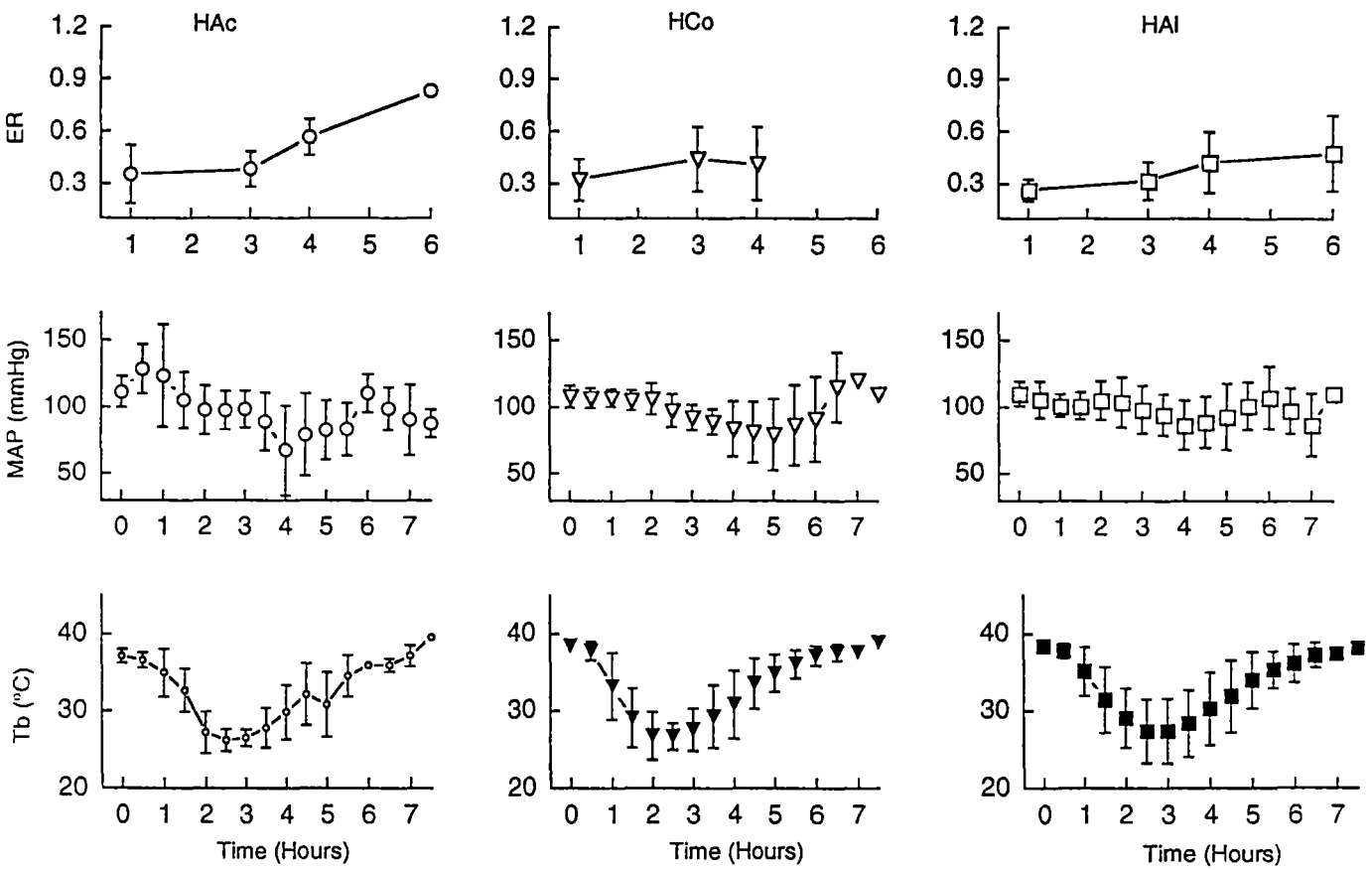


Fig 10.3.3 Changes of ER, MAP and CVP in three hypothermic groups at different body temperatures (Mean ± SD)

HAl animals had a higher initial value of PaO_2 and it remained at a higher level throughout cooling and rewarming phases ($P<0.05$). At the end of the experiment, HAl showed the highest value of those in three hypothermic groups although it failed to reach a significant level ($P=0.094$).

In brief, oxygen delivery declined during hypothermia in all animals and barely recovered during and after rewarming. It was found that the changes of DO_2 in the HAl were mainly dependant on the blood flow. The changes of DO_2 in HAc, however, related to both oxygen content and blood flow. It has also been noticed that O_2 content and PO_2 were maintained at a comparatively higher level in HAl than in the other two groups. Oxygen extraction ratio (ER) was increased gradually before rewarming in all hypothermic groups. During rewarming ER increased linearly in both HCo and HAc, particularly in the HAc. In HAl, ER showed a slow increase only. The difference between HAl and, HCo and HAc was significant.

Chapter 11

Conclusion

11.1 Conclusion

The present study is the first to establish a successful anaesthetic rabbit model using fentanyl citrate and vecuronium bromide anaesthesia. The results show that heart rate, mean aortic pressure, central venous pressure, carotid blood flow and body temperature were very stable throughout the experiment. Fentanyl citrate would appear to be the anaesthetic of choice in cardiovascular research on rabbits. This study has also demonstrated that the muscle relaxant vecuronium bromide has no cardiovascular side effects on rabbits. An unexpected finding was that rabbits apparently tolerated and required large doses of fentanyl (750 μg/hr) during surgery and 375 μg/hr during the monitoring period, without significant changes in the level of HR, MAP, CrdBF, CVP and blood gases.

This anaesthetic rabbit model allowed continuous observation of six or more hours of hypothermic study. With this hypothermic model, body temperature could be reduced from normal to 25 ± 0.5°C and maintained at this level for one hour, and then the animal rewarmed to normal.

The application of pre-alkalinity by intravenous administration of carbicarb (in HAl) and pre-acidification (in HAc) with ammonia chloride on rabbits demonstrated that a higher pH during hypothermia from pre-alkalinization could reduce or eliminate anaerobic metabolism and subsequent acidosis during and after rewarming, and hence could be the optimum acid-base strategy after induced hypothermia. By contrast, using pre-acidification to produce a more normal pH during hypothermia produced deleterious effects on rabbits during and after rewarming, as suggested by the following findings in cardiovascular function, oxygen consumption and cerebral performance.

11.1.1 Acid-base status and oxygen consumption

11.1.1.1 Acid-base status

pH increased as body temperature dropped. Animals in HAl had a significantly higher pH during hypothermia and rewarming. After rewarming, normal pHa and pHv were retained. Animals in HAc showed a "normal pH" (pH = 7.4) during deep hypothermia and a serious acidosis after rewarming. PCO_2 decreased during hypothermia and was lower in HAl than the other hypothermic groups during and after hypothermia, and after rewarming.

11.1.1.2 Oxygen consumption

Oxygen delivery declined during hypothermia in all animals and rarely recovered completely during and after rewarming. HAl showed a tendency for an increase in DO_2 although this failed to reach statistical significance. CO_2 and PO_2 were maintained at a significantly higher level in HAl than in the other two groups. Oxygen extraction ratio (ER) increased gradually before rewarming in all hypothermic groups. During rewarming ER increased linearly in both HCo and HAc, particularly in HAc. In HAl, ER showed a slow increase only. A significant difference was found between HAl and, HCo and HAc.

11.1.2 Cardiovascular function

11.1.2.1 Haemodynamics

Haemodynamic performance was best in the pre-alkaline animals. HR fell during cooling but recovered well after rewarming; MAP was higher, stayed at a relatively constant level during hypothermia and returned to normal after rewarming; CrdBF dropped during hypothermia and exhibited an upward trend after rewarming. CVP decreased slightly when cooling started and then gradually increased during rewarming. Animals which were pre-acidified showed a dramatic fall in CVP during cooling and thus stayed at a very low level after rewarming, possibly due to an acidotic dilatation on peripheral vessels; MAP was unstable during hypothermia and declined linearly after rewarming; HR

showed a slow increase after rewarming started then gradually returned to normal; CrdBF showed a trend of decline after rewarming. These indicated a beneficial effect of pre-alkalinization upon circulatory function during and after an induced hypothermia. However, the present study did not investigate the mechanisms of any such benefit. It is probable that alkalinity preserves cardiac contractility by resisting a decrease in myofilament Ca^{2+} sensitivity with decreased temperature in cardiac ventricular muscles (Thomson 1990) and increases the tone of small vessels.

11.1.2.2 Electrocardiograph

All intervals were prolonged in both groups during cooling and rewarming periods. Animals in the HAl group retained normal intervals when the body temperature returned to normal.

Depression of ST segments and "tent" T waves were common during hypothermia and rewarming. As temperature increased, the depression of ST segments became less apparent and T waves tended to have a normal pattern. Animals in the alkalosis group achieved a normal configuration of ST segment and T wave. Conduction disturbances and rhythm abnormalities occurred more frequently and severely in the acidosis group than in the alkalosis group. J, or Osborn, waves were occasionally seen in the acidosis group during rewarming. When temperature returned to normal, animals of the alkalosis group showed no sign of conduction disturbances and arrhythmia while acidotic animals showed severe ventricular arrhythmia.

The results indicate that pre-alkalinization causes less disorder in myocardial electrical activities than pre-acidification, which implies that the myocardium functions better under pre-alkalinization than pre-acidification. The results also imply that the sinus node is easily affected by hypothermia and ventricular fibrillation appears much less frequently in rabbits than in human beings during deep hypothermia.

Depressions of S-T segment, prolongations of Q-T interval and peaked T waves are obvious during hypothermia, which shows that repolarisation is more affected than other parts of ECG by low temperature.

11.1.3 Cerebral function

11.1.3.1 Electroencephalograph

During cooling, all degrees of suppression started earlier in HAc than in HCo and occurred latest in HAl. After rewarming, HAl recovered faster than the other two groups. All tested animals in HAl reached a stage of slight suppression but only one of three in HCo group and one of six in HAc reached the same stage. Three of six in HAc failed to recover after showing a complete suppression or a marked suppression. The lower pH in HAc and HCo appeared to more severely inhibit cerebral electric activity during and after deep hypothermia than did the higher pH in HAl. There was better recovery of the EEG of alkalised animals after rewarming. Acidosis caused a deterioration of cerebral function and led to depression after rewarming.

11.1.3.2 Cerebral blood perfusion and oxygen metabolism

The blood samples from the inferior vena cava and aorta and the data of carotid blood flow show that cerebral oxygen delivery (DO_2) dropped in all groups during cooling and remained at a low but stable level during rewarming. Thereafter it increased, particularly in HAl. An adequate cerebral blood supply ensures a proper oxygen delivery to the brain. Any changes in cerebral perfusion pressure and the cerebral peripheral resistance will change the cerebral blood supply. The change of DO_2 in HAl during rewarming appears to have been due mainly to a decrease of blood flow. This change of blood flow resulted from an increased peripheral resistance because MAP was increased from 86 ± 5 mmHg during hypothermia, to 107 ± 12 mmHg during rewarming ($p<0.05$). By contrast, the changes of DO_2 in HAc might have been caused by reduced myocardial contractility (Wang et al, 1965) and dilated peripheral vessels (Miki et al, 1983). MAP of this group was unstable and tended to decrease through the entire monitoring period, never reaching

the control value again. The different effects on vascular tone of acidosis and alkalinity may affect the distribution of blood flow in the microcirculation after rewarming. For instance, in the HAl, animals with a higher vascular tone could respond sensitively to accumulated local metabolic products ("initial vascular tone hypothesis", Fishman, 1980; Huang & Fang, 1987). This might contribute to blood recruitment after rewarming. As a result, there would be a better blood perfusion to the brain, and CNS dysfunction following CPB may happen infrequently.

In brief, results of this study showed that only the animals in HAl:

(a) Regained a normal S-T segment, T wave and sinus rhythm;

(b) Regained a stable and normal HR and MAP;

(c) Showed full recovery of EEG from hypothermia;

(d) Accumulated less oxygen debt (highest PO_2 and lowest oxygen ER).

This demonstrates that an alkalinity management could be an optimal option of acid-base management because alkalinity may reduce or eliminate anaerobic metabolism and subsequent acidosis. It presumably lessens hypothermic calcium overload in the myocyte therefore provides better myocardial and cerebral protection. It also leads to an optimum blood acid-base status and oxygen metabolism during induced hypothermia and after rewarming.

Table 11.1 Comparison of HAl, HAc and HCo on cerebral and cardiovascular function during and after induced hypothermia

	HAl	HCo	HAc
ECG			
1. Hypothermia	1. ST↓, T↑	No records	1. ST↓, T↑
2. During rewarming	2. ST↓, T↑		2. ST↓↓, T↑↑
3. After rewarming	3. Normal ST, T & rhythm		3. Arrhythmias & conduction disturbances
HR			
1. Hypothermia	1. ↓	1. ↓	1. ↓
2. During rewarming	2. ↑ steady	2. ↑ steady	2. ↑ slowly
3. After rewarming	3. Stable	3. Stable	3. Unstable
BP			
1. Hypothermia	1. ↑	1. ↓	1. Unstable ↓
2. During rewarming	2. ↑ steady	2. ↓ slowly	2. ↓ slowly
3. After rewarming	3. Return to normal	3. Slowly return	3. Fail to return
EEG			
1. Hypothermia	1. Suppressed latest	1. Suppressed later	1. Suppressed earlier
2. During rewarming	2. Recover earliest	2. Recover earlier	2. Recover slowly
3. After rewarming	3. Slight suppressed	3. Less suppressed	3. Recover rarely
ER (Oxygen)			
1. Hypothermia	1. No change	1. No change	1. No change
2. During rewarming	2. ↑	2. ↑↑	2. ↑↑↑
3. After rewarming	3. 0.47	3. 0.70	3. 0.83

11.2 Further Work

Results from this work suggest several areas for further research. First, mechanisms of myocardial, cerebral and oxygen metabolic preservation resulting from pre-alkalinization need evaluation. This could lead to a clearer picture of cellular activities under various acid-base states and help the clinical practitioner to apply pre-alkalinization effectively in preserving myocardial and cerebral functions.

Second, since post-operative neuropsychological dysfunction has emerged as an increasingly important aspect of cardiac surgical morbidity, further research is needed to reveal mechanisms of cerebral blood flow and metabolism regulation under different acid-base strategies during induced hypothermia. The dependence of whole body oxygen consumption over a wide range of oxygen deliveries is also worth further investigation.

Finally, animals sustained more injury during cooling and rewarming, particularly rewarming, than during profound hypothermia. Presumably this is related to a flow-metabolism mismatch during cooling and rewarming, which could cause postoperative neuropsychological dysfunction. Further investigation should lead to more precise control of acid-base states during rewarming, with discussion of possible differences of effects between respiratory alkalosis (reduced $PaCO_2$) and metabolic alkalosis (increased HCO_3^-) and between respiratory acidosis (increased $PaCO_2$) and metabolic acidosis (reduced HCO_3^-). The speed of rewarming also requires careful consideration.

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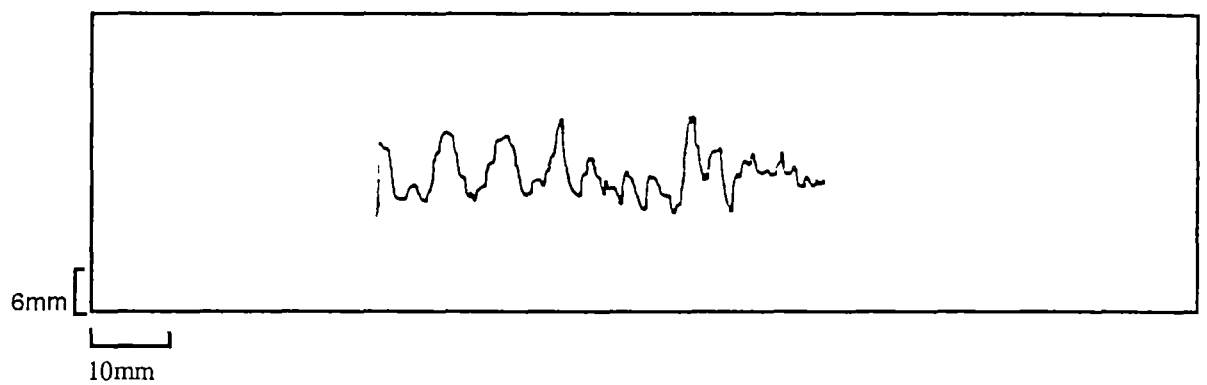
Appendix

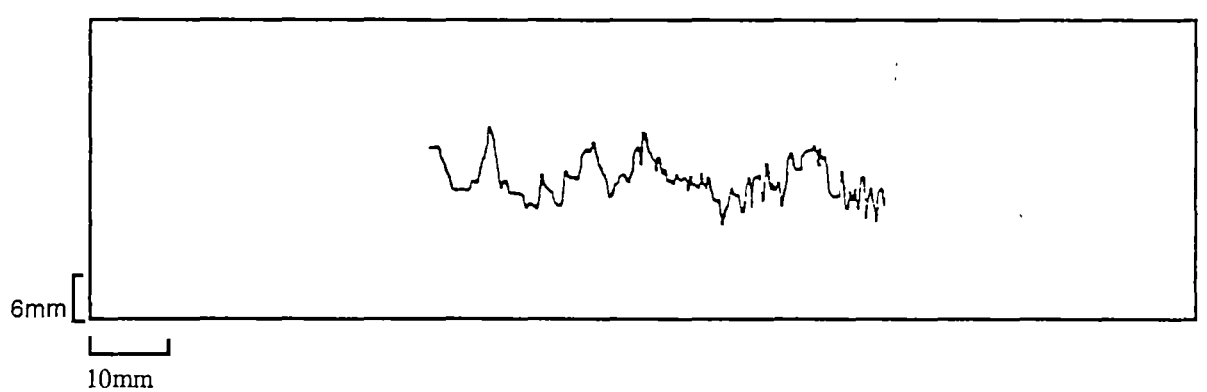
The following are the copies of some original records that showed EEG changes during control, cooling, profound hypothermia, rewarming and after rewarming in three hypothermic groups. Recording paper speed was 25 mm·second^{-1} and the sensitivity of the recorder set so that a calibration signal of 50 μv produced a 6 mm deflection (Sadove et al, 1967).

1. HCo group

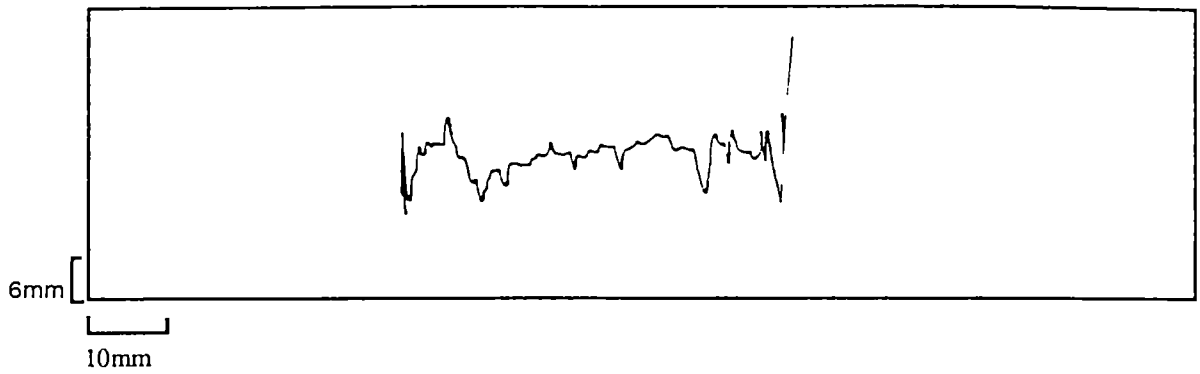
Rabbit 1803. Female. Recorded on March 18 1992



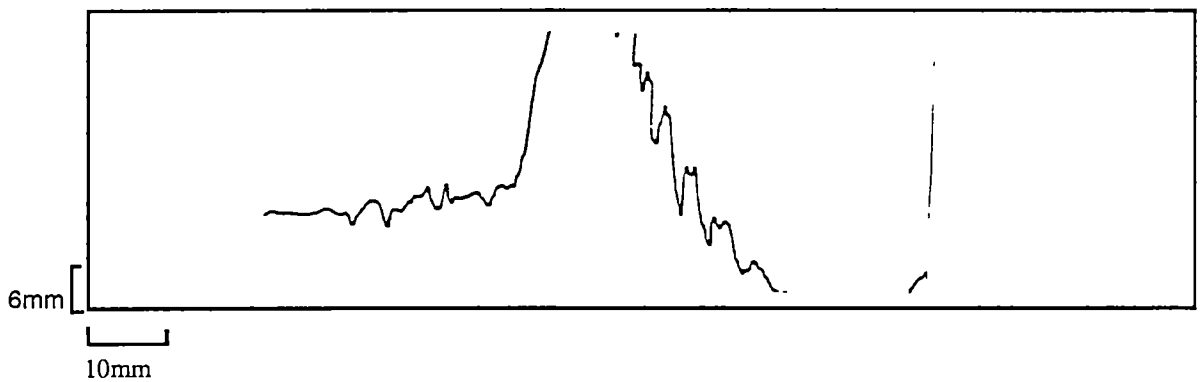
Record 1. (Time 0) EEG: High voltage irregular activity. MAP: 110 mmHg. CrdBF: 30 ml·min^{-1}. Tb:37.5°C



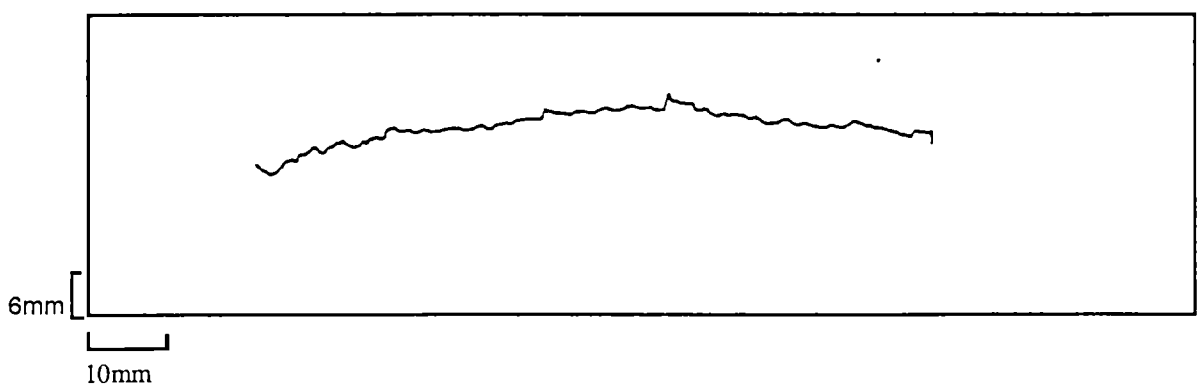
Record 2. (+60 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 105 mmHg. CrdBF: 21 ml·min^{-1}. Tb:37.0°C.



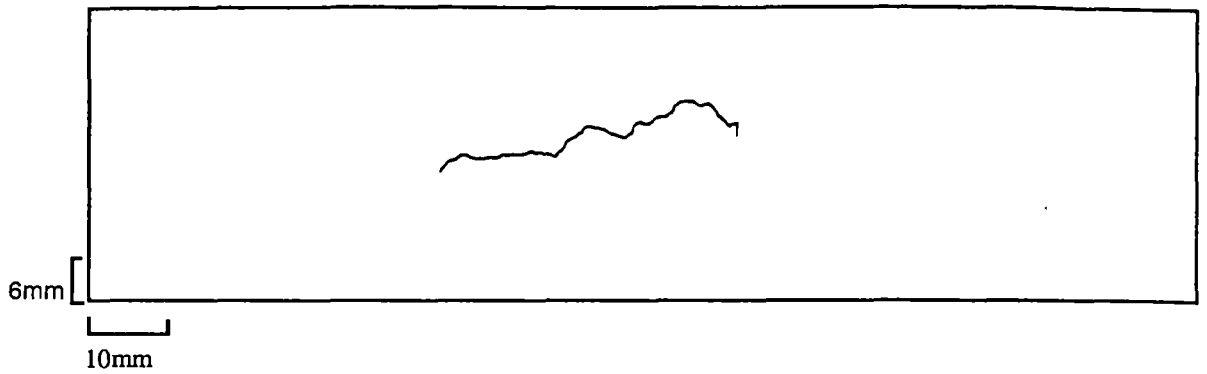
Record 3. (+80 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 19 ml·min^{-1}. Tb:35.2°C.



Record 4. (+90 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 22 ml·min^{-1}. Tb: 33.8°C.

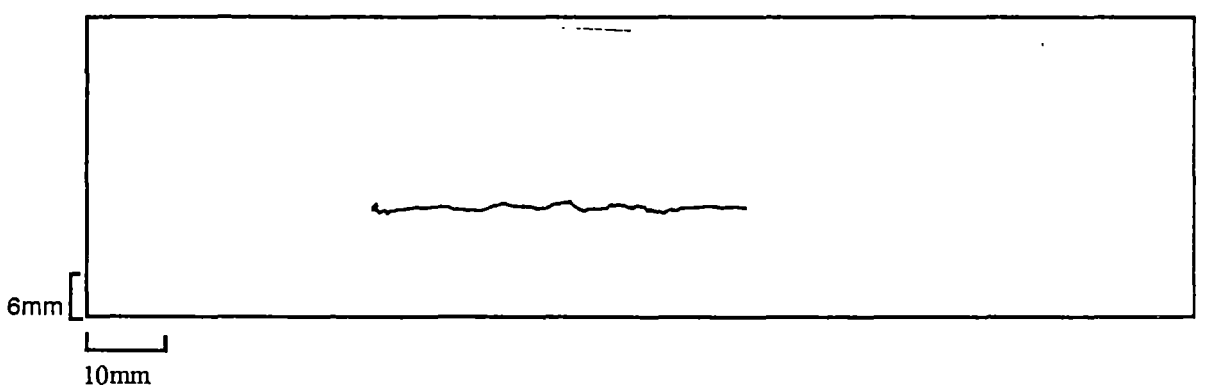


Record 5. (+100 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 23 ml·min^{-1}. Tb:32.2°C.

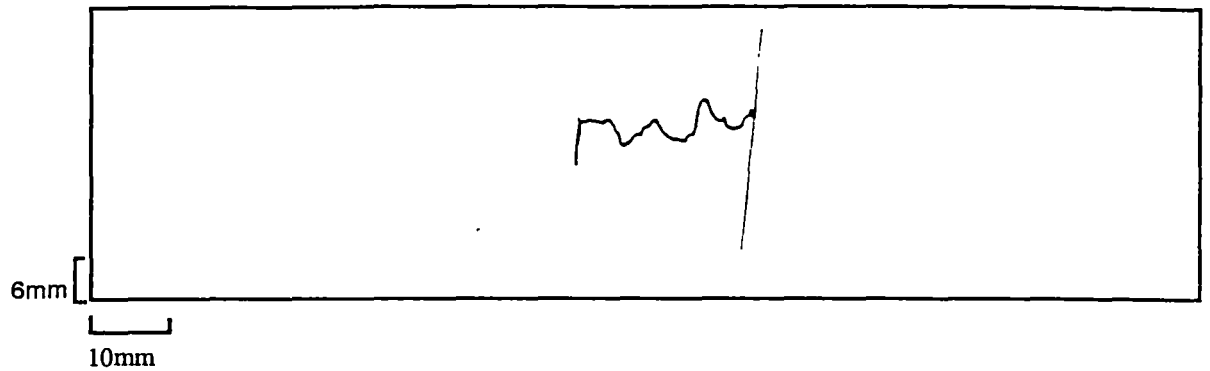


Record 6. (+130 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 19 ml·min^{-1}. Tb: 29.4°C.

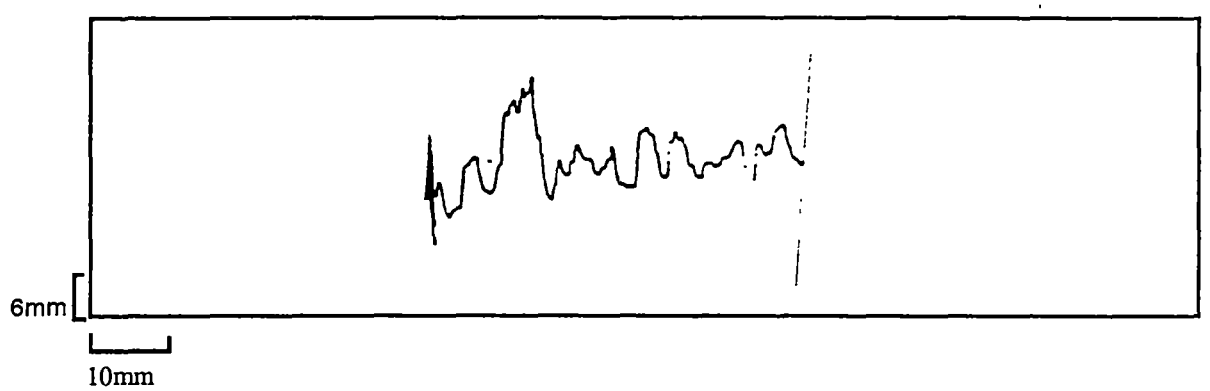
Record 7. (+170 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 8 ml·min^{-1}. Tb: 26.2°C.



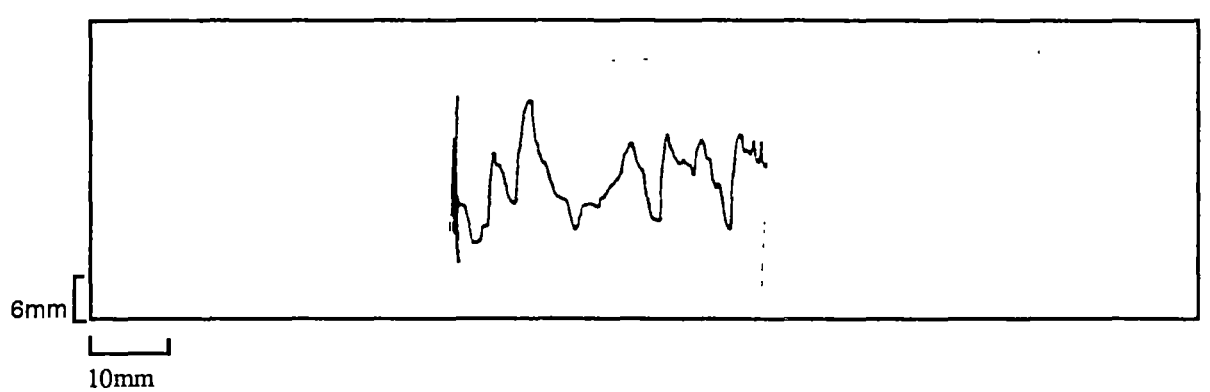
Record 8. (+200 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 95 mmHg. CrdBF: 7 ml·min^{-1}. Tb: 25.6°C.



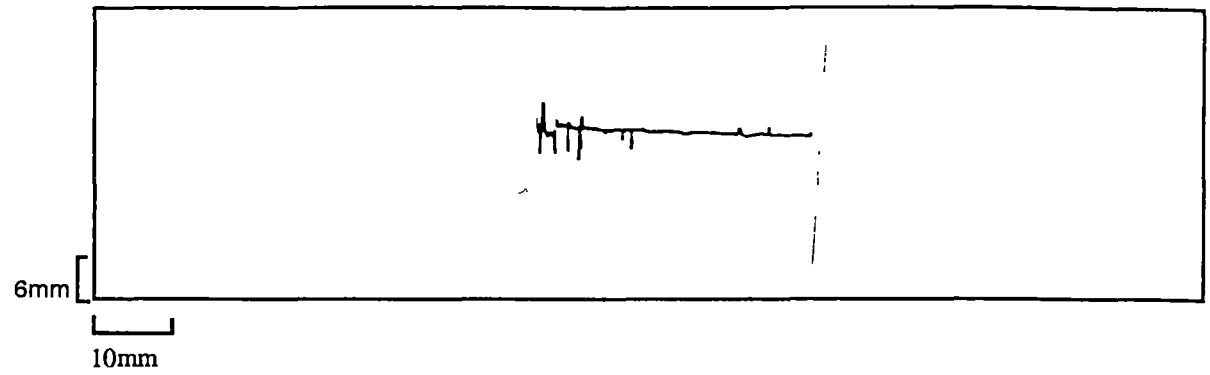
Record 9. (+260 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 95 mmHg. CrdBF: 4 ml·min^{-1}. Tb: 27.1°C.



Record 10. (+290 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 95 mmHg. CrdBF: 3 ml·min^{-1}. Tb: 31.7°C.

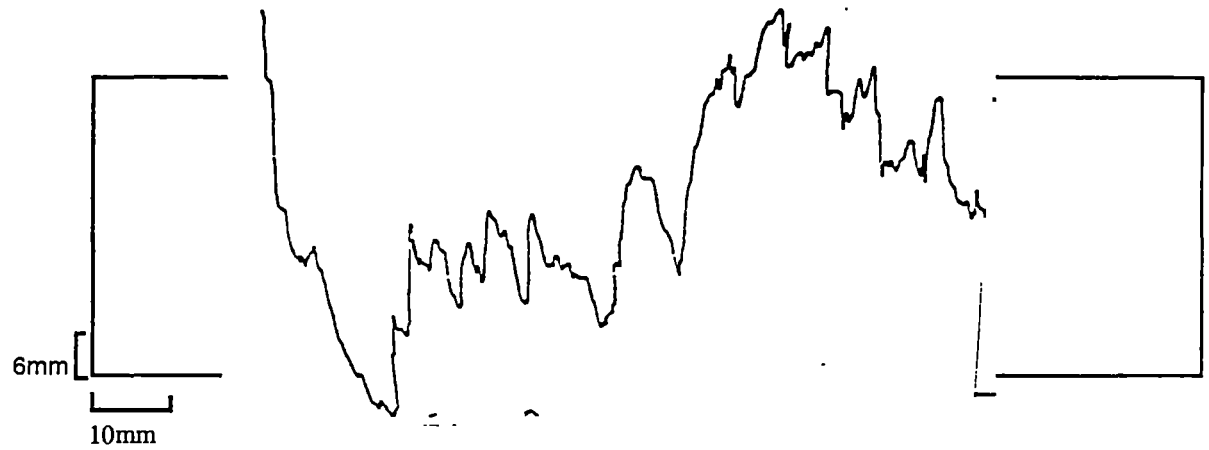


Record 11. (+310 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 70 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 34.8°C.

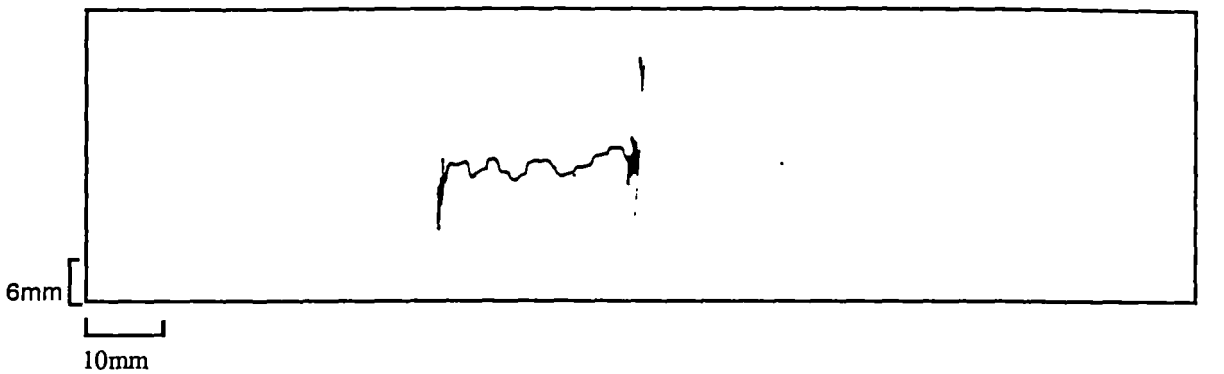


Record 12. (+335 min) EEG: Complete suppression. Isoelectric state. MAP: 70 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 34.8°C.

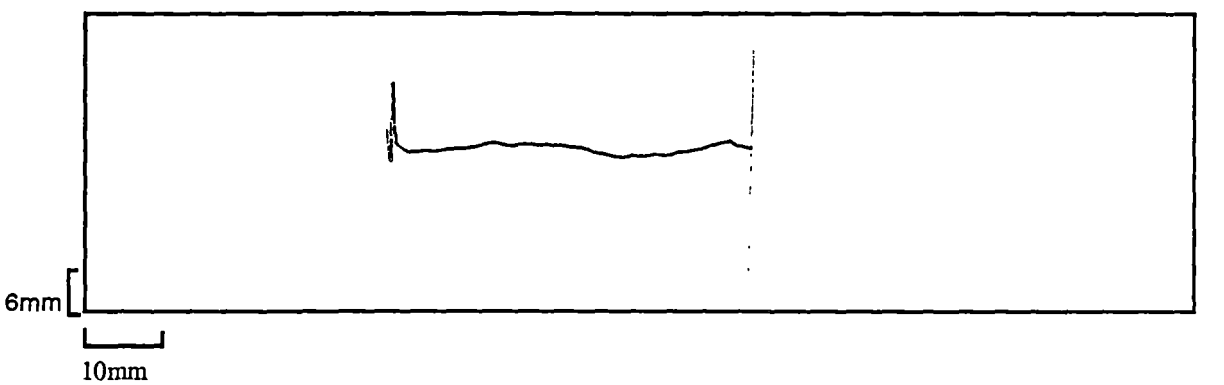
Rabbit 2503. Female. Recorded on March 25 1992.



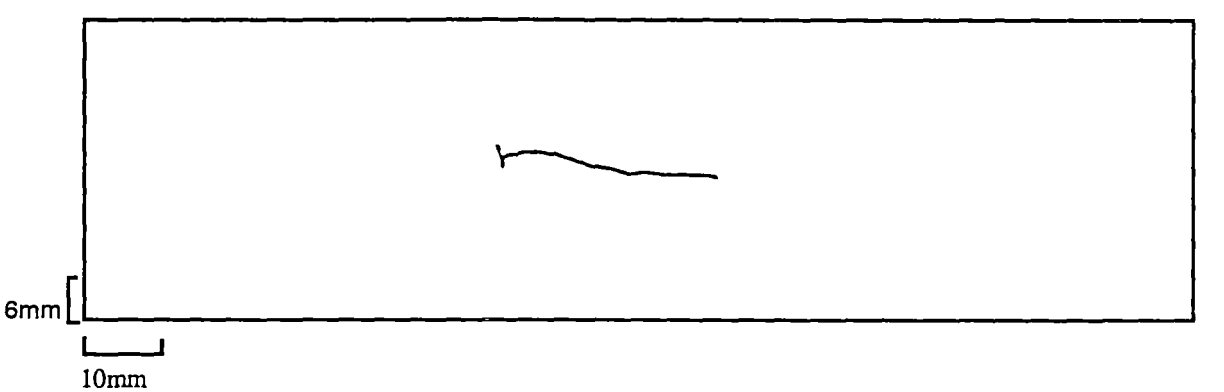
Record 1. (Time 0) EEG: High voltage irregular activity. MAP: 110 mmHg. CrdBF: 60 ml·min^{-1}. Tb: 37.5°C



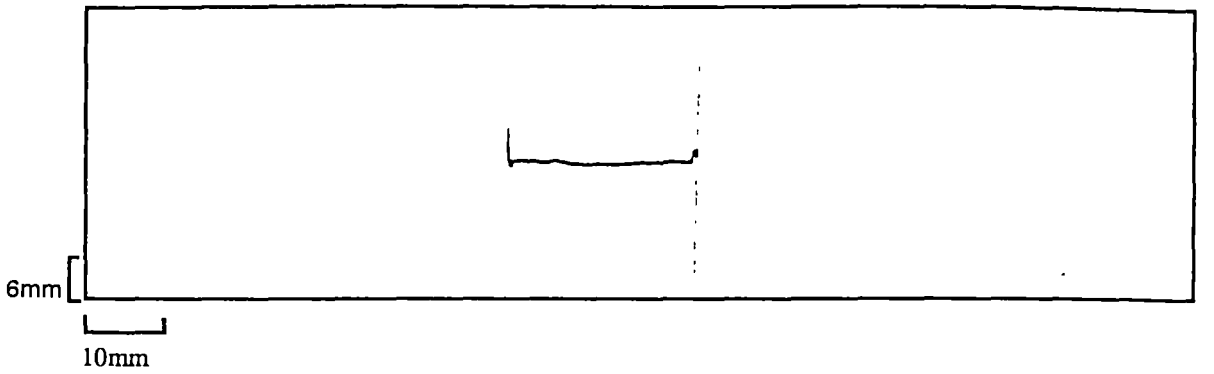
Record 2. (+50 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 110 mmHg. CrdBF: 45 ml·min^{-1}. Tb: 36.2°C.



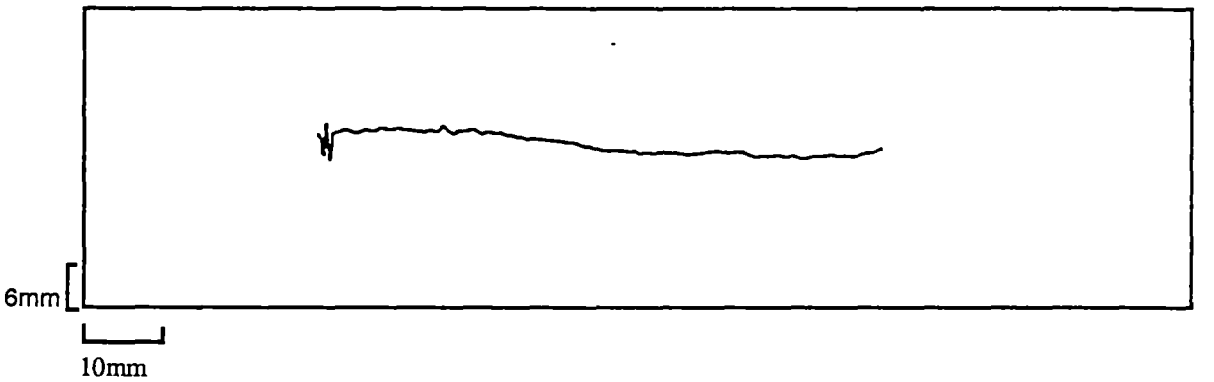
Record 3. (+90 min) EEG: Marked suppression. Low voltage. MAP: 110 mmHg. CrdBF: 38 ml·min^{-1}. Tb: 30.6°C.



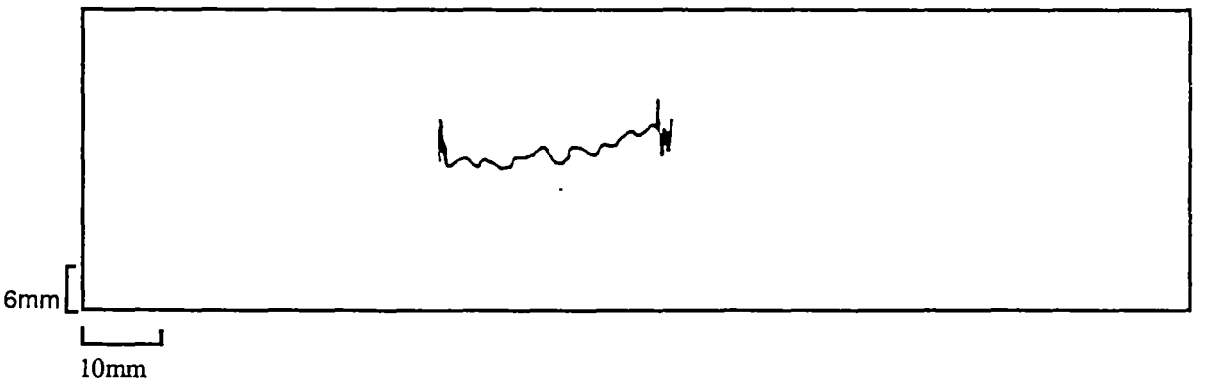
Record 4. (+110 min) EEG: Complete suppression. Isoelectric state. MAP: 120 mmHg. CrdBF: 34 ml·min^{-1}. Tb: 28.5°C.



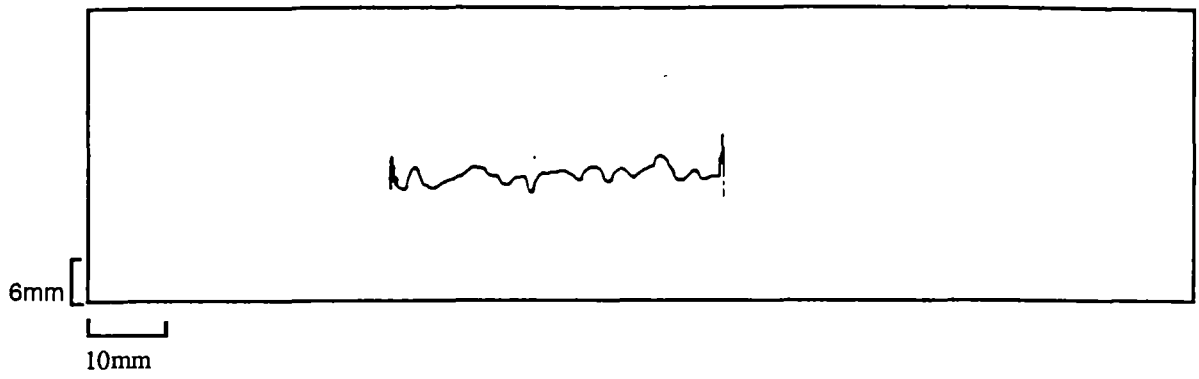
Record 5. (+140 min) EEG: Complete suppression. Isoelectric state. MAP: 140 mmHg. CrdBF: 29 ml·min^{-1}. Tb:26.0°C.



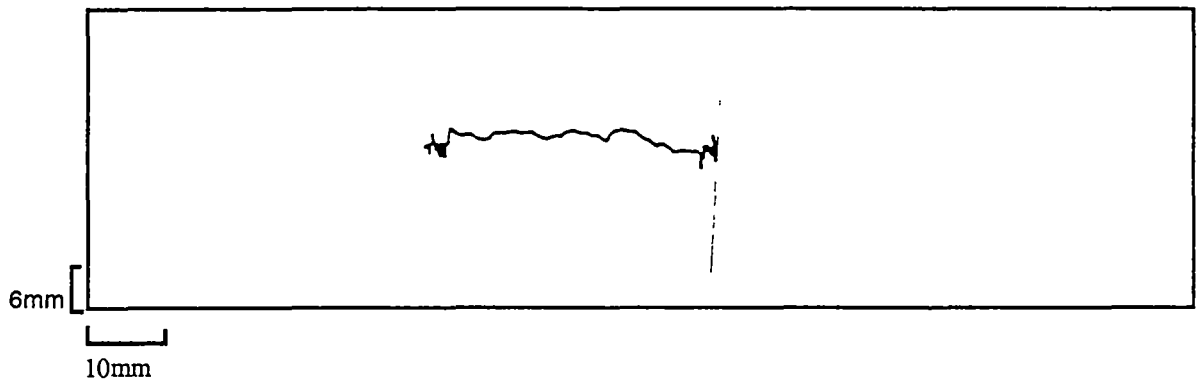
Record 6. (+170 min) EEG: Complete suppression. Isoelectric state. MAP: 110 mmHg. CrdBF: 17ml·min^{-1}. Tb: 25.2°C.



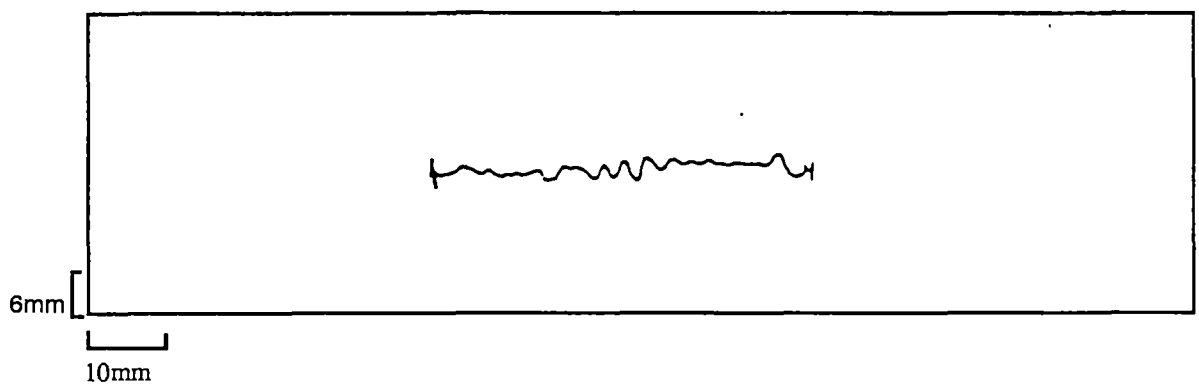
Record 7. (+260 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 110 mmHg. CrdBF: 15 ml·min^{-1}. Tb: 28.9°C.



Record 8. (+320 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 130 mmHg. CrdBF: 15 ml·min^{-1}. Tb: 34.0°C.



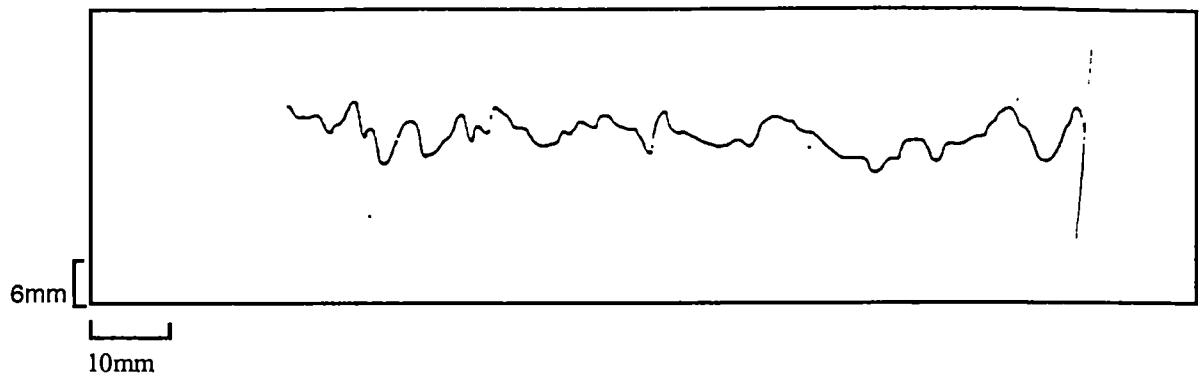
Record 9. (+370 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 140 mmHg. CrdBF: 22 ml·min^{-1}. Tb: 37.0°C.



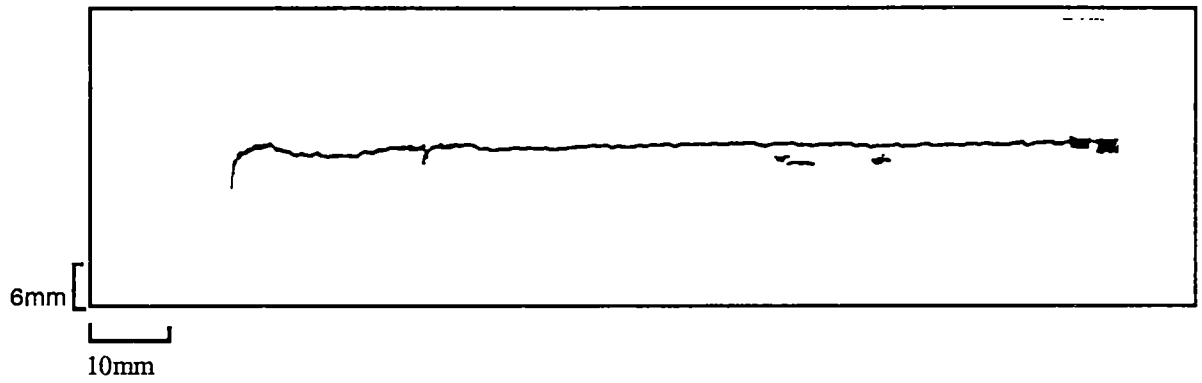
Record 10. (+400 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 140 mmHg. CrdBF: 20 ml·min^{-1}. Tb: 37.5°C.

2. HAc group

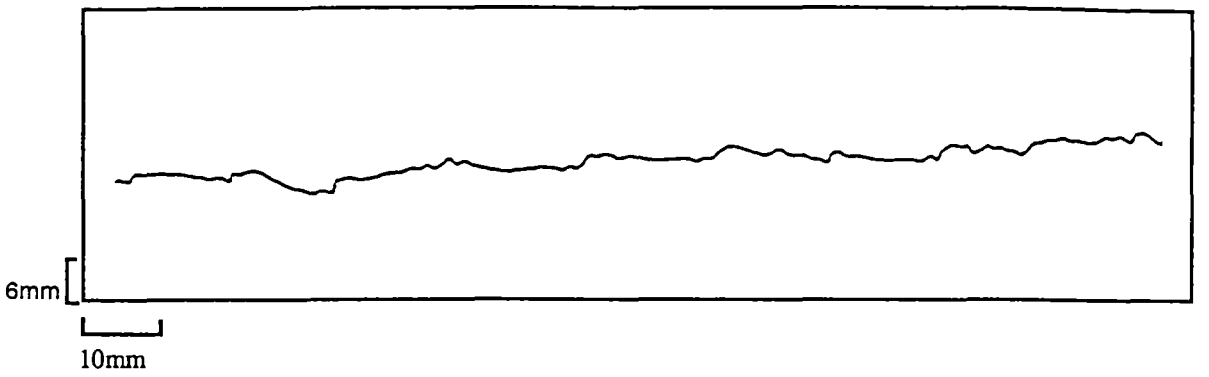
Rabbit 1504. Female. Recorded on April 15 1993



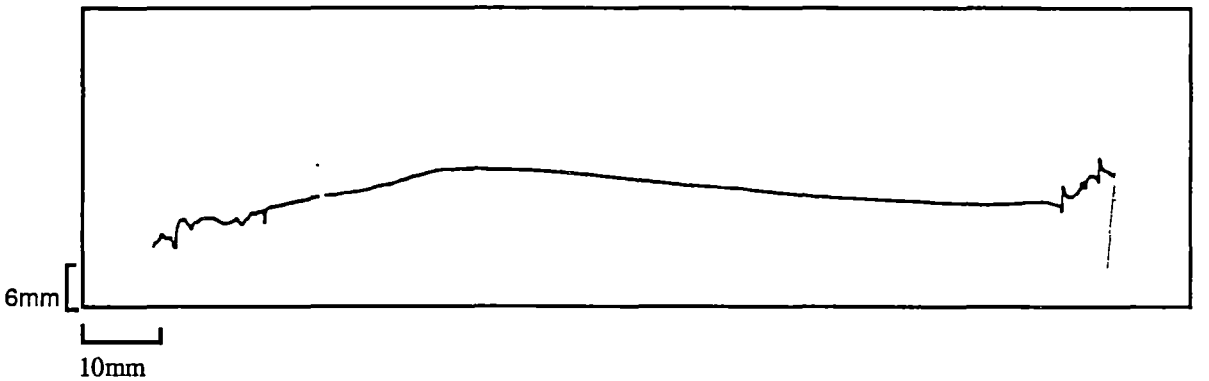
Record 1. (Time 0) EEG: Slight suppression, high voltage, slow waves interrupted by flattening. MAP: 120 mmHg. CrdBF: 29 ml·min^{-1}. Tb: 35.9°C



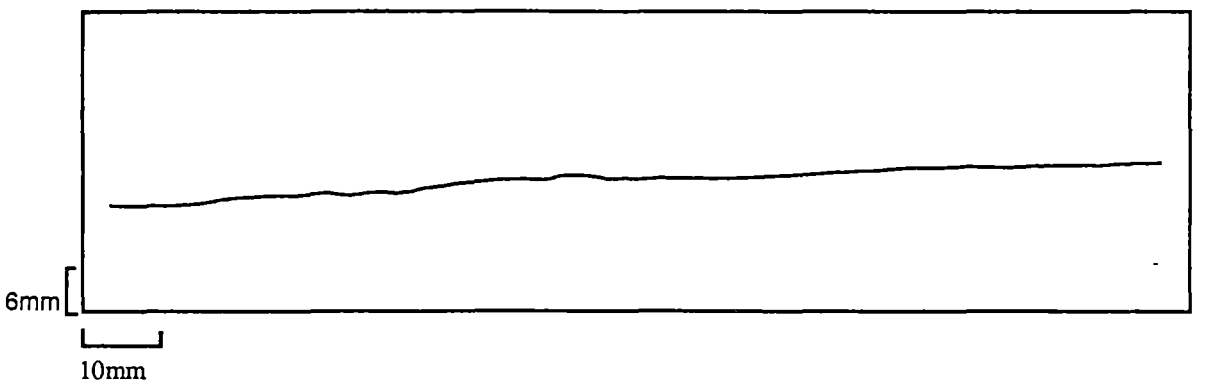
Record 2. (+20 min) EEG: Marked suppression. Low voltage. MAP: 140 mmHg. CrdBF: 17 ml·min^{-1}. Tb: 35.5°C.



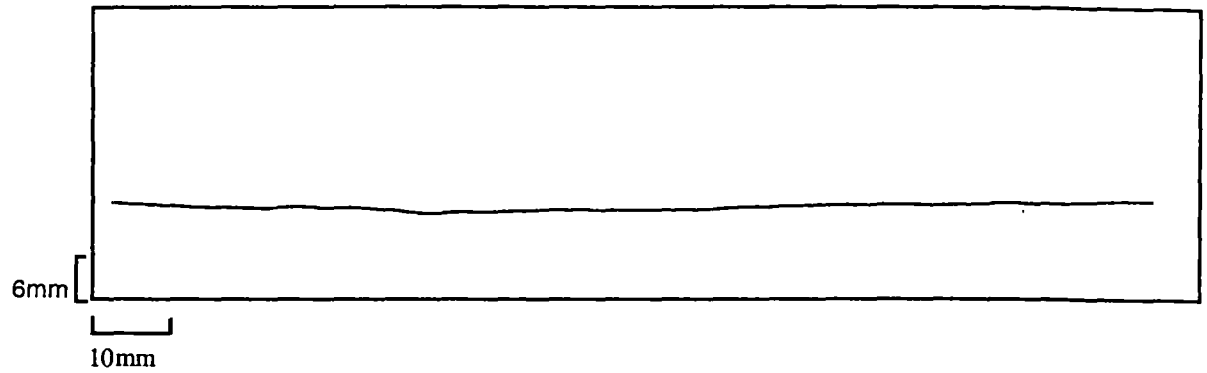
Record 3. (+50 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 105 mmHg. CrdBF: 11 ml·min^{-1}. Tb: 30.3°C.



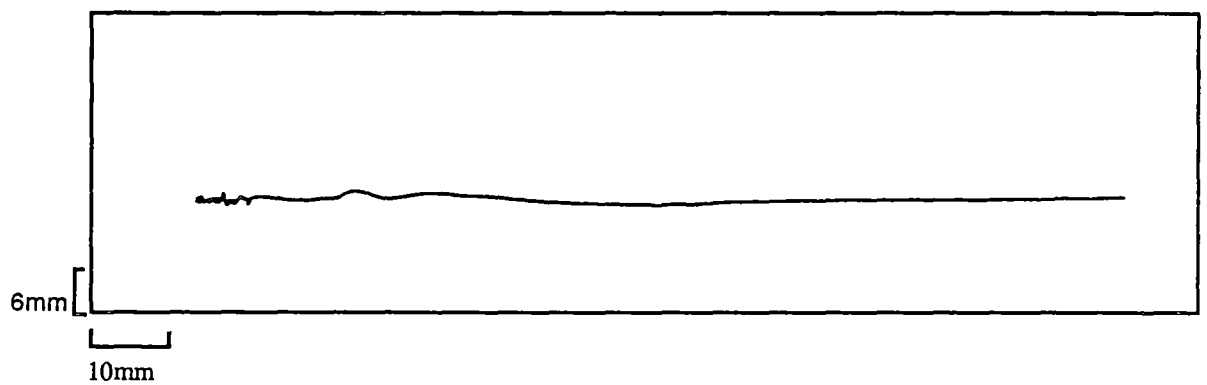
Record 4. (+70 min) EEG: Complete suppression. Isoelectric state. MAP: 75 mmHg. CrdBF: 7 ml·min^{-1}. Tb: 26.2°C.



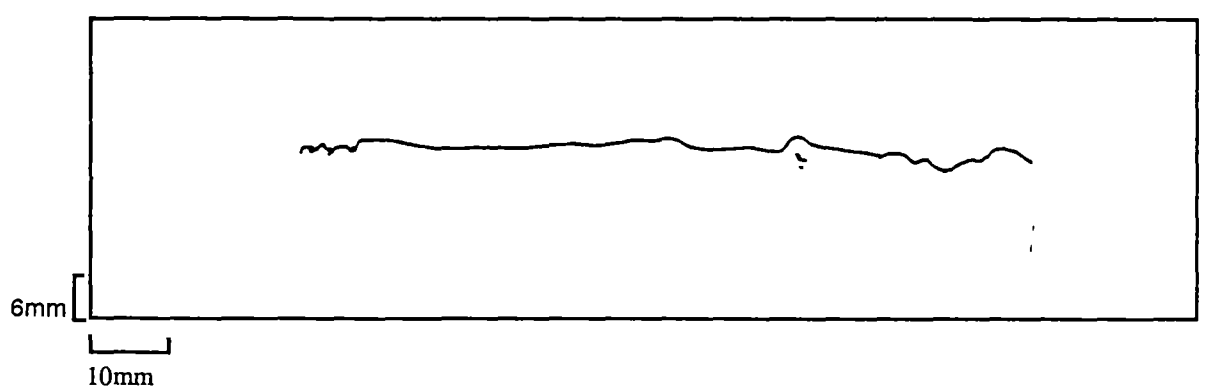
Record 5. (+75 min) EEG: Complete suppression. Isoelectric state. MAP: 50 mmHg. CrdBF: 6ml·min^{-1}. Tb: 25.8°C.



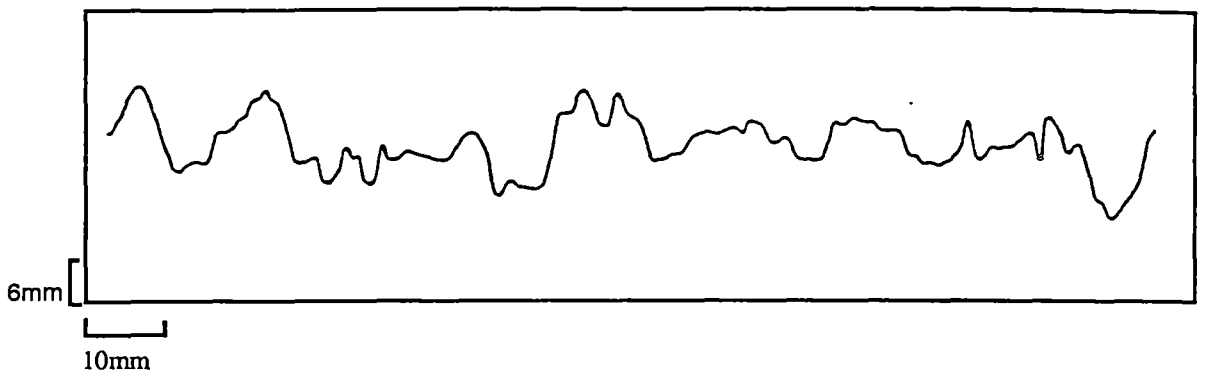
Record 6. (+120 min) EEG: Complete suppression. Isoelectric state. MAP: 40 mmHg. CrdBF: 2 ml·min^{-1}. Tb: 25.3°C.



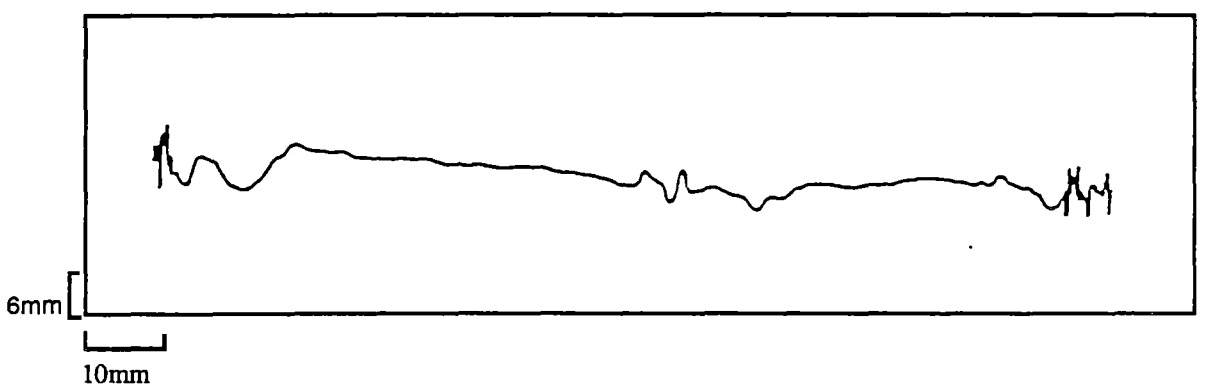
Record 7. (+150 min) EEG: Complete suppression. Isoelectric state. MAP: 60 mmHg. CrdBF: 4 ml·min^{-1}. Tb: 26.3°C.



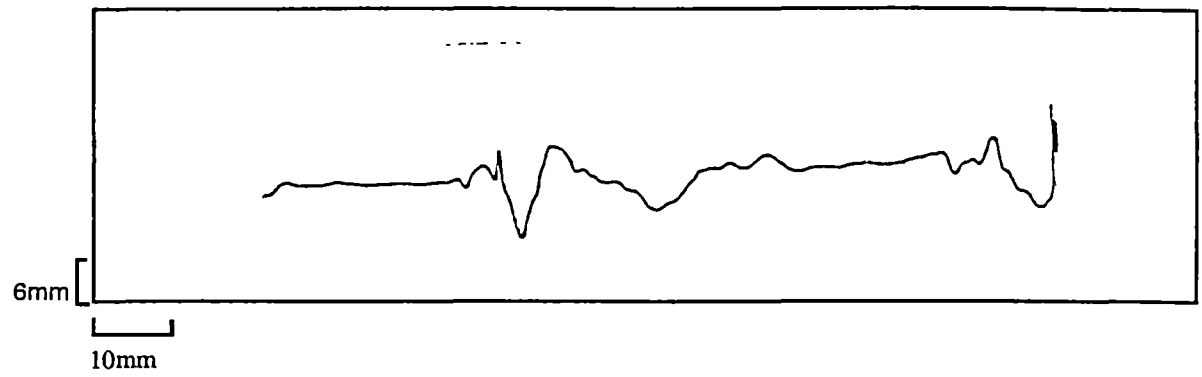
Record 8. (+170 min) EEG: Complete suppression. Isoelectric state. MAP: 50 mmHg. CrdBF: 4 ml·min^{-1}. Tb: 30.4°C.



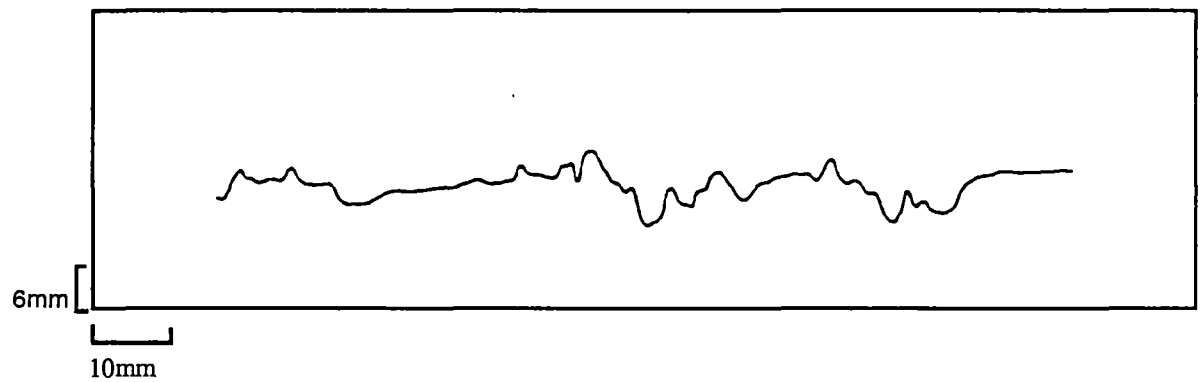
Record 1. (Time 0) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 120 mmHg. CrdBF: 26 ml·min^{-1}. Tb: 36.5°C.



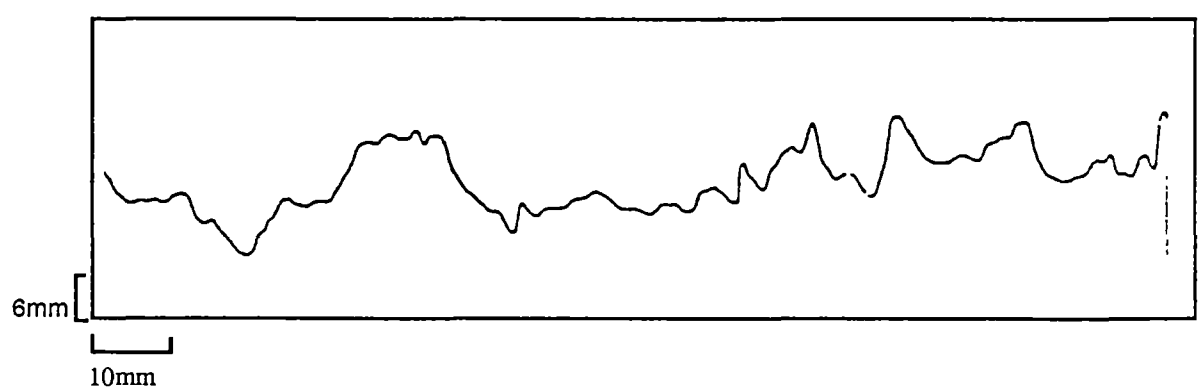
Record 2. (+20 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 80 mmHg. CrdBF: 11 ml·min^{-1}. Tb: 36.2°C.



Record 3. (+25 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 24 ml·min^{-1}. Tb: 36.0°C.

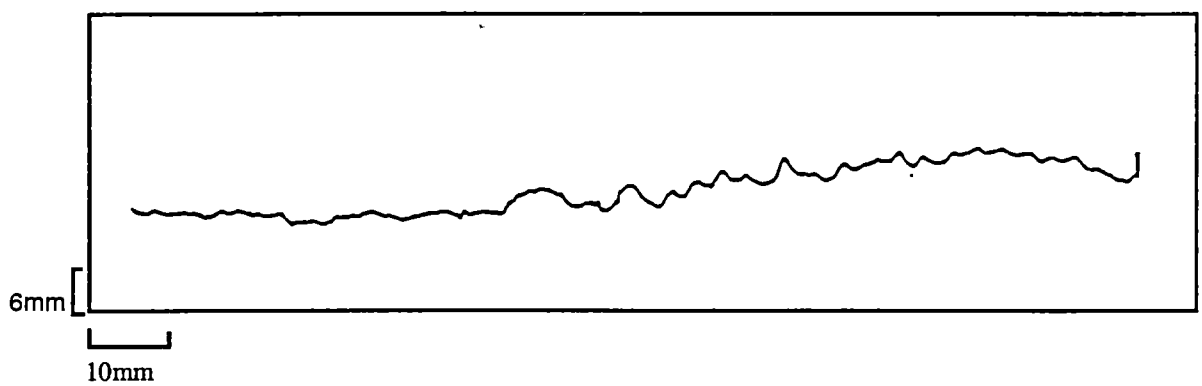


Record 4. (+30 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 23 ml·min^{-1}. Tb: 35.7°C.

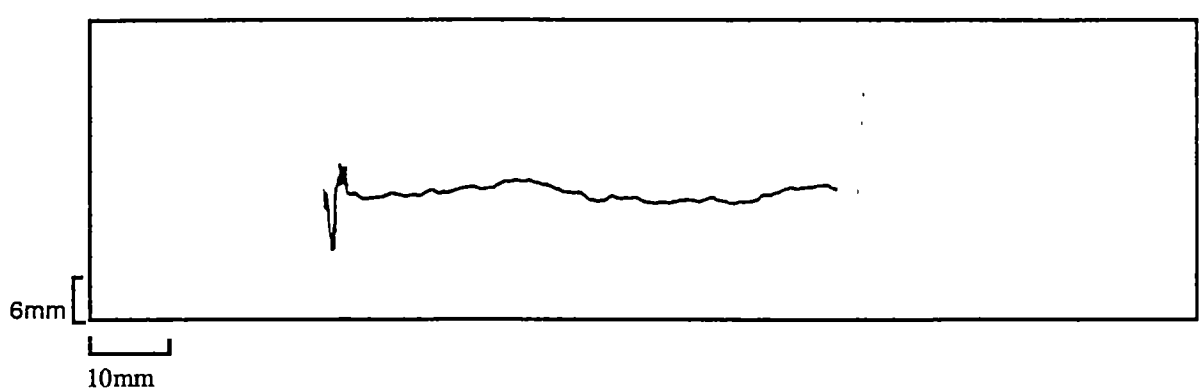


Record 5. (+60 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 21 ml·min^{-1}. Tb: 32.2°C.

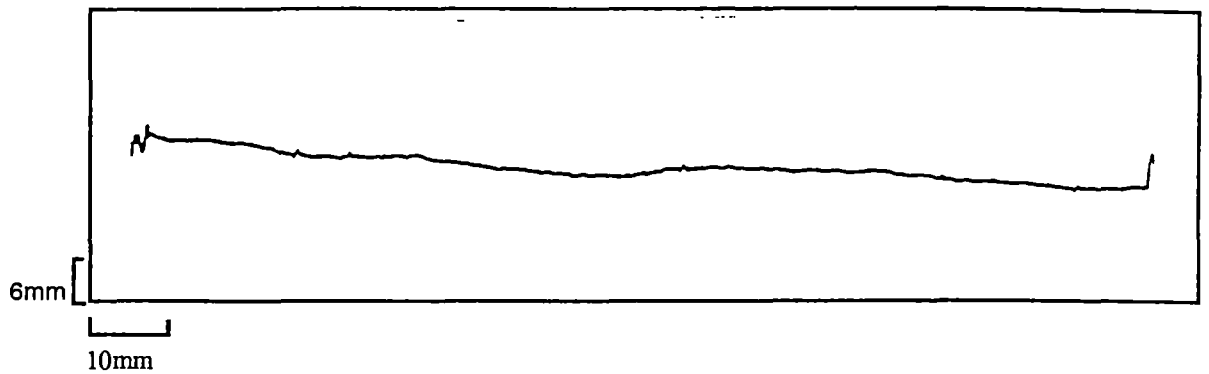
Record 6. (+100 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 28.2°C.



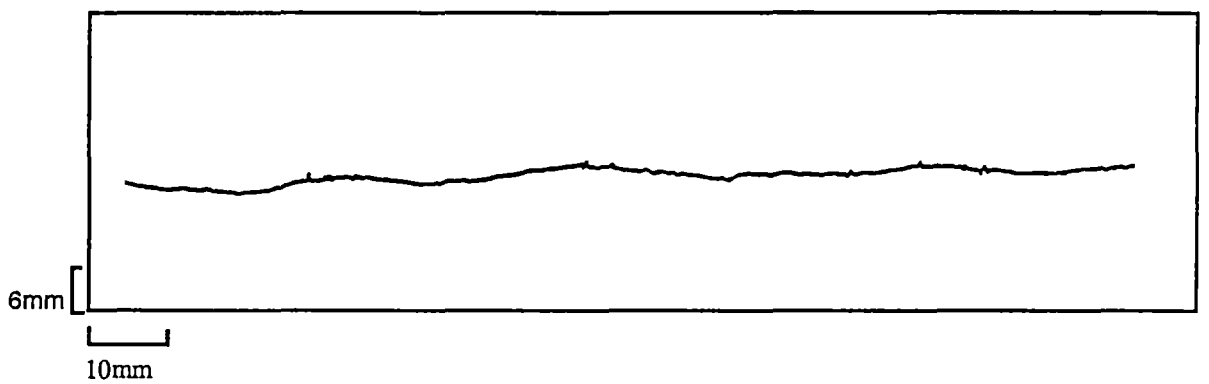
Record 7. (+120 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 95 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 26.8°C.



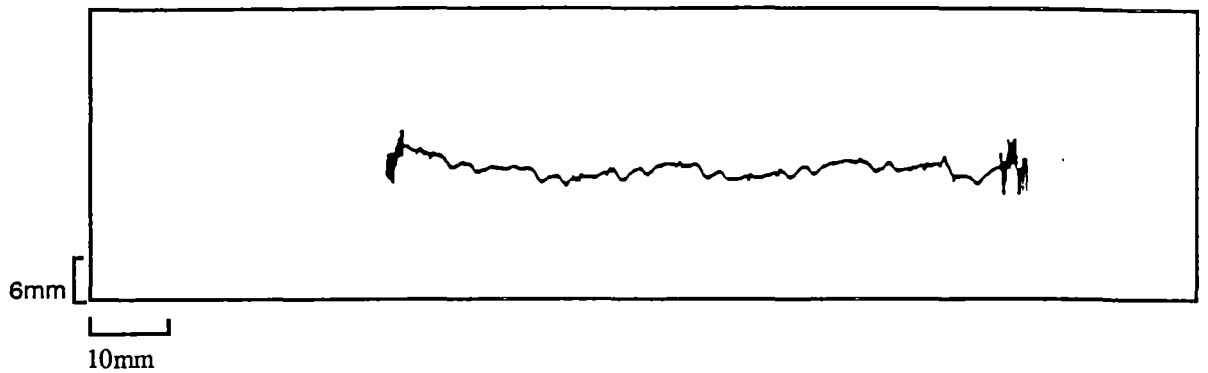
Record 8. (+135 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 80 mmHg. CrdBF: 3 ml·min^{-1}. Tb: 25.5°C.



Record 9. (+195 min) EEG: Complete suppression. Isoelectric state MAP: 65 mmHg. CrdBF: 2 ml·min^{-1}. Tb: 25.0°C.



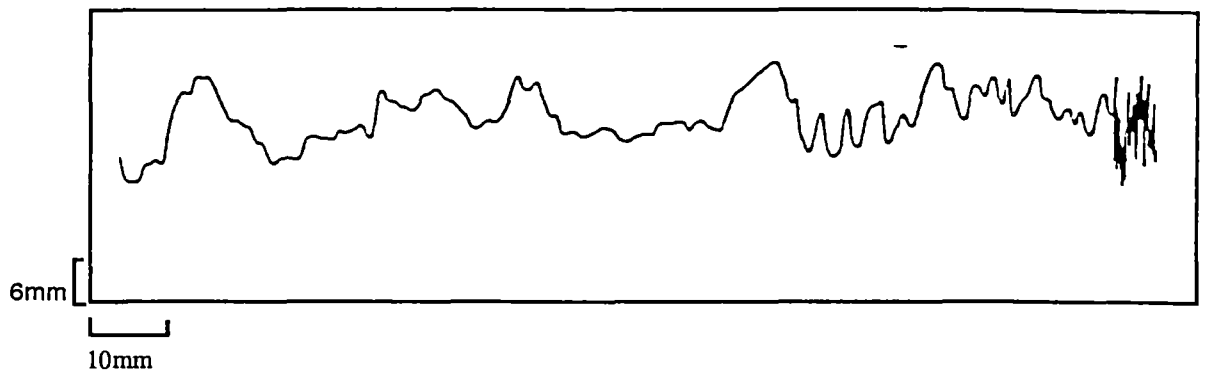
Record 10. (+230 min) EEG: Complete suppression. Isoelectric state. MAP: 70 mmHg. CrdBF: 2 ml·min^{-1}. Tb: 28.7°C.



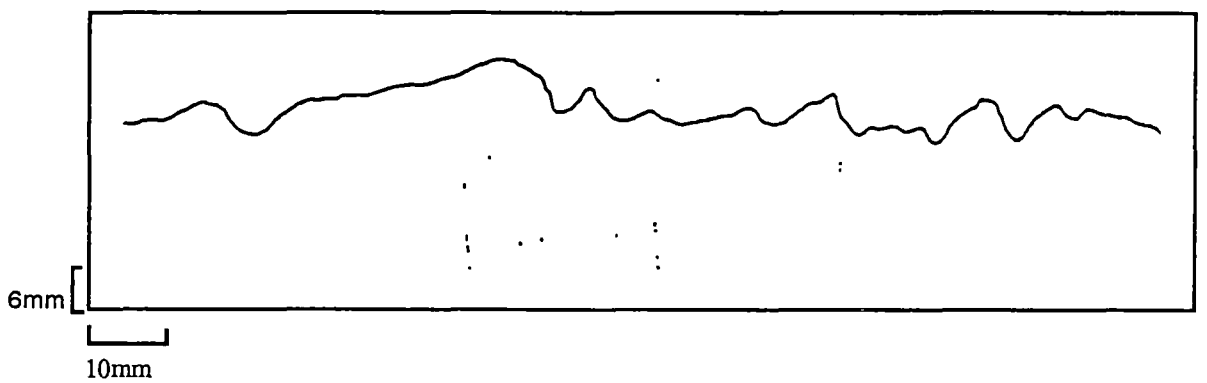
Record 11. (270 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 80 mmHg. CrdBF: 3 ml·min^{-1}. Tb: 32.0°C.

Record 12. (+340 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 80 mmHg. CrdBF: 2 ml·min^{-1}. Tb: 34.9°C.

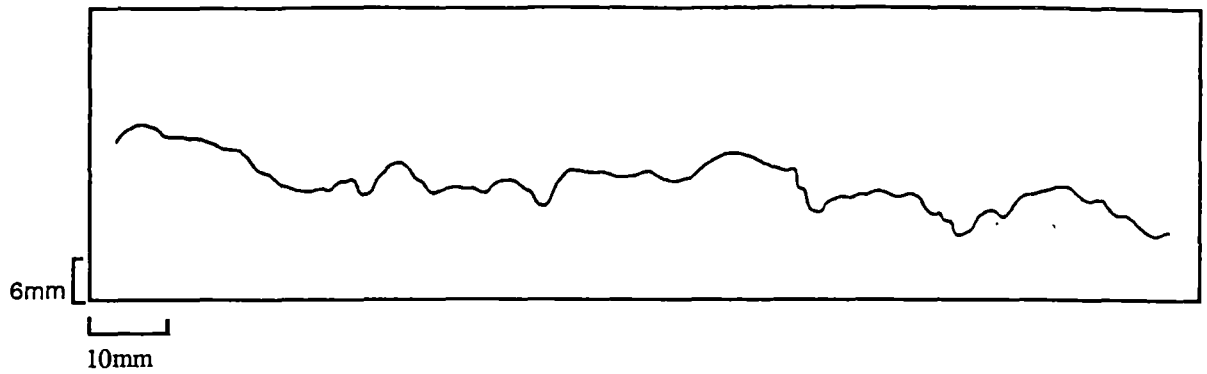
Record 13. (+360 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 60 mmHg. CrdBF: 1 ml·min^{-1}. Tb: 35.5°C.



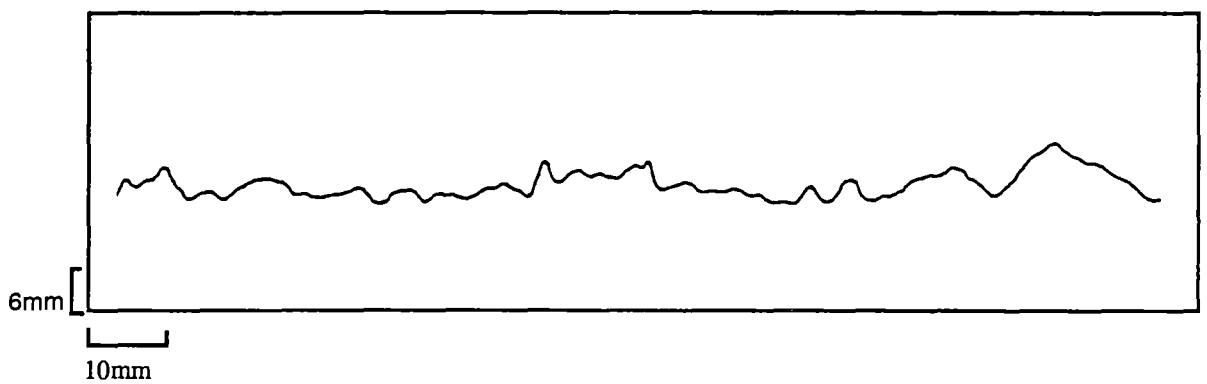
Record 1. (Time 0) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 120 mmHg. CrdBF: 24 ml·min^{-1}. Tb: 36.5°C



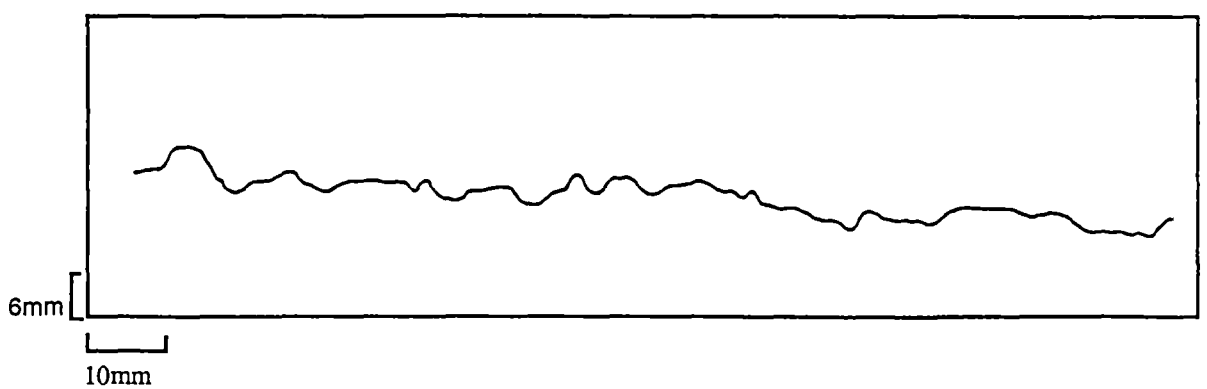
Record 2. (+5 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 170 mmHg. CrdBF: 28 ml·min^{-1}. Tb: 36.0°C.



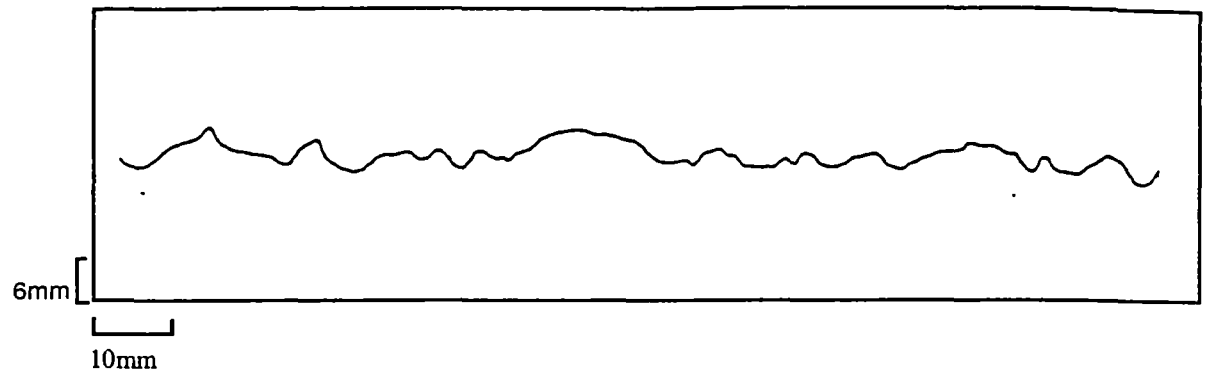
Record3. (+30 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 120 mmHg. CrdBF: 14 ml·min^{-1}. Tb: 35.6°C.



Record 4. (+40 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 110 mmHg. CrdBF: 14 ml·min^{-1}. Tb: 35.5°C.

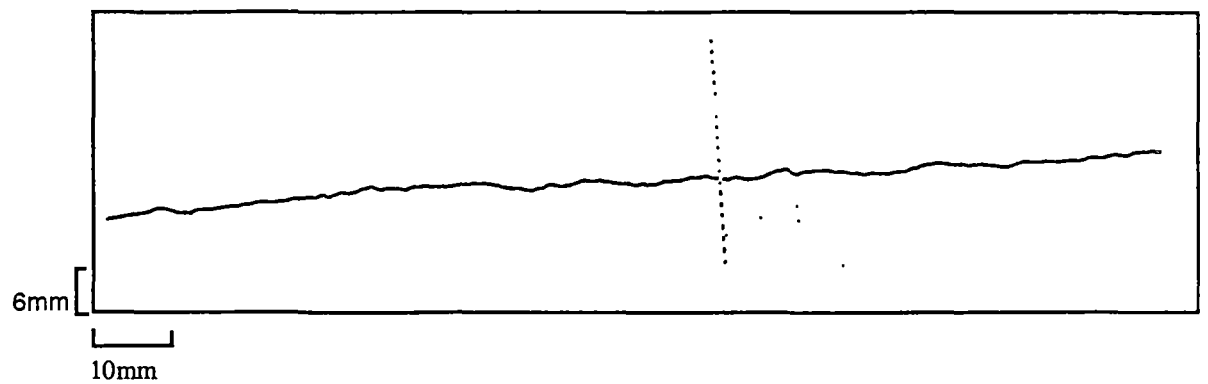


Record 5. (70 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 110 mmHg. CrdBF: 9 ml·min^{-1}. Tb: 26.4°C.

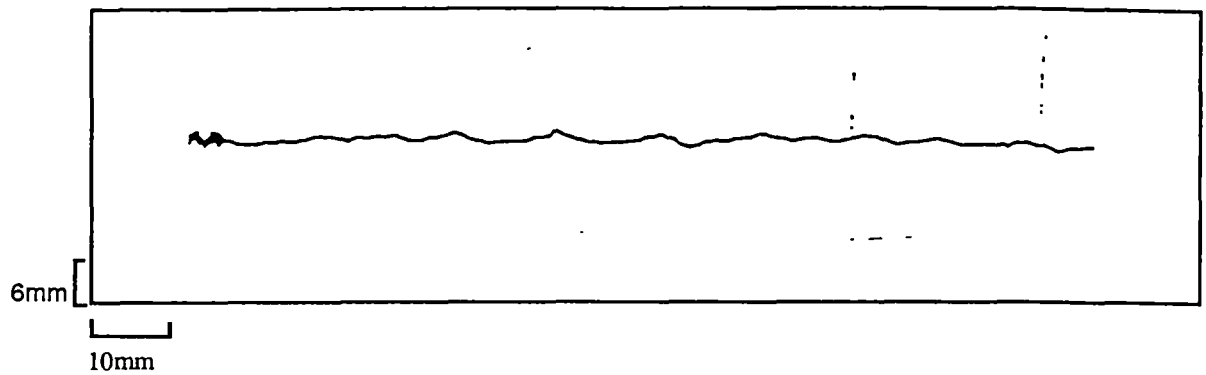


Record 6. (+80 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 9 ml·min^{-1}. Tb: 25.4°C.

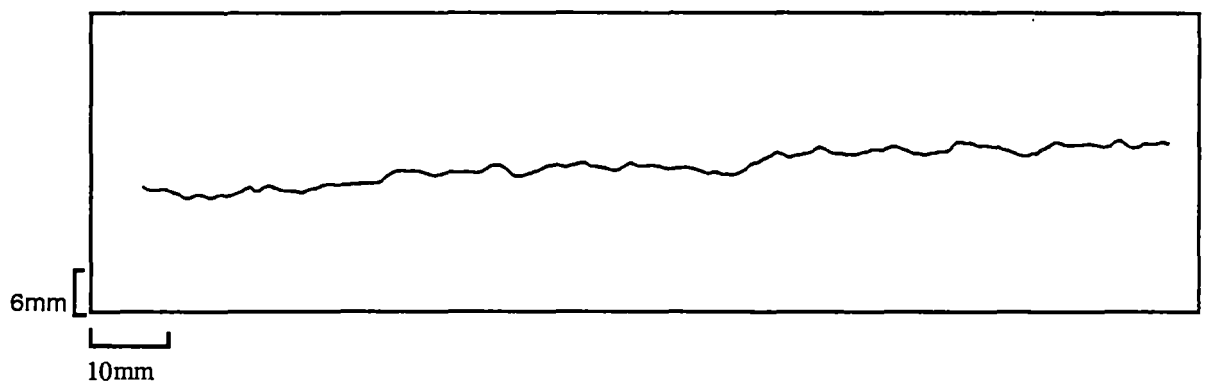
Record 7. (+100 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 90 mmHg. CrdBF: 8 ml·min^{-1}. Tb: 24.7°C.



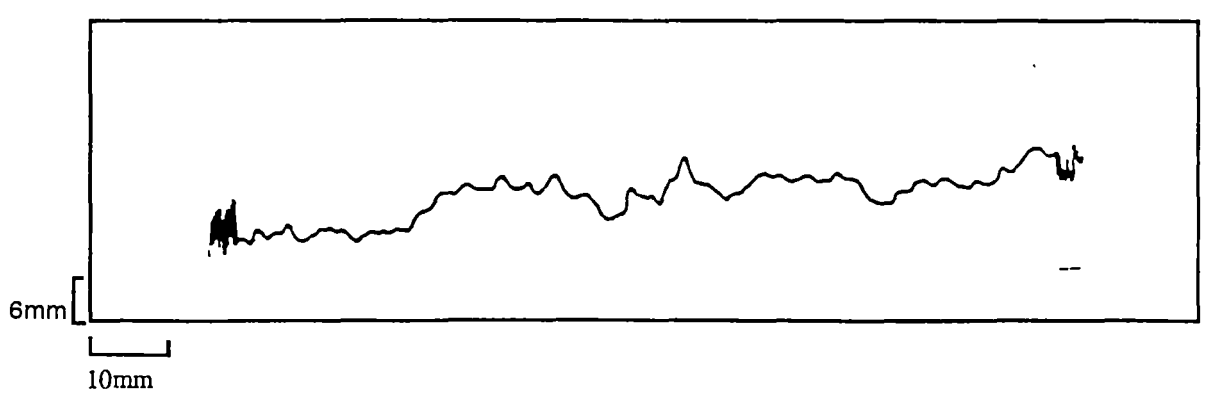
Record 8. (+160 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 80 mmHg. CrdBF: 6 ml·min^{-1}. Tb: 25.3°C.



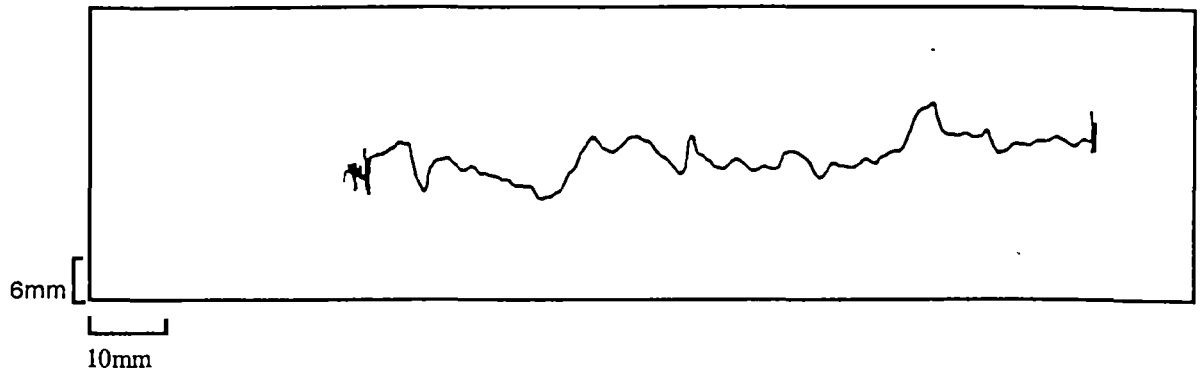
Record 9. (+200 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 70 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 28.9°C.



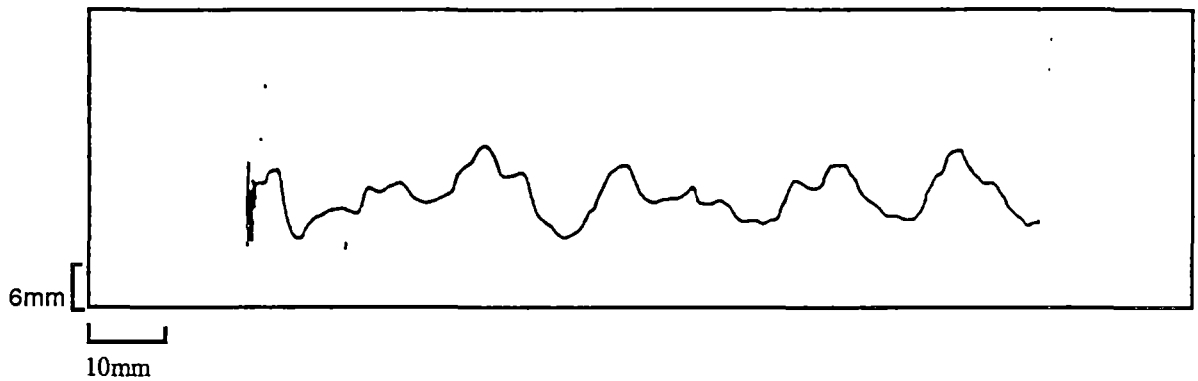
Record 10. (+210 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 70 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 31.7°C.



Record 11. (+230 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 60 mmHg. CrdBF: 5 ml·min^{-1}. Tb:32.8°C.

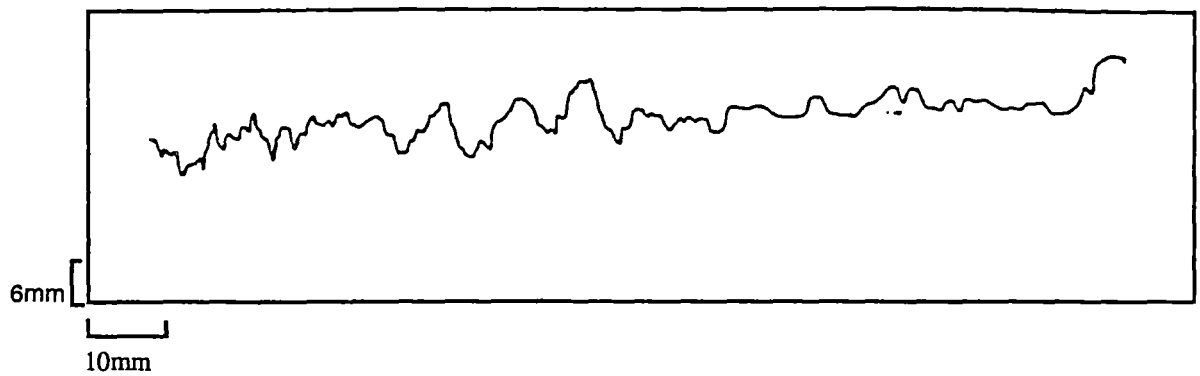


Record 12. (+290 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 70 mmHg. CrdBF: 6 ml·min^{-1}. Tb: 35.8°C.

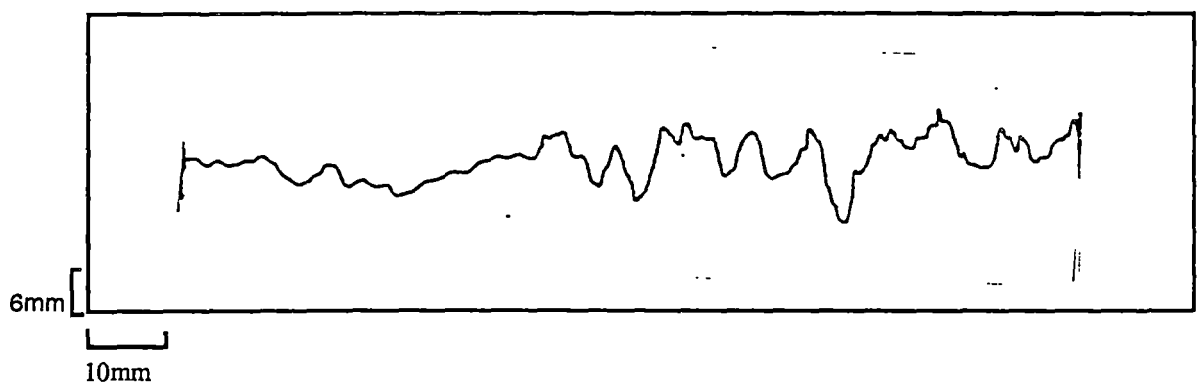


Record 13. (300 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 60 mmHg. CrdBF: 7 ml·min^{-1}. Tb: 36.3°C.

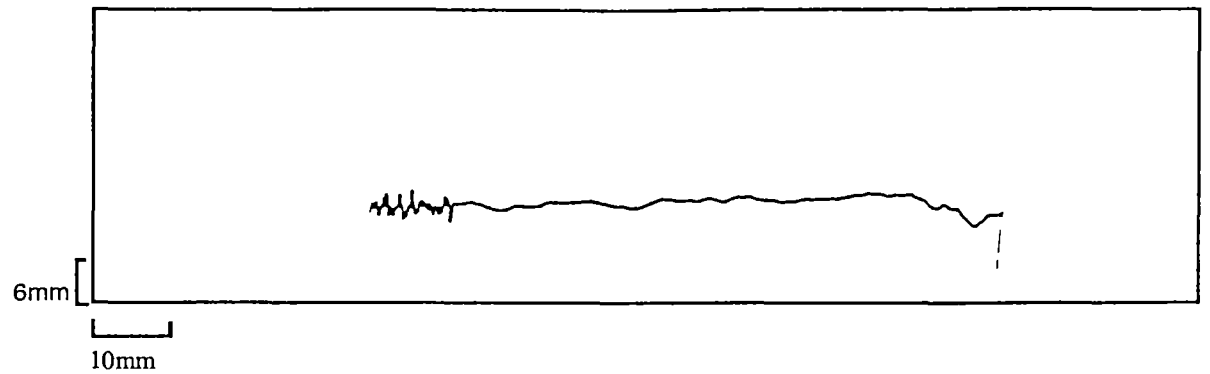
Rabbit 0610. Male. Recorded on October 6 1992



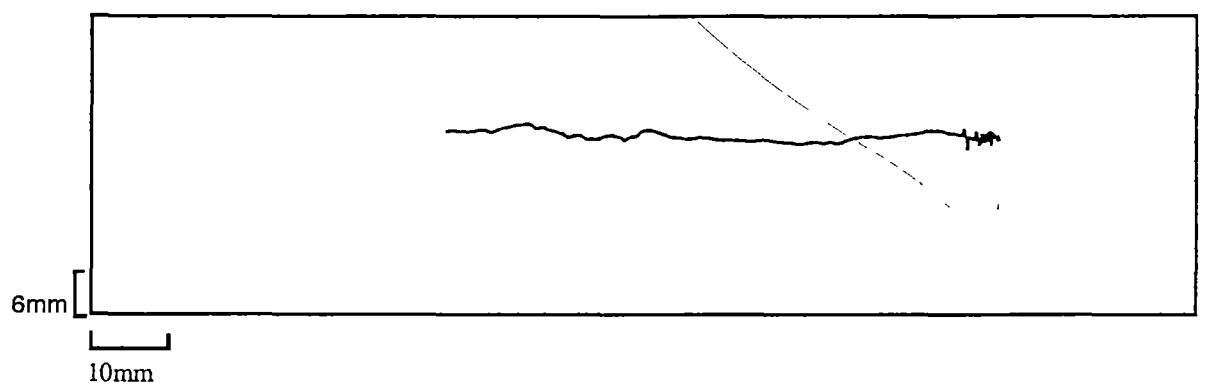
Record 1. (Time 0) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 105 mmHg. CrdBF: 50 ml·min^{-1}. Tb: 36.5°C



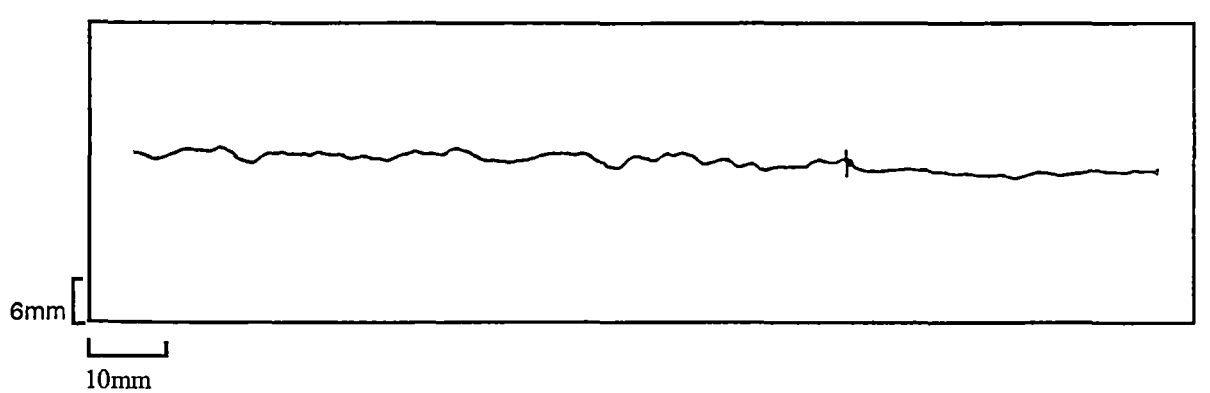
Record 2. (+20 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 100 mmHg. CrdBF: 55 ml·min^{-1}. Tb: 36.0°C.



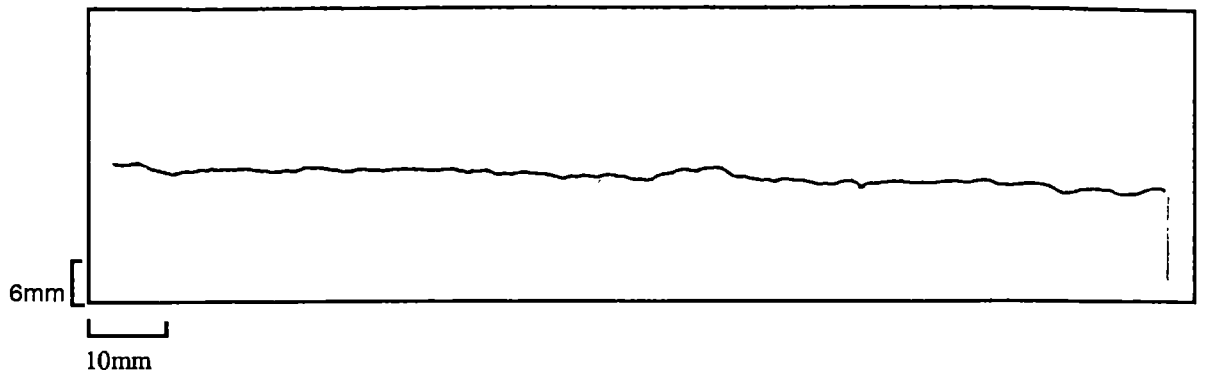
Record 3. (+120 min) EEG: Marked suppression. Low voltage. MAP: 120 mmHg. CrdBF: 13 ml·min^{-1}. Tb: 28.4°C.



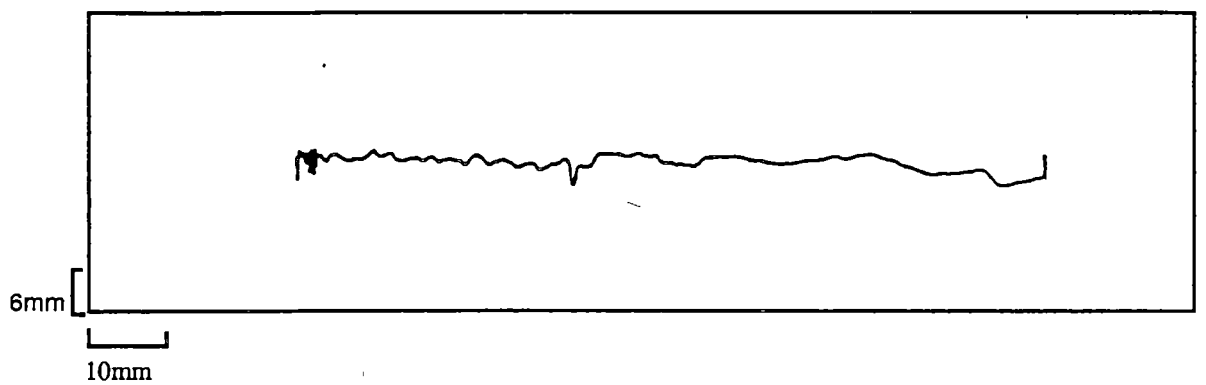
Record 4. (+150 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 120 mmHg. CrdBF: 8 ml·min^{-1}. Tb: 25.4°C.



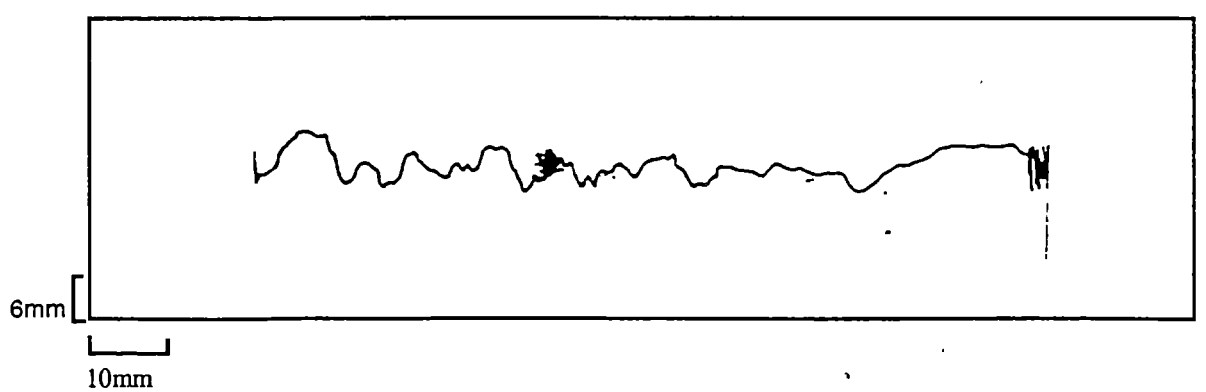
Record 5. (+170 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 115 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 24.8°C.



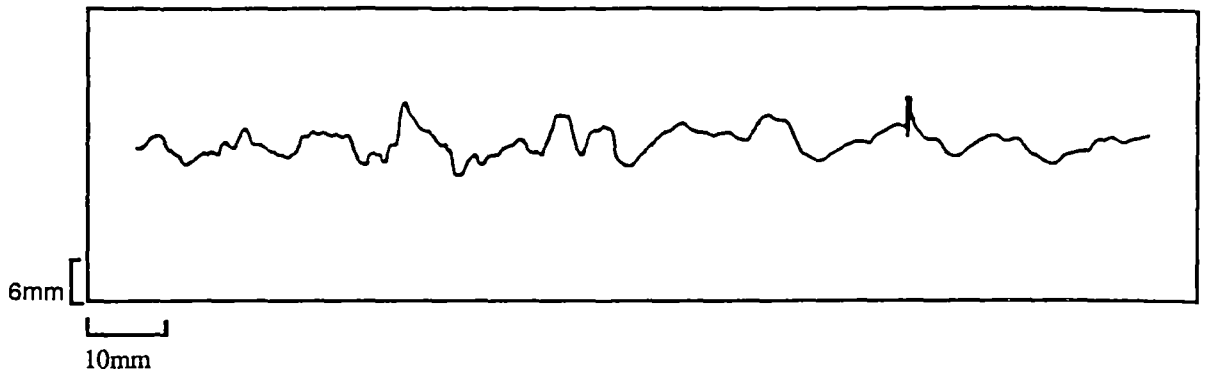
Record 6. (240 min) EEG: Marked suppression. Low voltage. MAP: 90 mmHg. CrdBF: 6 ml·min^{-1}. Tb: 26.0°C.



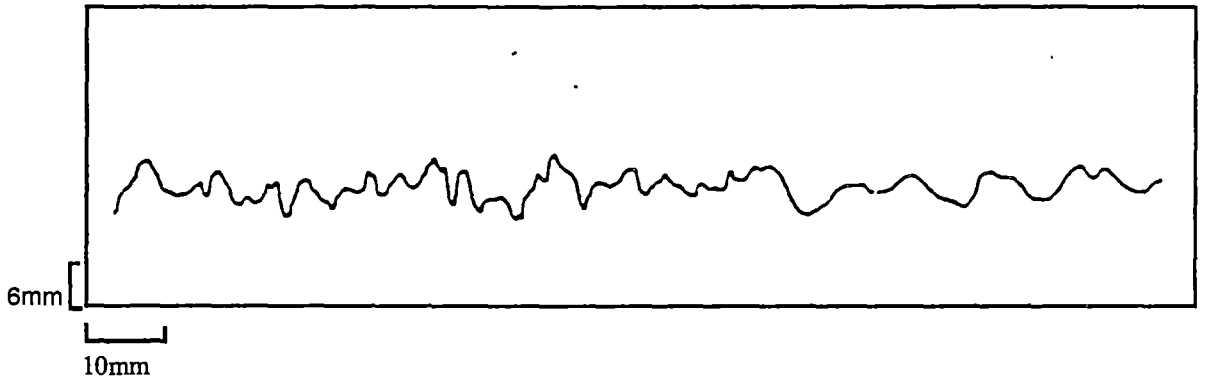
Record 7. (+260 min) EEG: Marked suppression. Low voltage, slow waves interrupted by flattening. MAP: 95 mmHg. CrdBF: 5 ml·min^{-1}. Tb: 28.3°C.



Record 8. (+300 min) EEG: Moderate suppression. Low voltage, slow waves interrupted by flattening. MAP: 105 mmHg. CrdBF: 11 ml·min^{-1}. Tb: 31.6°C.



Record 9. (+350 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 105 mmHg. CrdBF: 15 ml·min^{-1}. Tb: 35.0°C



Record 10. (+410 min) EEG: Slight suppression. High voltage, slow waves interrupted by flattening. MAP: 110 mmHg. CrdBF: 11 ml·min^{-1}. Tb:37.5°C.