A STUDY OF TRANSIENT CHANGES IN PLASMA POTASSIUM DURING HAEMORRHAGIC HYPOTENSION USING ION-SELECTIVE ELECTRODE CATHETERS

by

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DECLARATION

This thesis comprises my own original studies, and has not been previously presented as a thesis in any form.

All experiments have been performed by me.

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November, 1987

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ABIOTONA SOKARI, M.B., B.S.

FORMAT OF THE THESIS

The thesis consists of three sections, each describing a separate but related study. A General Introduction of the whole study is given on page 7 . Each section is treated in the following format:

Introduction

Materials & Method

Results

Discussion & Conclusion

Summary

A General Conclusion is drawn at the end, followed by references and additional papers published.

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SECTION ONE

ABSTRACT

Potassium ion-selective electrode catheters have been recently devised to continuously monitor changes in plasma K^+ levels in animals including man, in response to various physiological and pharmacological stimuli. In this thesis, such electrode catheters have been used to continuously monitor the plasma K^+ changes during haemorrhagic hypotension in deeply anaesthetised cats. Similar transient changes in plasma K^+ produced by either catecholamines, morphine, hyperventilation or asphyxia, were also studied to throw some light on the mechanisms of plasma K^+ changes during and after haemorrhage. The continuous monitoring of mean arterial blood pressure, lead II electrocardiogram, and end-tidal CO_2 simultaneously with changes in plasma K^+ levels enabled information to be obtained about the role of hyperkalaemia (plasma K levels above 5.5 mmol/l) in the reversibility of cardiac function after prolonged haemorrhagic hypotension.

The insertion of the K^+-selective electrode into different sites in the animal, viz: the inferior vena cava with the electrode tip above the entry of the hepatic vein, and either the aorta in the abdominal region, or the low inferior vena cava with the electrode tip below the entry of the hepatic vein, permitted the assessment of any contribution to the plasma K^+ levels by the liver, the heart or the hind skeletal muscles, as well as the contribution to plasma K^+ from the splanchnic region via the hepatic vein.

Results showed that plasma K^+ levels increased in all the sites examined with greatest increases in the high inferior vena cava during haemorrhagic hypotension.

Early rises in plasma K^+ during haemorrhage were accompanied by hyperventilation-induced respiratory alkalosis. If hypotension continued without reinfusion of the shed blood, plasma K^+ rose further with accompanying metabolic acidosis.

Hyperventilation *per se* was found to contribute to the rise in plasma K^+ levels via the release of either catecholamines or endogenous opioids or both during haemorrhagic hypotension. Beta-adrenoceptor and opiate receptor blockade reduced the hyperventilation-induced hyperkalaemia. Alpha adrenoceptor blockade did not significantly prevent the hyperkalaemia produced by hyperventilation *per se*.

The alpha-adrenergic receptors and opiate receptors were found to be involved in the release of K^+ after haemorrhage, and the release was mainly from the region drained by the hepatic vein. Adrenaline or morphine injections produced a rise in plasma K^+. In the presence of the alpha-adrenoceptor blockers, phentolamine or prazosin, the rise in plasma K^+ following haemorrhage was significantly reduced. Naloxone, an opioid receptor blocker did not prevent the rise in plasma K^+ but significantly lowered the raised K^+ after haemorrhage. The effects of naloxone depended on the time of injection, the duration and the severity of

haemorrhage. Propranolol, a beta-adrenoceptor blocker did not prevent the haemorrhage-induced rise in plasma K^+. The action of naloxone appears to be either potentiating the effects of circulating catecholamines in transiently changing the plasma K^+ concentration and raising the mean arterial blood pressure, or rendering the body tissues more sensitive to catecholamines. Naloxone was also found to antagonise the depressor effects of opioids released during haemorrhage and increased the mean arterial blood pressure, as well as reducing the rise in plasma K^+ resulting from haemorrhagic hypotension. The mechanism of raising the blood pressure by naloxone following haemorrhage, seems to be different from that of lowering the plasma K^+ for even after keeping the mean arterial blood pressure low by further withdrawal of blood, naloxone still lowered the raised plasma K^+.

The vagi were found to play a significant role in diminishing the rise in plasma K^+ as bilateral cervical vagotomy caused an increased rise in plasma K^+ with an accompanying rise in mean arterial blood pressure. Less severe haemorrhage after vagotomy caused quantitatively similar rises in plasma K^+ to those produced by severe haemorrhage with the vagi intact.

It appears that two levels of increased plasma K^+ occur during prolonged haemorrhagic hypotension. The end of the first level and the beginning of the second, may serve as an indicator of generalised hyperkalaemia from global ischaemia following haemorrhage.

This indicator heralds the beginning of irreversibility in shock resulting from haemorrhage. The sudden deaths in animals under prolonged anaesthesia and hypotension with moderate hyperkalaemia (6 to 7 mmol/l) were attributed to respiratory and cardiac failure resulting from the increasing shortage of oxygen supply (hypoxia) to the cardiac muscle and the body tissues, as well as lack of wash-out of accumulating metabolites, increased plasma K^+ and acidosis with gross base deficits.

Hyperkalaemia per se, following haemorrhage was not found to be the cause of death but contributed to the worsening state of cardiac function before cardiac and respiratory arrest occurred. Reinfusion of the shed blood or the volume expander, dextran 110, restored the plasma level of K^+ to normal in some cases and did not in others. However, in all cases during the second level of raised plasma K^+ following haemorrhage reinfusion produced severe acidosis and gross base deficit resulting in death whether hyperkalaemia was present or not.

The uptake of plasma K^+ may take place in the heart, the lungs, the skeletal muscles or all three as well as the dilution of HIVC blood by superior vena cava and vena azygos blood as shown by the consistent greater levels of plasma K^+ in the high inferior vena cava than in the aorta, and the low inferior vena cava (personal communication, MacKenna, 1985) following haemorrhage. The prevention of the fall in plasma K^+ following reinfusion of the shed blood by the presence of propranolol, the beta-

adrenoceptor blocker, and the sustained greater level of plasma K^+ following haemorrhage or injection of adrenaline in the presence of propranolol suggest that beta-adrenoceptors are involved in the uptake of plasma K^+.

This study reveals that:

1. The degree of haemorrhage is the major factor that determines the outcome.
2. Plasma changes in acid-base status vary.
3. Ventricular fibrillation does not always precede death, nor does it always supervene when K^+ concentration reaches a particular level; anoxaemia and plasma pH are co-determinants.

The findings suggest that ionic and myocardial effects of ischaemia in patients may be equally variable.

The growing usefulness of the potassium ion-selective electrode catheters as a new and sensitive tool for the continuous monitoring of plasma potassium in experimental laboratories and intensive care units cannot be overemphasized. The continuous recording of plasma K^+ with the potassium-selective electrode is as impressive as the continuous monitoring of

arterial blood pressure, electrocardiogram or electrocorticogram, blood pH and oxygen tensions using the respective electrodes.

SECTION ONE

INTRODUCTION

1. POTASSIUM

1.1 DISTRIBUTION IN THE BODY

In a healthy adult the total amount of potassium in the body is approximately 50 mmol/kg body weight or about 3500 mmol for a 70 kg man (Edelman & Liebman, 1959; Moore et al., 1963; Miller & Remenchik, 1963; Barter & Forbes, 1963).

Most of this potassium is intracellular, and most is in muscles since this tissue makes up the largest proportion of the body mass (Fig. 1.1). In plasma the concentration of potassium (K^+) is 3.5 to 5.2 mmol/l. The intracellular K^+ has been estimated in various tissues: in skeletal muscle it is about 140 mmol/l (Conway, 1957), 151 mmol/l in heart muscle of the cat and 139 mmol/l in that of the dog (Leonard & Hajdu, 1962), and 102 mmol/l in human erythrocytes (Mayer & Starkey, 1977). Though the values vary from one species to another and in different tissues, the intracellular K^+ usually exceeds the extracellular level by about 30:1. The maintenance of this high intracellular to extracellular K^+ concentration is in part dependent on the Na^+-K^+-ATPase pump (MacKnight, 1977 & Maffly, 1981) as well as hydrogen ion balance, plasma insulin, epinephrine, and aldosterone concentrations (Bia & Defonzo, 1981). Small changes in the intracellular:extracellular K^+ concentration ratio lead to severe disturbances in neuromuscular functions, particularly of the heart. Gain or loss from the whole body of an amount of K^+ equal to only one percent (35 mmol) of the total body K^+ content can cause a twenty five percent

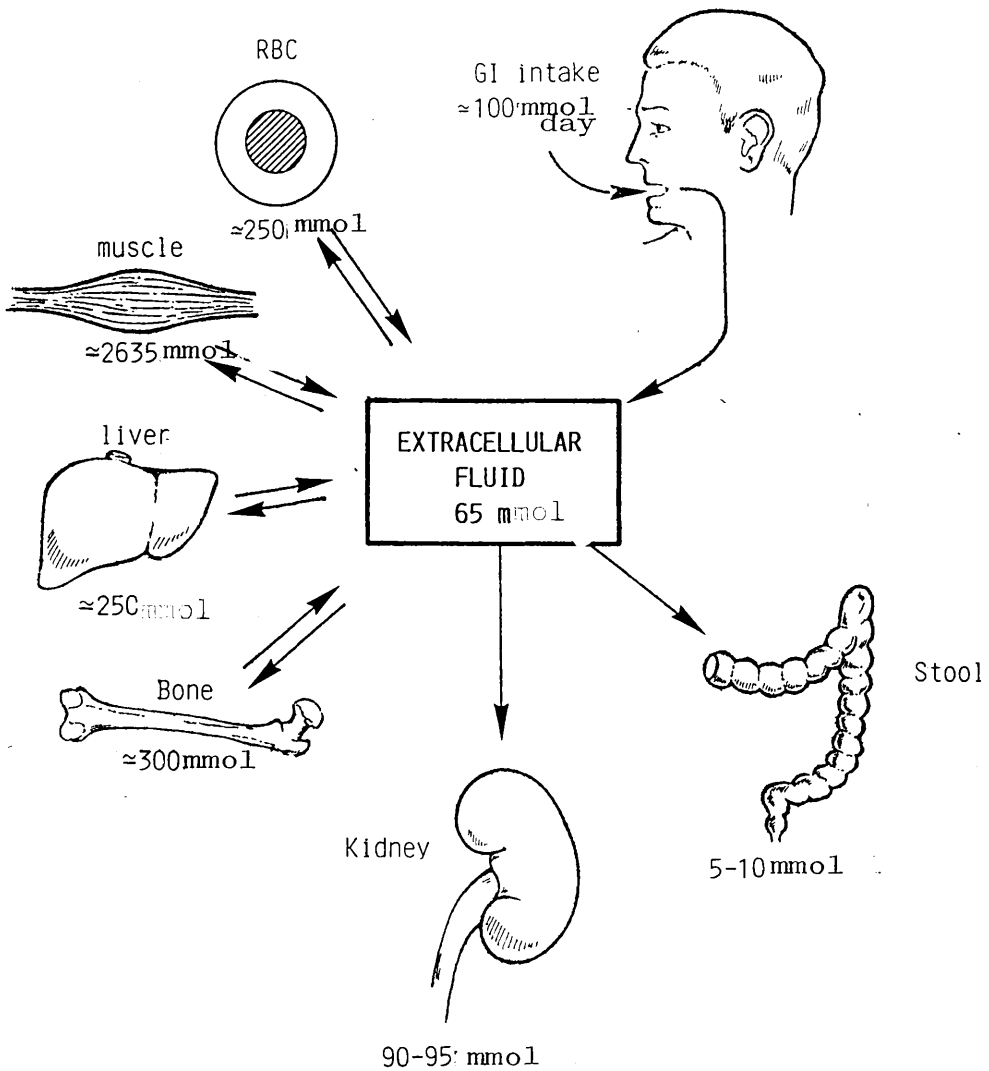


Fig.1.1

Internal and external potassium balance in man.
(from Smith. Bia & de Fronzo [1985] in fluid and electrolyte disorders).

increase or decrease in the plasma K^+ concentration and would be expected to have similar effects on neuromuscular excitability as would an equal net gain by, or loss from, the extracellular fluid.

There is an even greater inequality in the absolute amounts of K^+ in the fluid compartments. The plasma water is about 7% of the total body water content while the cells contain about 55% of the total body water. For example, taking a 70 kg man's total body water as $\simeq$ 42 litres, plasma volume $\simeq$ 4.5% body weight, i.e. = 3.15 litres. Therefore plasma water = 0.93 kg/l = 2.93 kg $\simeq$ 7% total body water. K^+ is 30 times more concentrated in the cells and therefore about 90% of the body's 3,500 mmol of K^+ is intracellular, while only about 2% is in the extracellular fluids with a fraction of this, only 0.4% of the total, in the plasma. It has therefore been argued in some quarters that to measure K^+ in a fluid which contains less than 1% of the body's content of the ion is likely to produce unreliable information about the concentration of K^+ in the body as a whole (Walker & Johnstone, 1971). Changes in distribution may occur quite rapidly in minutes or seconds in association with metabolic changes or abnormalities of acid/base balance. Various methods of measuring total body content or intracellular K^+ have been devised because of the uncertainty about the relationship between the plasma measurement and the state of K^+ in the body as a whole.

Corsa et al. (1950), used dilutions of radioactive ^{42}K, and determination of the naturally occurring radioisotope ^{40}K as methods of measuring total body K^+. On an outpatient basis, Walsh et al. (1974) measured total body K^+ by whole body counting of naturally occurring ^{40}K in diabetics. Graham et al. (1967) have described muscle biopsy as a means of measuring body water and intracellular electrolytes including K^+. Graham and his colleagues analysed small biopsy samples of skeletal muscle to obtain information about the amounts of water and electrolyte in cells, neglecting the amount of fat in such small biopsies. Flear et al. (1968) criticized the method of Graham and his colleagues because the method did not take "into account possible alterations in the cellular content of chloride in pathological states", and because fat-free samples of other tissues like smooth muscle of the gut, cardiac muscle, and brain needed to be analysed to show whether a low potassium content in biopsy samples of skeletal muscle necessarily proved depletion in tissues like the heart, since the total potassium content of the heart is only a small part of the total body potassium. Flear and Florence (1963) described a method using small samples of dried and defatted slices of fresh human skeletal muscle (taken at operation) and extracted in 0.1N nitric acid which gave the true chloride content.

Scribner and Burnell (1956) have studied the relationship between measured total body K^+ and the plasma levels, claiming that they are related arithmetically with a 1 mmol/l change in serum K^+ being equivalent to 100-200 mmol total body K^+ (Fig. 1.2). This relationship holds until the serum

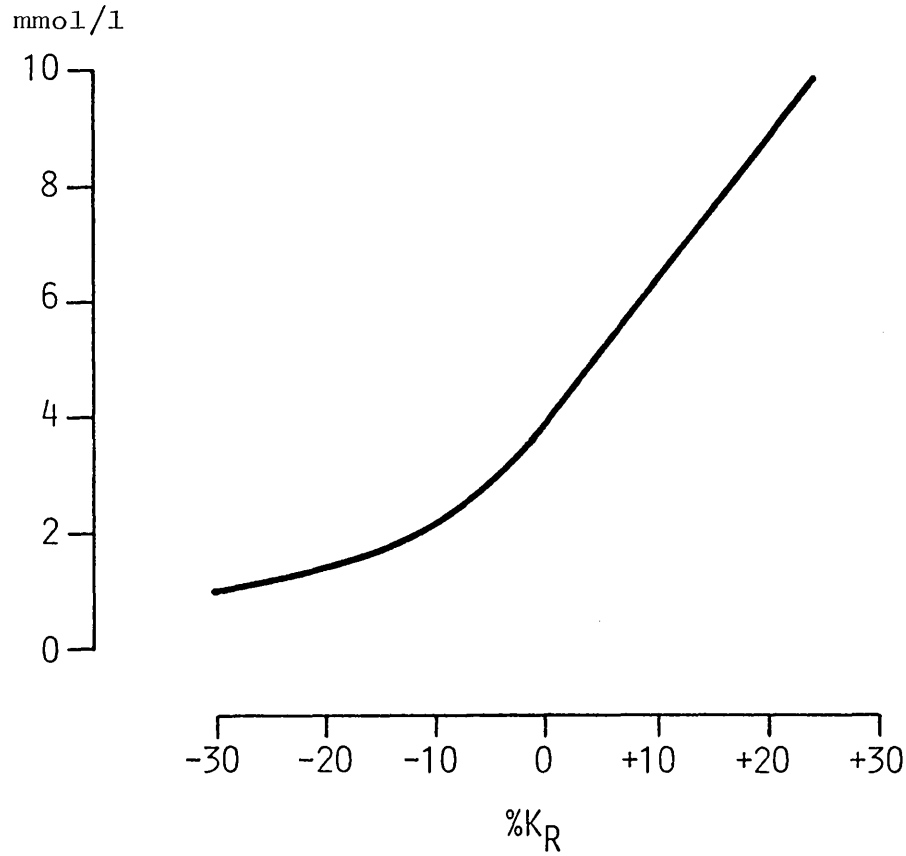


Fig. 1.2 An attempt by Scribner and Burnell (1956) to illustrate the relationship between serum potassium and total body potassium. They use the term K_R, the potassium content-capacity ratio, to express net gains or losses of potassium. $\%K_R$ therefore represents deviations from normal total body potassium. They suggest that a measurement of serum potassium can thus be used to determine the magnitude of K imbalance from this graph. This graph is based largely upon the calculations from unpublished data by Scribner & Burnell and from data by Schwartz & Relman (1953), to visualize an idealized curve to illustrate the above stated relationship.

K^+ is below 2 mmol/l when the level is maintained in spite of a falling total body K^+. Flear *et al.* (1957) in a study consisting of 110 simultaneous observations on total exchangeable ^{42}K and serum K^+ failed to show any correlation and pointed out that a good correlation only existed with acute experimental depletion. Flear's findings are not surprising, considering the earlier discussion about K^+ distribution. Chronic K^+ abnormalities, more commonly depletion, can therefore co-exist with a range of plasma levels.

In this thesis, the K^+ measurements described have been carried out in blood. This is because it is the distribution rather than the total body content of K^+ that is affected. The plasma K^+ serves as the best indicator of the rapid changes in haemorrhagic shock which are described in this thesis.

1.2 FORMS OF K^+ IN BODY FLUIDS

Theoretically there are four forms in which K^+ can exist in the plasma.

An ion selective electrode responds to the activity of the ionised form in the test solution. The K^+ electrodes described in this thesis can only measure K^+ existing in this form and it is therefore essential to review the physical and chemical states of K^+ in body fluids.

The four states in which K^+ can exist include:

i) ionised

ii) simple solution but non-ionised

iii) complexed with undissociated ion pairs

iv) protein bound.

The relative importance of these is considered below:

i) Ionised state

The extent to which an electrolyte is dissociated in solution

$$AB = A^+ + B^- \quad \text{..... (1)}$$

is defined in terms of the law of Mass Action:

$$\frac{c_{A^+} + c_{B^-}}{c_{AB}} = K^1 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where K^1 is the dissociation constant and c denotes concentration in moles per litre of solution. A strong electrolyte is considered to be fully ionised (e.g. KCl and NaCl). As a result of interaction between the charged particles with each other in solution, and the solvent molecules, the observed behaviour of the solution will fail to agree with the predicted behaviour if concentration units only are used for calculations. The relationship between the "activity" of the ion (designated a) and the concentration (c) is expressed by:

$$a = cf \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where f is a molar activity coefficient (Mattock, 1961). In an infinitely dilute solution the activity is considered to be equal to concentration. Thus when a = c, f, the activity coefficient, equals 1. As the solution becomes more concentrated, the activity coefficient which has no dimensions, being a ratio, becomes smaller than unity. In Figure 1.3 the activity coefficients for KCl, NaCl and $CaCl_2$ are plotted against the square root of the concentration (data from Robinson and Stokes, 1970). In the range 0 to 1 molar the activity coefficients for NaCl and KCl fall to around 0.7 that of $CaCl_2$ having fallen to 0.43 begins to rise again and becomes greater than unity at 2.5M but is then well outside the present range of interest. The ionic activities in mixed solutions can become very difficult to define (Robinson and Stokes, 1970). However, when

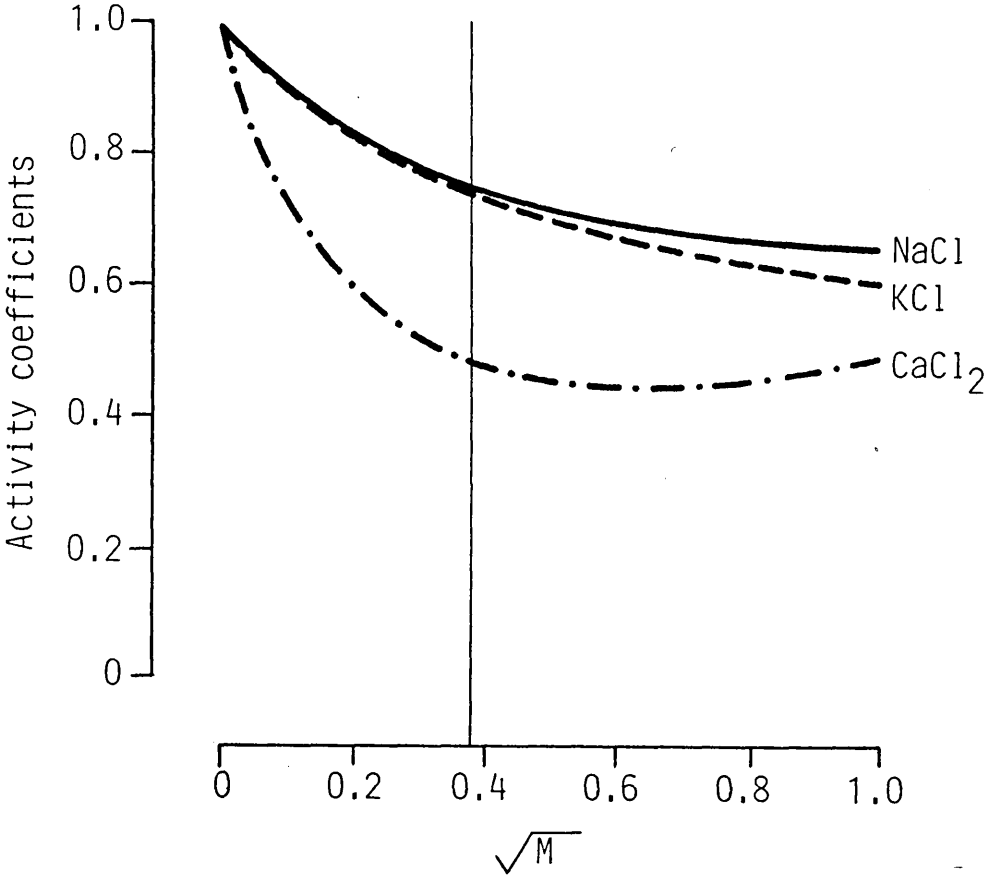


Fig. 1-3 The activity coefficients for sodium, potassium and calcium chloride plotted against the square root of the molarity. Data from Robinson and Stokes 1970. The line indicates the expected values in plasma where the molarity approximates to 0.15 M/1. $\sqrt{0.15} = 0.387$

(Robinson & Stokes, Fig. 8-12 pg. 219 refers).

considering a small concentration of KCl in a solution containing an excess of NaCl it is reasonable to assume an activity coefficient for the K^+ that would be appropriate for a pure solution of KCl of the same total ionic strength (Thomas and Moody, 1975).

The behaviour of ions in generating a membrane potential either in a biological system or across an artificial ion-selective membrane depends on activity rather than concentration. This immediately makes the ion selective electrode interesting to the biological scientist. In medical practice it is important to remember that this is one of a number of ways in which an electrode and flame photometer measure different quantities. Considering an aqueous solution of 4 mmol/l KCl in a background of 140 mmol/l NaCl, the K^+ electrode will respond to the activity:

$$a = cf$$

$$a = 0.74 \times 4$$

$$= 2.96$$

Since this is the chemical activity of K^+ which would be "sensed" by a cell membrane it is a biologically important measurement (Moore and Wilson, 1963; Rechnitz, 1975).

As the ionic composition of plasma is relatively constant it is possible to calibrate the electrode using standards made up to a similar ionic strength as plasma (e.g. with 140 mmol NaCl/l). Since the activity coefficient should be virtually identical in the standards and plasma it can be ignored and the results quoted as concentrations

(Covington & Robinson, 1975; Bates, 1973).

ii) Simple solution, non-ionised

Where dissociation is incomplete

$$AB \longrightarrow A^+B^-$$

the undissociated fraction AB remains in solution and is not measured by the ion-selective electrode. For KCl the dissociation is complete

$$KCl \longrightarrow K^+ + Cl^-$$

(Robinson & Stokes, 1970; Bates, 1973).

iii) Complexed and

iv) Protein bound

The distinction between these two states rests upon the size of the complexed form. Complexation with organic anions leads to a non-ionised but ultra-filtrable fraction. Protein binding leads to a non-diffusible fraction (Moore, 1969a).

Kilburn (1965) has found that during severe respiratory acidosis in man venous K^+ rises to above 7 mmol/l while Blesa et al. (1965) found a reduction of K^+ concentration in muscular venous blood with acute respiratory alkalosis. In view of the evidence of the lability of K^+ in muscular venous blood cited above and provided by other writers (e.g. Mohrman & Sparks, 1974; Kilburn, 1966; Hnik et al., 1974) the statement that K^+ is protein bound (Maas et al., 1985) requires some critical examination, since this is one of the ways in which the concentration and potentiometric measurements could differ in a variable and unpredictable way.

Band, Kratochvil, Wilson and Treasure (1978) found that the slope of the regression line for the ion-selective electrode

determined plasma K^+ against whole blood K^+ approaches unity and so it is assumed that K^+ is unbound, fully ionised and has an activity coefficient in plasma and whole blood very much like that in an aqueous solution of NaCl. Treasure (1977) concluded that K^+ was not buffered and any control of its activity must be by redistribution rather than by a change in its chemical nature.

It is essential to consider how ion-selective electrode measurements compare with the more traditional measurements by flame photometry. The discrepancies between the two measurements are always significantly large and particularly when the plasma contains increased quantities of lipids (Treasure 1977) because the mass concentration of water in plasma is decreased. ISE measurements of K^+ and Na^+ are reduced by an increase in HCO_3^- in the test concentration (Coleman & Young, 1981; Flear *et al.*, 1985). Coleman & Young suggested that these reduced ISE Na^+ and K^+ concentrations resulted because Na^+ and K^+ complexed with bicarbonate. Czaban *et al.* (1982) suggested that an increase in the residual liquid junction potential also reduced the ISE measured Na^+ and K^+ concentrations. Flear *et al.* (1985) suggested that reduced ISE Na^+ and K^+ concentrations resulted from ion-pair formation between Na^+ and K^+ and the "third form" of carbonate ($H_3C_2O_6^-$).

It is clear therefore that (a) changes in ionic strength markedly affect ISE-determined concentrations of Na^+ and K^+ (Flear et al., 1986a). (b) Increasing PCO_2 at constant total substance concentrations of Na^+ and K^+ *increases* concentrations determined by ISE, at any given HCO_3^- concentration (Flear *et al.*, 1985).

(c) Change in total plasma protein concentration also affects ISE-determined Na^+ and K^+ concentrations (Flear et al., 1986b; Maas et al., 1985).

Thus while flame photometer measurement of K^+ may be artefactually low due to the fact that each ml of plasma contains less than 1 ml of water, ISEs although giving the biologically more important information of the activity of K^+ in plasma water can be subject to errors because of the presence of HCO_3^-, PCO_2, plasma protein and pH. These interferences have to be borne in mind because PCO_2, pH, HCO_3^- and plasma protein concentrations may change during blood loss and shock. It is also clear that the accuracy of measurement of K^+ activity determined by ISE is controversial in the literature. While this may throw some doubts on some of the interpretations of the findings in this thesis, the findings are nonetheless valid and full interpretation will have to await the outcome of further investigations about the functioning of ISE which this investigation was not designed to study. Indeed Covington, Flear and Lockie (1983) have suggested that a proper understanding of the interactions of blood electrolytes will be held up until the development of a satisfactory bicarbonate ion-responsive electrode.

1.3 MEASUREMENT WITH ION SELECTIVE ELECTRODES, THE PRINCIPLES

THE MEMBRANE POTENTIAL

In using ion-selective electrodes, the essential measurement is that of a potential difference established across a membrane separating two dissimilar solutions. The measurement is made under conditions where the current drawn across the membrane is negligible, by using a high impedance voltmeter or electrometer. The voltage generated by the different solutions acting across the membrane is due to a potential generated at each of the two solution interfaces: the salt bridge and the test, apart from the potentials generated at the solid-liquid interfaces of the reference electrodes.

Various mathematical analyses of the problem are possible. However, the simplest approach which satisfactorily describes the main operative characteristics of membranes when used as sensing electrodes is to consider a semi-permeable membrane, permeable predominantly to the ion in question. Figure 1.4 shows diagrammatically a K^+ selective membrane with KCl solutions on either side. The membrane is permeable to K^+ which can enter the membrane and tend to diffuse through it

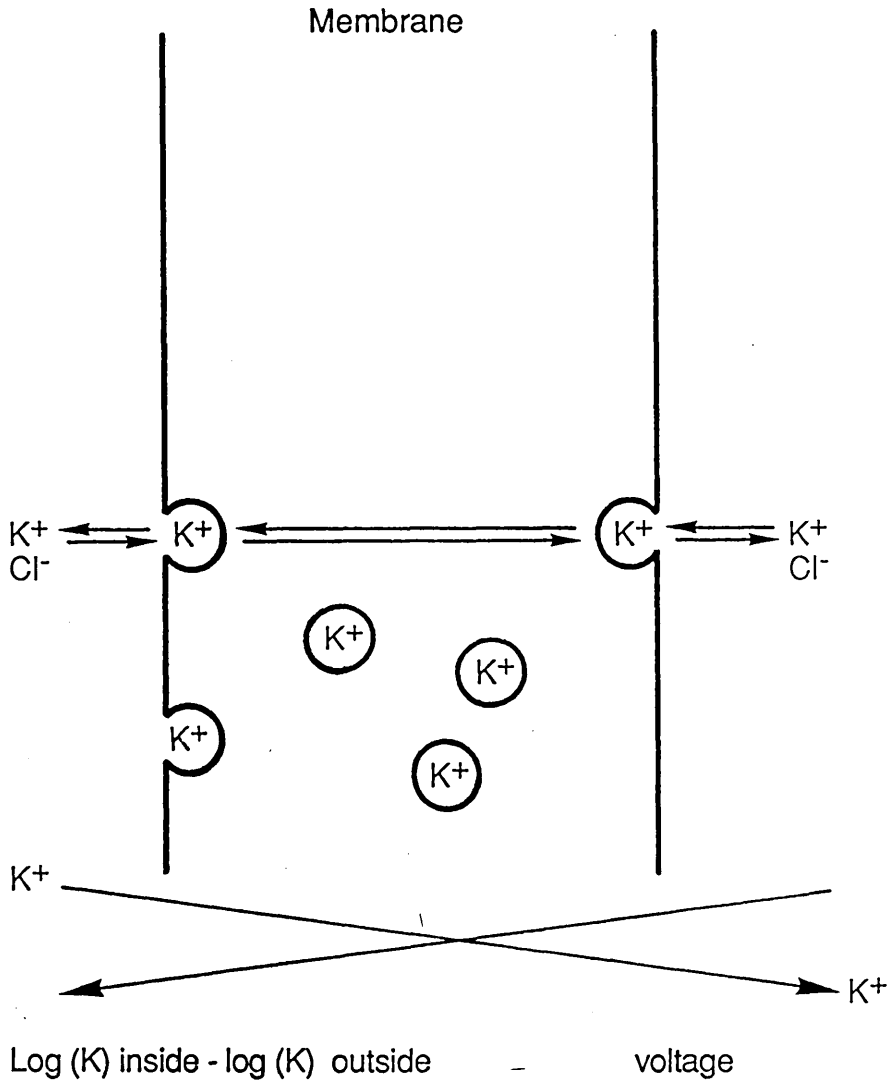


Fig. 1-4
A schematic representation of the PVC membrane considered as a semi-permeable membrane predominantly to potassium. If it separates solutions of unequal concentrations the tendency for the potassium ions to diffuse down their concentration gradient is resisted because the chloride ions cannot enter the membrane. A potential is established and at equilibrium is proportional to the ratio of the potassium activities on the two sides, and hence to the logarithm of potassium in the sample if the internal reference solution remains unchanged. (See text).

down their concentration gradient. However, chloride ions are unable to enter the membrane and since electro-neutrality is maintained in both solutions and current cannot pass in the external circuit, net movement of K^+ is prevented. A potential difference is established across the membrane which is proportional to the ratio of activities in the two solutions. This is expressed by the Nernst equation

$$E = \frac{RT}{zF} \log n \frac{fc_1}{fc_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where E is the potential across the membrane

R = gas constant 8.31439 Joules/^oC_c/mole

T = absolute temperature (absolute zero = -273.16^oC)

F = Faraday's number 96493.1 coulomb/g equivalent

z = valency

f_c = activity of potassium (see Eqn.(3) on p.11) inside (c_1) and outside (c_2) cell

log n = natural logarithm.

The natural logarithm can be converted to a common logarithm by multiplying by the modulus 2.30259. For potassium the valency, z = 1, so the expression becomes

$$E = 2.30259 \frac{RT}{F} \log \frac{fc_1}{fc_2} \quad \ldots\ldots\ldots\ldots (5)$$

at 37^oC the value of $2.30259 \frac{RT}{F}$ is 61.4 mV.

The potential difference would therefore be 61.4 mV for each decade difference in the ratio of the activities on the two sides.

In practice the activity on one side of the membrane is maintained constant at some arbitrary value by a filling solution in contact with an internal reference electrode and only the solution on the other side of the membrane is changed when standardizing or measuring. The e.m.f. is measured between the internal reference electrode and an external reference electrode which makes contact with the standard or test solution via a suitable salt bridge (usually 140 mmol/l NaCl solution in this study). The e.m.f. generated by this complete cell therefore includes the potentials contributed by the internal and external reference electrodes, but ideally only the voltage generated across the membrane should change when the test or standard solutions are changed. The behaviour of this cell can be described by

$$E = E_0 + 2.30259 \frac{RT}{F} \log fc_1 \quad \ldots\ldots\ldots\ldots \quad (6)$$

where E_0 is the e.m.f. of the complete cell filled with a standard solution of activity 1. The cell voltage will therefore start at some value determined by the choice of reference electrodes and will then change by 61.4 mV (at 37°C) for each decade change in activity of the test or standard solutions. It is necessary to standardize the cell not only to check that the slope factor approaches theoretical value of $2.30259 \frac{RT}{F}$, but to back off the standing voltage of the cell E_0 so that a suitable scale can be used.

The response of the cell is strictly in terms of activity. However, as has been noted when the activity coefficient is the same for both standard and test solutions the results can be read as simple concentrations (by cancellation of the activity coefficient "f").

1.4 SELECTIVITY OF THE MEMBRANE

The description of the potential established across a membrane so far has dealt with pure KCl solutions. Membranes should not be considered as ion-specific but ion-selective. The usefulness of a membrane depends on its selectivity for the species of interest over interfering ions at the concentrations found in the solution under investigation. For example, to the biologist a K^+ sensor for use in plasma must be highly selective for K^+ over Na^+ but selectivity over rubidium and caesium would not contribute to its usefulness. A modification of the Nernst equation including terms for activity of the primary and interfering ion is called the Nikolski equation:

$$E = E_O + \frac{RF}{F} \ln(a_{K^+} + k_{KNa}.a_{Na^+}) \quad \ldots\ldots\ldots\ldots \quad (7)$$

(Ryba et al., 1973) where in addition to the expressions already met

a_{K^+}, a_{Na^+} are activities of potassium and sodium,

k_{KNa} is the selectivity coefficient for the electrode.

It is a ratio and therefore has no dimension. It has a value of less than 1 when the electrode is selective for potassium (the primary ion) over sodium (the interfering ion). Thus when used as in the Nikolski equation given

above ($^{k}_{KNa} \cdot a_{Na}$), it predicts the extent to which a given sodium activity will contribute to the potential across the membrane.

There are a number of ways of obtaining and quoting this important piece of information about electrode function (Moody & Thomas, 1971) but it has been recommended by the International Union of Pure and Applied Chemists (IUPAC, 1975) that a symbol of the form K_{ij} should be used to represent the selectivity coefficient where "i" represents the primary ion and "j" the inferfering ion.

1.5 REFERENCE ELECTRODES

The potential established across the membrane is measured with suitable reference electrodes which complete the electrochemical cell. The reference electrodes used in the present experiments were silver/silver chloride electrodes which are anion reversible and are dealt with in detail by several writers (Mattock, 1961; Covington, 1969, 1973).

The reaction is

$$AgCl + e^- \rightarrow Ag + Cl^-$$

The internal reference electrode is exposed to an unchanging concentration of chloride ions and its potential is determined by the expression

$$E = E_0 - 2.30259 \frac{RT}{F} \log a_{Cl^-} \quad \ldots\ldots\ldots\ldots \quad (8)$$

where E_0 is the standard potential of the reference electrode compared with the hydrogen electrode at a given temperature and chloride ion concentration. If ion activity increases the electrode potential becomes less positive, hence the

minus sign in the expression above. This is in keeping with I.U.P.A.C. convention (IUPAC, 1970).

The external reference electrode is in contact with the solution via a liquid junction and the complete cell is described by:

Ag; AgCl/KCl/MEMBRANE/SAMPLE//NaCl/AgCl; Ag

where the sign conventions and symbols adopted for description of electrochemical cells are as follows: electrode-solution interfaces are signified by a single stroke. Liquid junctions are indicated by a double stroke. A semicolon is used to distinguish other phase boundaries. The ion-selective half cell is written first and is therefore on the left. The external reference electrode is written on the right. This convention applies irrespective of whether the cell e.m.f. is positive or negative since the cells used are designed to function near zero e.m.f. and sign depends on the sample at that moment.

1.6 THE HISTORY OF THE POTASSIUM ION SELECTIVE ELECTRODE

1.6.1 GLASS ELECTRODES

The best known and most widely used cation sensors are pH glass electrodes. Eisenman, Rudin, and Casby (1957) experimented with a range of glass compositions and found that the addition of aluminium oxide to sodium silicate glasses increases the sensitivity of hydrogen glass to other cations in the order $Na^+ K^+ Li^+$. A glass of the ternary system Na_2O-Al_2O_3-SiO_2 (NAS_{11-18}) had sodium selective characteristics. It produced a 58 mV change in potential

(near theoretical Nernst response of 61.4 mV) at 37°C, for a decade concentration change when tested in single cation solutions, and in the biological pH range had a selectivity for Na^+ over K^+ of over 100:1 ($^kNaK^{0.01}$). Their attempts to produce a K^+ selective glass were less successful and the best selectivity they achieved was 6:1 ($^kKNa^{0.16}$). Portnoy et al. (1962a) described a glass KAS_{20-5} with a K^+ selectivity of 10:1 over sodium ($^kKNa^{0.1}$). By using sodium glass electrodes at the same time they attempted to correct the sodium error which was inevitably considerable when measurements were being made in serum. Even with these corrections the errors in K^+ measurement could be as large as 0.25 mmol/l. Dahms (1967) incorporated a K^+ glass electrode in an automated electrolyte analyser. He used a glass NAS_{27-4} obtained from Corning Glass Works with a quoted selectivity coefficient (^kKNa) of 0.1 presumably at neutral pH. He claimed good reproducibility of results and precision within 0.1 mmol/l for K^+.

The use of pH glass to make micro-electrodes for intracellular use was first described by Caldwell (1954, 1958). Hinke (1959) used Eisenman's solution (NAS_{11-18}) and K^+ selective glasses (NAS_{27-8}) to study intracellular activities of these cations in the large muscle fibres of crabs and lobsters. The poor selectivity ($^kKNa = 0.125$) necessitated calculation of the K^+ activity from the Nikolski equation ...(7).

Armstrong and Lee (1971) used NAS_{27-5} potassium glass to study intracellular activities in frog skeletal muscle using Hinke's technique, and Khuri (1974) has applied a similar technique in his studies on renal tubular fluid. However, the poor selectivity of potassium glasses has limited their usefulness.

1.6.2 LIQUID ION EXCHANGERS

Corning introduced a liquid ion-exchanger used as a liquid membrane for analysis of serum (Wise et al., 1970). The liquid membrane was covered by a cellophane dialysis membrane to avoid poisoning of the exchanger that otherwise occurred. The Corning liquid ion exchangers were based on potassium tetraphenylborate and closely related compounds. Potassium tetraphenylborate is an insoluble compound and its precipitation is a standard method of K^+ determination in quantitative inorganic analysis (Vogel, 1961). Although insoluble in water it is lipophilic. Corning Exchanger 477317 contains potassium tetra-p-chlorophenylborate (Davies et al., 1973) and has a selectivity for K^+ over Na^+ of 80:1 ($k_{KNa}^{1.2} \times 10^{-2}$). This was a great improvement on the glass electrodes and was particularly useful where K^+ was in excess over Na^+ such as in intracellular measurement. Walker (1971) used liquid membrane microelectrodes and his method has been used since to follow extracellular fluxes in neurophysiological studies (Kris et al., 1974, 1975; Lothman and Somjen, 1975; Lothman et al., 1975), venous K^+ in exercising muscle (Hnik et al., 1975) and in intracellular studies (Walker and Ladle, 1973; Khuri et al., 1972).

However, it was the recognition of the cation complexing behaviour of the macrocyclic antibiotics that led to the first highly selective membranes. The first to be used was nonactin (Stefano and Simon, 1967) which gave a selectivity of 100:1 over Na^+ ($^{k}_{KNa}0.01$) in a liquid membrane (Pioda and Simon, 1969). Reports soon followed of the use of the more selective valinomycin to measure K^+ in aqueous solutions (Pioda *et al.*, 1969) and serum (Pioda *et al.*, 1970). With selectivity of 5000:1 ($^{k}_{KNa}2 \times 10^{-4}$) this was a very great improvement on all K^+ sensors which had been available until that time. They reported the results of measurements on 15 serum samples compared with flame photometry and the results agreed within 0.05 mmol/l.

Valinomycin in aromatic solvents such as nitrobenzene or diphenylether were used by Frank and Ross (1970) and formed the basis of the Orion potassium liquid membrane electrodes ($^{k}_{KNa}1 \times 10^{-4}$). The principal ligand used in the work described in this thesis was valinomycin, and a brief account of its history is therefore given.

Valinomycin was first discovered by Brookman and Schmidt-Kastner (1965) who extracted it from cultures of streptomyces fulvissimus and obtained a pure crystalline sample. Its effect on the membrane permeability of mitochondria (Moore & Pressman, 1964) and red cell ghosts (Pressman & Heeb, 1974) was studied. When crystals of valinomycin were added into the physiological solutions bathing mitochondria or red cell ghosts the membranes of these structures were rendered more permeable. An early

application was in the study of ion permeability of bilayer membranes by Mueller and Rudin (1967). Using lecithin they made membranes about 70 Å thick which is approximately equal to twice the length of a lecithin molecule. Lluger (1972) used membranes of this type to study nonactin and valinomycin. He found that the bilayer membrane has a high electrical resistance of about 108 ohms cm^{-2}. The addition of valinomycin reduced the membrane resistance. He noted in addition that if a bilayer membrane containing valinomycin was used to separate dissimilar solutions of KCl a potential was established across the membrane reaching nearly 58 mV for an activity ratio of 10:1 between the two sides. The high selectivity over Na^+ by an order of 10^3 was noted in these membranes and the conductivity was proportional to the amount of valinomycin combined in a 1:1 ratio.

It seems that valinomycin acts as a mobile carrier within these membranes for geometrical reasons. The dimensions of the valinomycin molecule are 12 x 16 Å which make pore formation unlikely in its 70 Å membranes. Similar studies were performed in membranes of up to a millimetre in thickness (Leu et al., 1973) which behaved in a similar fashion. Eyal and Rechnitz (1971) reached a similar conclusion about the unlikelihood of pore formation in their experiments and were able to stop conduction by freezing the membrane.

Shemyakin et al. (1963) described the structure of valinomycin and a number of studies have been made of its various configurations (Pinkerton et al., 1969; Rothschild, 1973). Shemyakin's group went on to study valinomycin and

other cyclodepsipeptides (Shemyakin et al., 1969). They pointed out that the cation complexing ability of these compounds correlates with their antimicrobial activity. They considered that the valinomycin/potassium complex had the configuration shown in Figure 1.5 and that there was a one-to-one relationship between valinomycin and K^+. The work of Ohnishi and Urry (1970) establishing that there is a 4.5 Å cavity within the valinomycin ring supported this idea.

Although it had been demonstrated by Mueller and Rudin (1967) that valinomycin in a membrane would complex selectively with K^+ rather than Na^+, the first report of its incorporation into an ion-selective membrane for analytical purposes was that by Pioda, Stankova and Simon (1969). In 1970, Simon's group (Pioda et al., 1970) and Orion workers (Frant and Ross, 1970) separately reported the successful application of valinomycin in liquid ion exchangers to the measurement of K^+ in serum.

1.6.3 POLYMER MEMBRANES

In the same year (1970), Moody, Oke and Thomas reported the successful incorporation of a calcium liquid ion exchanger in a polyvinyl-chloride (PVC) matrix. This overcame the problems of supporting the liquid membrane, its indefinite interface and its poor mechanical characteristics. Moody and Thomas' group later incorporated the potassium tetra-p-chlorophenylborate liquid ion exchanger (Corning 477317) in PVC membranes (Davies et al., 1973) and investigated the PVC composition for these membranes (Griffiths et al., 1972).

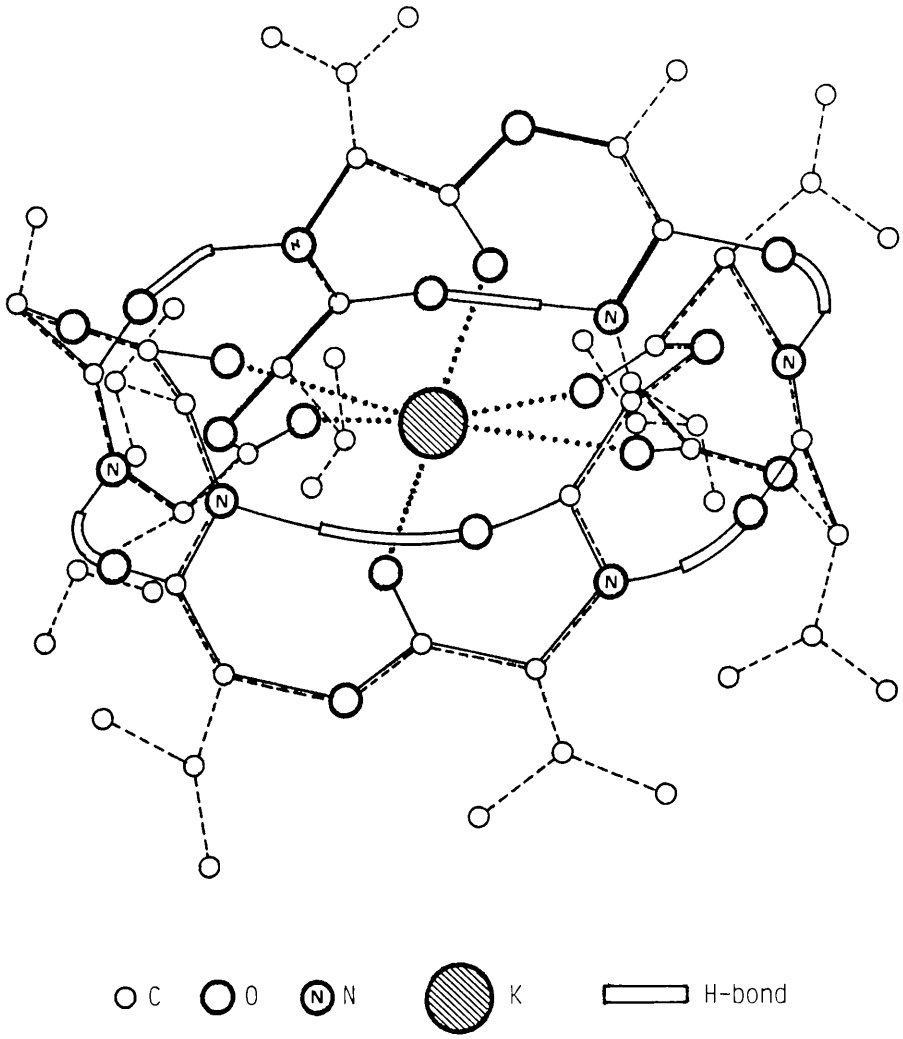


Figure 1.5 conformation of the K^+ complex of valinomycin. From Shemyakin et al. 1969.

1.7 SELECTION OF A MEMBRANE COMPOSITION

While the polymer matrix is generally considered to be inert there is evidence that some materials are more satisfactory than others. Mascini and Pallozzi (1974) reported that PVC was a particularly satisfactory matrix but that selectivity was also influenced by the plasticiser and that Nernstian responses were poor or absent without inclusion of a suitable plasticiser. For valinomycin they found dibutylphthalate, dipentylphthalate and bis-2-ethylhexyladipate (di-octyl adipate, DOA) most satisfactory. Following the work of Band and Kratochvil (1974) this combination of valinomycin dissolved in nitrobenzene and potassium tetraphenyborate in DOA was used as the mixed ion exchanger system. This mixture was included in the PVC polymer matrix.

1.8 ELECTRODE CONFIGURATION

Various modifications have been made in the configuration of K^+ selective electrodes. Attempts have been made to dispense with the internal reference solution and make solid state electrodes by casting these membranes directly onto platinum wires. Early reports of success were made with this technique (Cattrall and Freiser, 1971) but it became clear that the poorly defined interface between the polymer and the metal led to troublesome drift. This interface potential was neither fixed nor did it respond in a predictable way to the test solution (James, Carmack & Freiser 1972). Electrodes made in this way were only usable if repeated recalibration was accepted (Hopirtean et al., 1974; Cattrall

et al., 1974) and the problem of drift has been remarked upon by subsequent writers (Ryba & Petransk, 1975; Fleat, Bound & Sanbach, 1976). In an attempt to overcome this problem, Smith, Genshaw and Greyson (1973) coated a silver chloride wire with polyvinyl alcohol (PVA) containing KCl. The PVC membrane was then cast on the PVA gel producing an ion-selective half cell of more conventional form

Ag; AgCl/KCl in PVA/MEMBRANE/Sample

However, the concentration of the KCl/PVA gel was subject to change due to water passing through the membrane from the sample and again the potential of the internal reference system was subject to drift.

Höper, Kessler and Simon (1976) claimed success with a membrane cast directly on to silver and PVC (carbon solid state electrode had also been tried (Ansaldi and Epstein, 1973)). Moss, Janata and Johnson (1975) did cast a membrane directly on to the metal oxide gate of a field effect transistor. Though this did not in any way overcome the interface problems outlined above, it did however avoid the interference caused by carrying a high impedance signal to the amplifier. The signal is delivered straight to the gate of a field effect transistor which is the input stage of a high impedance amplifier.

Several different configurations of membrane were attempted by Treasure & Band (1977). PVC membranes were used throughout this present work and the new method of casting and supporting the membranes as developed by Treasure & Band (1977) was adopted in this laboratory.

1.9 CIRCULATORY SHOCK

1.9.1 EARLY DESCRIPTIONS OF SHOCK

Terms such as consussion, commotion, collapse, prostration, syncope, and torpor, were frequently used by early writers to label the effects of trauma. Le Dran (1743) is credited with using the word choc for the first time, but he used it to designate the act of collision rather than the resulting functional damages. Guthrie came near to the modern use in 1815 when he suggested postponement of operations "until the alarm and shock have subsided". In discussing gunshot wounds of the Crimean War, Cooper stated in 1838 that many wounded soldiers had died without significant loss of blood, severe pain, or serious injury. He wrote: "Surgeons were in the habit of saying men died of shock without asking themselves very strictly what they meant by the term. It is however, a convenient name, although not perhaps a philosophical one". Indeed the term "shock" was accepted untranslated into the German and French languages (see Heineman, 1938). Morris (1867) was the first to use the word in the title of a monograph.

1.9.2 CONCEPTS OF SHOCK

In view of the inadequate state of physiological knowledge at the early descriptive period, it is not surprising that the sudden collapse of vital processes attributable to no apparent pathological state remained a mystery (Morris, 1867). The profound apathy, reduced sensibilities, extreme motor weakness, reduced cerebration, and impaired reflexes suggested a primary defect of the nervous system, whereas the feeble

heart action, weak and rapid pulse, soft arteries and pallor pointed towards cardiac failure.

In 1864 Goltz demonstrated an interesting experiment on a frog. It consisted of tapping the abdomen sharply with a blunt instrument, as a result of which the muscles immediately became flaccid, the reflexes disappeared, and the heart stopped or slowed markedly. When the heart resumed its beat it was relatively empty, the reason being that the blood had collected in the abdominal viscera and so did not return to the heart. Goltz attributed this pooling to a reflex dilatation of abdominal vessels. This was later supported by observations attributed to Salathé (1877) that acute circulatory failure, syncope, and even death can be produced in rabbits by tilting them suddenly from a horizontal to a vertical position. This is essentially the gravity shock that occurs in certain human subjects as a result of prolonged standing or suspension in a vertical position.

The most comprehensive analysis of the nineteenth century was made by Groeningen in 1885. This author attempted to apply existing physiological knowledge to the interpretation of shock. After a careful consideration of evidence he concluded (1) that vagal depression of the heart could not explain the circulatory signs, and
(2) that depression of blood pressure did not account for the reduction of motility and sensibility or the impairment of reflexes. His analysis led to the conclusion that shock was due to a fatigue or exhaustion of the spinal cord and

medulla which resulted from intensive stimulation of sensory nerves or from direct concussion of the central nervous structures. The circulatory failure due to vasodilatation was regarded as only a part of the general picture produced by central nervous exhaustion.

Today, the term shock is applied clinically to a fairly characteristic and yet somewhat varied syndrome following anaesthesia, surgical operations, wounds, and various other injuries including burns. The clinical signs and symptoms indicate that while the functions of many organs are deranged, impairment and progressive failure of the circulation is of paramount importance.

The following major types of shock have been defined over the decades from experimental and clinical studies, each of which is briefly reviewed below:
Hypovolaemic shock, Neurogenic shock, Anaphylactic shock, and Septic shock.

1.9.3 HYPOVOLAEMIC SHOCK: The haemorrhagic form of hypovolaemic shock is the model employed in the present study and is therefore fully discussed later.

Loss of plasma from the circulatory system even without the loss of whole blood can be severe enough to reduce the total blood volume to mimic hypovolaemic shock caused by haemorrhage. In civilian closed injuries without external loss of blood from the body, blood loss from the circulatory system has been estimated by limb swelling measurements (Clarke et al., 1955). Severe plasma loss occurs in such conditions as intestinal obstruction, severe burns, and loss of fluid from all fluid compartments of the body, a condition called dehydration. A characteristic sign that differentiates the shock caused by plasma loss and that by haemorrhage is the one additional complicating factor - the blood viscosity increases greatly as a result of plasma loss, and this further

exacerbates the sluggishness of blood flow.

Trauma to the body is one of the most common causes of circulatory shock, and the shock may result simply from haemorrhage caused by the trauma. But it can also occur even without haemorrhage, for contusion of the body can often damage the capillaries sufficiently to allow excessive loss of plasma into the tissues and so reduce plasma volume with resultant hypovolaemic shock. Thus the blood volume can be markedly reduced whether or not haemorrhage occurs when a person is severely traumatized.

1.9.4 NEUROGENIC SHOCK: Neurogenic shock occasionally results without any loss of blood volume whatsoever. The vascular capacity instead, increases so much that even the normal amount of blood becomes incapable of adequately filling the circulatory system. A major cause of this is the loss of vasomotor tone throughout the body. Venous pooling of blood occurs and reduces the mean systemic filling pressure with a consequent reduction in the venous return to the heart. Some of the factors that can produce loss of vasomotor tone resulting in neurogenic shock include, deep general anaesthesia, spinal anaesthesia, brain damage, depression of the vasomotor centre which frequently occurs during fever, excessive loss of sleep or metabolic disturbances, all of these often lead to "fainting". Fainting results from excessive blood pooling in greatly dilated peripheral blood vessels thereby causing a large fall in cardiac output.

Vaso-vagal syncope - Emotional Faintin :

This is a circulatory collapse that is caused not by

vasomotor failure but instead by strong emotional excitation of the parasympathetic nerves to the heart and of the vasodilator nerves to the skeletal muscles, thereby slowing the heart and reducing the arterial pressure.

1.9.5 ANAPHYLACTIC SHOCK: Anaphylaxis is an allergic condition which results primarily from an antigen-antibody reaction that takes place all through the body immediately after an antigen to which the subject is allergic has entered the circulatory system. If such a reaction takes place in direct contact with the vascular walls or cardiac musculature, damage to these tissues result directly, or the cells damaged anywhere in the body by the antigen-antibody reaction can release several highly toxic substances, such as histamine or histamine-like substances mainly from the circulating basophils and the mast cells, into the blood. The histamine in turn causes venous dilatation and thus an increase in vascular capacity, arteriolar dilatation resulting in greatly reduced arterial pressure, and greatly increased capillary permeability with rapid loss of fluid into the tissue spaces. The sum total of these is a great reduction in venous return and often serious circulatory shock ensues (Hershey, 1977).

1.9.6 SEPTIC SHOCK: This is the condition formerly referred to as "blood poisoning", which means widely disseminated infection in many areas of the body with the infection being borne through the blood causing extensive damage to the tissues. Because of the many different types of bacterial infection that can cause septic shock there are many different varieties of it. In the early stages of septic shock, the subject or patient does not have signs of circulatory collapse. High

fever and marked vasodilatation throughout the body, especially in the infected tissues, and perhaps high cardiac output in some patients caused by high metabolic rate from high body temperature, might be some of the special features of septic shock. The end stages of septic shock are not greatly different from the end stages of haemorrhagic shock, even though the initiating factors are different in the two conditions.

1.10 HAEMORRHAGIC SHOCK

When blood flow throughout the body is inadequate to the extent that there is too little delivery of nutrients especially of oxygen to the tissue cells, circulatory shock is said to be present. This condition becomes progressively worse as the cardiovascular system itself begins to deteriorate. As all degrees of shock, from the mildest reduction of cardiac output to almost complete cessation of output can result from haemorrhage, it is the model chosen to study the transient changes in plasma potassium (continuously monitored) with ion-selective electrodes as the shock progresses. Therefore haemorrhagic shock is considered in some detail below.

For simplicity, haemorrhagic shock will be divided into three major stages (Guyton *et al.*, 1973):
(i) a non-progressive stage (sometimes called the compensated stage) in which tissue perfusion is deficient but not deficient enough to cause a vicious cycle of cardiovascular deterioration,
(ii) a progressive stage in which the shock has progressed to a point that the circulatory system begins to deteriorate,

thus leading to a vicious cycle that leads to death eventually unless treatment is instituted, and

(iii) the irreversible stage in which the shock has progressed to the point that all forms of therapy will not be adequate to save the life of the patient even though the patient is at the moment still alive (see Figure 1.6).

1.10.1 CARDIAC OUTPUT AND ARTERIAL PRESSURE IN SHOCK

Haemorrhage decreases the mean systemic filling pressure and as a consequence decreases venous return. Cardiac output falls below normal as a result and this is the cause of inadequate tissue perfusion in circulatory shock. This contrasts with some forms of septic shock in which the cardiac output itself may be normal or even greater than normal but may still be inadequate to supply the tissue needs. This condition may result from too high a rate of metabolism in the tissues, or from abnormal flow patterns in the peripheral vasculature that prevents adequate diffusion of nutrients and other substances between the circulatory system and the tissue cells. There are times when a person is in severe shock and still has a normal arterial pressure because of nervous reflexes which keep the pressure from falling. At other times the arterial pressure can fall to as low as one-half normal but the person can still have normal tissue perfusion and not be in shock.

In haemorrhagic shock the arterial pressure does usually decrease at the same time as the cardiac output decreases.

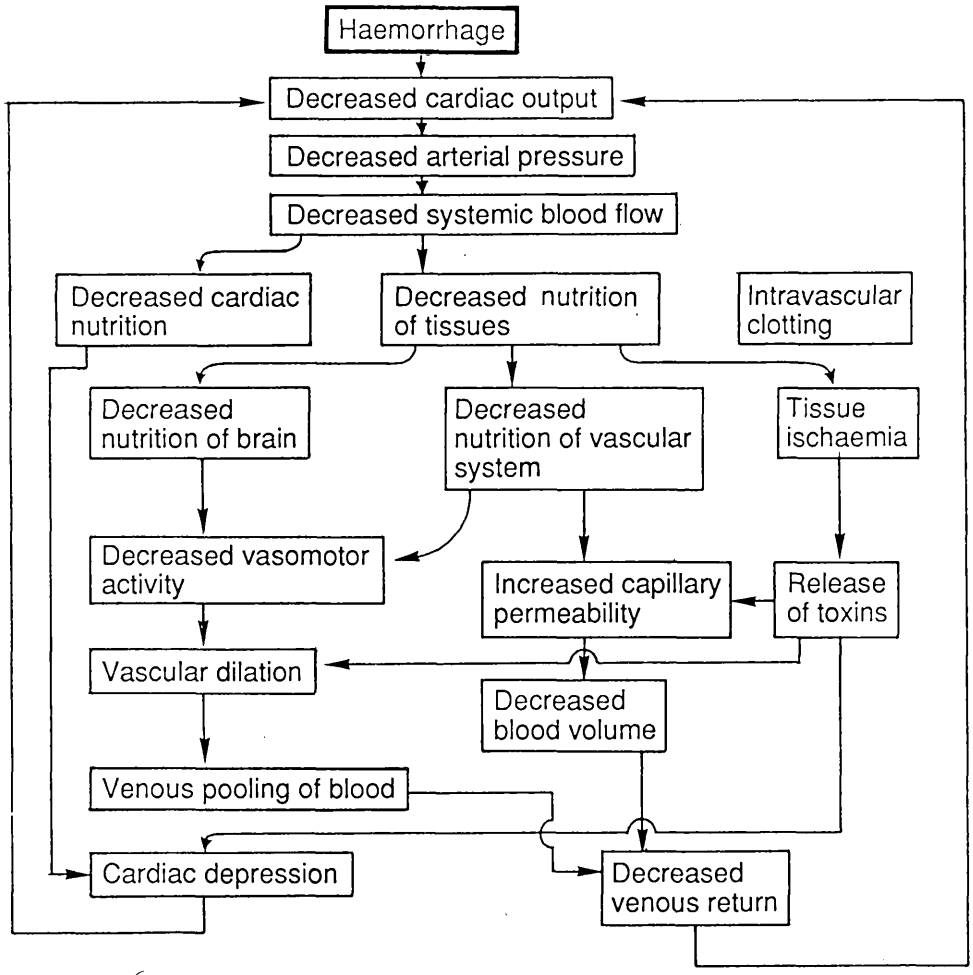


Fig. 1- 6
Types of positive feedback that can lead to progression of haemorragic shock (from A.C. Guyton in circulatory shock 1981).

Although the measurement of arterial pressure may not be the best index of cardiovascular well-being, it is usually of major value in assessing the degree of shock (Guyton & Crowell, 1961).

1.10.2 BLEEDING VOLUME RELATIONSHIP TO CARDIAC OUTPUT AND ARTERIAL PRESSURE

Guyton and Crowell (1961) illustrated the effects of removing blood from the circulatory system over a period of about half an hour on both cardiac output and arterial pressure. They showed that approximately 10 percent of the total blood volume can be removed with no significant effect on arterial pressure or cardiac output, but greater loss of blood usually diminishes the cardiac output first and later the pressure, both of these falling to zero when about 35 to 40 percent of the total blood volume has been removed.

1.10.3 REFLEX COMPENSATION IN SHOCK, ROLE OF THE SYMPATHETIC NERVES

The decrease in arterial pressure caused by blood loss initiates powerful reflexes (initiated mainly by the baro-receptors) that stimulate the sympathetic vasoconstrictor system throughout the body, resulting in three important effects (Chien, .. 1967): (i) The arterioles constrict in most parts of the body, thereby greatly increasing the total peripheral resistance,

(ii) the veins and venous reservoirs constrict, thereby helping to maintain adequate venous return despite diminished blood volume, and

(iii) the heart activity increases markedly, sometimes increasing the heart rate from the normal value of 72 beats per minute to as much as 200 beats per minute.

In the absence of the baroreceptor reflexes, if 15 to 20 percent of the blood volume is removed over a period of half an hour death will occur. This is in contrast to 30 to 40 percent when the reflexes are intact. The reflexes therefore extend the amount of blood loss that can occur without causing death, to about twice that which would be possible in their absence.

Guyton and Crowell (1961), and Guyton (1973) have shown that the arterial pressure is maintained at or near normal levels in the haemorrhaging person longer than is the cardiac output. The reason given for this is that the sympathetic reflexes are geared more for maintenance of arterial pressure than for output. They increase the arterial pressure to a great extent by increasing the total peripheral resistance, which has no beneficial effect on cardiac output. The sympathetic constriction of the veins is important to reduce the drop in venous return and cardiac output.

The second plateau in the arterial pressure curve in their studies is especially interesting and is thought to be caused by activation of the CNS ischaemic response which causes extreme stimulation of the sympathetic nervous system. This effect of the CNS ischaemic response can be called the "last ditch stand" of the sympathetic reflexes in their attempt to keep the arterial pressure from falling too low.

1.10.4 REFLEX PROTECTION OF CORONARY AND CEREBRAL BLOOD FLOW

In the face of decreasing cardiac output a special importance of the maintenance of normal arterial pressure is protection of blood flow through the coronary and cerebral circulatory systems (Kovach and Sandor, 1976). Sympathetic stimulation does not cause significant constriction of either the cerebral or cardiac vessels. In addition, in both these vascular beds local autoregulation is excellent, and prevents moderate changes in arterial pressure from significantly affecting their blood flows. Blood flow through the heart and brain is maintained essentially at normal levels as long as the arterial pressure does not fall below about 70 mm Hg, despite the fact that blood flow in many other areas of the body might be decreased almost to zero because of vasospasm.

1.10.5 RECOVERY TO NON-RECOVERY IN EXPERIMENTAL BLEEDING

Jones (1974), Crowell (1965), Crowell and Guyton (1961) demonstrate on dogs the effects of different degrees of haemorrhage on the subsequent course of arterial pressure. The dogs were bled rapidly until their arterial pressures fell to different levels. These dogs whose pressure fell immediately to 45 mm Hg all eventually recovered; the recovery occurred rapidly if the pressure fell only slightly but occurred slowly if it fell almost to the 45 mm Hg level. However when the arterial pressure fell below 45 mm Hg, all the dogs died, though many of them lingered between life and death for many hours before the circulatory system began to deteriorate. This is a demonstration that the circulatory system can recover as long as the degree of haemorrhage is

no greater than a certain critical amount, crossing which by even a few millilitres of blood loss makes the difference between eventual life and death. Thus haemorrhage beyond a certain critical level causes shock to become progressive whereby the shock itself causes still more shock, the condition becoming a vicious cycle that leads to death following complete deterioration of the circulation. There are therefore some factors that allow recovery from moderate degrees of shock, and those that prevent recovery. These two aspects are briefly discussed below with a view to defining the role of elevated plasma potassium in the cause of irreversibility in shock.

1.10.6 RECOVERY FROM NON-PROGRESSIVE SHOCK

Non-progressive shock is frequently called compensated shock because baroreceptor reflexes and other factors have compensated enough to prevent deterioration of the circulation. The factors that cause such recovery are the negative feedback control mechanisms of the circulation that tend to return cardiac output and arterial pressure to normal levels.

These include:

(i) The baroreceptor reflexes, which elicit powerful sympathetic stimulation of the circulation, (Chien, 1967: Rothe, 1963).

(ii) the central nervous system (CNS) ischaemic response, which elicits even more powerful sympathetic stimulation throughout the body but is not activated until the arterial pressure falls below 50 mm Hg (Rothe, 1963; Lewis, 1977).

(iii) reverse stress-relaxation of the circulatory system, which causes the blood vessels to contract down around the diminished blood volume so that the blood volume that is available will more adequately fill the circulation (Moyer & Butcher, 1967).
(iv) formation of angiotensin, which constricts the peripheral arterioles and causes increased conservation of water and salt by the kidneys, both of which help to prevent progression of the shock (Weil & Shubin, 1967).
(v) formation of vasopressin (anti-diuretic hormone), which constricts the peripheral arterioles and veins and acts on the distal nephron to produce increased retention by the kidneys.
(vi) compensatory mechanisms that return the blood volume back to normal, including absorption of large quantities of fluid from the intestinal tract, from the interstitial spaces of the body, conservation of salt and water by the kidneys and increased thirst and appetite for salt which makes the subject drink water and eat salty foods if able (Zweifach, 1974).

The sympathetic reflexes become maximally activated within 30 seconds after haemorrhage to provide immediate help toward bringing about recovery. The reverse stress-relaxation requires some 10 minutes to an hour to occur completely, while the adjustment of blood volume by the other compensatory mechanisms may require from 1 to 48 hours, but recovery eventually takes place provided the shock does not become enough to enter the vicious cycle.

1.10.7 THE VICIOUS CYCLE OF CARDIOVASCULAR DETERIORATION - PROGRESSIVE SHOCK

Various types of positive feedback develop once shock has become severe enough to cause deterioration of the structures of the circulatory system themselves. These cause a vicious cycle of progressively decreasing cardiac output. Figure 1.7 illustrates different types of feedback that can lead to progressive shock and the evidence leading to myocyte death. The major aspects of these are:

Cardiac Depression

Early in the stages of shock progressive deterioration of the heart plays very little role partly because deterioration of the heart itself is not very severe during the first hour or so of shock but mainly because the heart has a large reserve that makes it normally capable of pumping 300 to 400 per cent more blood than is required by the body for adequate nutrition (Crowell, 1965; Crowell & Guyton, 1961). In the late stages of shock however, deterioration of the heart is probably the most important factor in the further progress of the shock. Apart from the myocardial depression caused by poor coronary blood flow in shock, the myocardium can also be depressed by toxic factors transported to the heart from other parts of the body especially by a factor called myocardial toxic factor (Lefer *et al.*,1967; Lefer & Martin, 1970) and also by such substances as excess lactic acid (Suteu, 1977), bacterial toxins from the gut, and degeneration products from dying tissues.

Vasomotor Failure

There comes a point at which diminished blood flow to the vasomotor center itself so depresses the centre that it becomes progressively less active. For instance, if there is complete circulatory arrest to the brain, during the first four to eight minutes, there is an intense sympathetic discharge, resulting in a typical CNS ischaemic response, but by the end of 10 to 15 minutes the vasomotor centre becomes so depressed that no evidence of sympathetic discharge at all can be demonstrated. (Kovach, 1976; Chien, 1967). Fortunately though the vasomotor centre does not usually fail in the early stages of shock, only in the late stages.

Acidosis in Shock

The poor delivery of oxygen to the tissues in shock greatly diminishes oxidative metabolism of foodstuffs. The cells obtain their energy by the anaerobic process of glycolysis that leads to excess lactic acid in the blood (Rosenblum, 1977). Normal removal of carbon dioxide is prevented by the poor blood flow through the tissues. Intracellularly the carbon dioxide reacts with water to form carbonic acid which in turn reacts with the various tissue buffers to form still other intracellular acidic substances. There is therefore generalized and local tissue acidosis as shock progresses.

Tissue Necrosis and Increased Capillary Permeability

The permeability of the capillaries increases after many hours of capillary hypoxia and large quantities of fluid begin

to transude into the tissues. This further decreases blood volume and cardiac output. Such capillary permeability occurs late in shock and therefore plays a significant role in few instances of shock (Guyton, 1973). Not all the cells of the body are equally damaged by shock because some tissues have better blood supply than others. For instance the tissues at the arterial ends of capillaries receive better nutrition than those adjacent to the venous ends of the same capillaries.

Crowell and Smith (1964) demonstrated the first sign of damage in the liver as swelling of the liver cells which compresses the sinusoids thus causing total blockade of blood flow in their central ends. Similar punctate lesions occur in the heart muscle though here a definite repetitive pattern such as occurs in the liver cannot be demonstrated. Nonetheless, the cardiac lesions probably play an important role leading to the final irreversible stage of shock (Guyton & Crowell, 1961).

1.10.8 IRREVERSIBLE SHOCK

With the progression of shock to a later stage, transfusion or any other type of therapy becomes incapable of saving the life of the subject. The subject is therefore said to be in the irreversible stage of shock. In some cases, ironically, even in this irreversible stage therapy can still return the arterial pressure and even the cardiac output to normal for short periods of time, but the circulatory system nevertheless continues to deteriorate and eventually death ensues in another few minutes or few hours (Jones et al., 1968; Crowell, 1955).

Thus, there seems to be something that changes in the overall function of the circulatory system during shock that may not necessarily affect the immediate ability of the heart to pump blood but over a long period of time does depress this ability and results in death (Crowell, 1965; Crowell & Guyton, 1961).

With a view to answering the question of the factor or factors that lead to the eventual deterioration of the circulatory system that precipitates death, Crowell and Smith (1964); Lefer and Martin (1970); Jamieson and Greenwalt (1978) have demonstrated and incriminated the depletion of cellular high energy phosphate reserves of all cells, increasing failure of the heart and oxygen deficiency as the main factors in irreversible shock.

1.10.9 CELLULAR HIGH ENERGY PHOSPHATE RESERVE DEPLETION IN IRREVERSIBLE SHOCK

The high energy phosphate reserves in the tissues of the body, especially in the liver and in the heart, are greatly diminished in severe degrees of shock. All of the creatinine phosphate is essentially degraded, and almost all of the sarcoplasmic ATP has been degraded to ADP, AMP or adenosine. Much of the adenosine that is derived from degradation of the ATP diffuses out of the cells into the circulating blood and is converted into uric acid, a substance that cannot re-enter the cells to reconstitute the adenosine phosphate system. New ATP can be synthesized only at very low rates, about 2 per cent an hour, emphasizing the difficulty in replenishing

the high energy phosphate stores. This is one of the most important end results of deterioration in shock and perhaps the most significant in the development of the final state of irreversibility (Suteu, 1977).

1.10.10 A CAUSE OF IRREVERSIBLE SHOCK - DETERIORATION OF THE HEART

Deterioration occurs in many different organ systems in shock and the degeneration in any one of these systems could become so severe that it would eventually be incompatible with continued life. The following shows that it is the deterioration of the heart itself that makes the shock irreversible. Effective modern therapy is available to produce adequate venous return. Even in the most severe degrees of shock administration of blood and other blood substitutes can almost always provide adequate inflow pressure to the heart, yet the heart fails to pump this inflowing blood in the late stages of shock (Jamieson & Greenwalt, 1978; Moyer & Butcher, 1967; O'Riordan et al., 1978).

1.10.11 OXYGEN DEFICIENCY HAS A SPECIAL ROLE IN SHOCK IRREVERSIBILITY

From the studies of Cromwell (1958), and Cromwell and Smith (1964) it seems that the one most important nutrient necessary to prevent cellular deterioration and death during shock is oxygen. These workers measured the accumulated deficit of oxygen usage of animals in mild, moderate, severe and very severe shock. In each group of animals, when the average accumulated oxygen deficit reached 120 millilitres of oxygen per kilogram of body weight, 50 percent of the

animals died regardless of how long it took to accumulate this amount of oxygen deficit.

As one of the aims of the present study is to throw more light on the role of K^+ in the irreversibility of haemorrhagic shock, a brief review of the causes of K^+ release and uptake, and the metabolism of K^+ by the heart in health and disease is given below.

1.11 POTASSIUM HOMEOSTASIS

Measurement of the plasma level of potassium is the method used to determine potassium content in the extracellular fluid which contains 60 to 70 mmol or only 2 per cent of the body K^+. Approximately 3,500 mmol of K^+ are contained in the body of a 70 kg human subject, predominantly as an intracellular ion in muscle. And so this 2 per cent of the body potassium causes problems in the interpretation of total body content since the plasma level is affected by shifts of K^+ between the intracellular and extracellular fluids in addition to the total body balance. When losses are sustained, the decrease in plasma K^+ is rather gradual (Fig. 1.2). There must be a large K^+ loss, usually 200 mmol or more, before any hypokalaemia (plasma K^+ level below 3.0 mmol/l) develops (Valtin, 1979).

In fact, the plasma level will rise transiently about 1 mmol/l with a K^+ load of only 40 to 50 mmol in a 70 kg person who is already somewhat hyperkalaemic.

Both man and animals adapt to high K^+ diets after one week; once they are K^+ adapted, they can handle large K^+

loads without the development of severe hyperkalaemia (Silva, Brown & Epstein, 1977). K^+ adaptation involves both renal and extrarenal mechanisms of K^+ homeostasis.

1.11.1 RENAL POTASSIUM HOMEOSTASIS

Renal excretion of K^+ is modified by several factors, including mineralocorticoid levels, acid-base status, distal tubular flow rate, and distal tubular Na^+ delivery.

The adrenocorticoid hormones are critical to increasing secretion in the distal nephron (Adam, Goland & Wellard, 1984). Aldosterone secretion by the adrenal zone glomerulosa can be stimulated directly by K^+ in synergy with angiotensin II (Pratt, 1982). In this way, K^+ loading will increase mineralocorticoid levels and thereby enhance urinary K^+ excretion. Adam, Goland and Wellard (1984) have also shown that adrenal glucocorticoid hormones, together with mineralocorticoids, play an important role in the renal adaptation to increase K^+ excretion. Renal excretion of K^+ is affected by acid-base status. It is well known that alkalosis causes urinary K^+ loss (Gennari & Cohen, 1975). Less well appreciated is that acute acidosis actually decreases the excretion of urinary K^+. The K^+ losses seen in chronic acidosis, such as diabetic ketoacidosis, are dependent upon other factors, such as increased Na^+ delivery to the distal tubule that may often be accompanied by poorly reabsorbed organic anions, rather than due to the acidosis itself (Gennari & Cohen, 1975).

K^+ secretion in the distal nephron can be augmented by increased distal tubular delivery of Na^+ (Peterson & Wright, 1977), and by distal tubular fluid flow rate (Good & Wright, 1979). These factors generally work in concert to increase K^+ excretion when Na^+ and water intake is high. Conversely, the diminished ability of the kidney to excrete K^+ when distal tubular delivery of Na^+ and water is reduced in patients with prerenal accumulation of urea in blood (azotaemia) explains the severe hyperkalaemia often seen when the glomerular filtration rate is only moderately reduced but oliguria is present.

1.11.2 EXTRARENAL POTASSIUM HOMEOSTASIS

The intake of K^+ in a single meal can easily be greater than the K^+ content in the entire extracellular fluid. But such quantities will usually not cause hyperkalaemia, even during the time period before the load can be excreted in the urine. Maintenance of a normal level of extracellular K^+ under such conditions, or in exercise, trauma, acidosis, and in patients with renal failure when K^+ excretion is compromised, depends on extra-renal uptake or redistribution of K^+ into cells (Alexander & Levinsky, 1968; Silver, Brown & Epstein, 1977).

At least four factors are known to affect the distribution of potassium between the intracellular and extracellular fluids: acid-base status, insulin, mineralocorticoids, and adrenergic activity.

In acidosis, an increase in intracellular hydrogen ions increases cellular K^+ efflux causing hyperkalaemia. The opposite effect seen in alkalosis is frequently exploited therapeutically, whereby the administration of sodium bicarbonate to buffer intracellular hydrogen ions, even if extracellular pH remains unchanged, will enhance cellular K^+ uptake and correct hyperkalaemia (Fraley & Adler, 1977).

Insulin plays an important role in augmenting cellular uptake of K^+ in addition to its well-known cell membrane effect on glucose uptake. It appears that it is necessary to have a normal level of insulin for normal K^+ regulation (De Fronzo & Sherwin, 1978), although large K^+ loads are capable of stimulating increased insulin secretion to help prevent hyperkalaemia (Bia & De Fronzo, 1981). Insulin lack is one of the factors that predisposes juvenile diabetic patients to hyperkalaemia.

Mineralocorticoids are known to augment colonic secretion of K^+ (Bia & De Fronzo, 1981). Aldosterone also appears to have a significant effect on the extrarenal disposal of K^+ (Bia, Tyler & De Fronzo, 1982), but the magnitude of its effect and the conditions under which cellular K^+ uptake is enhanced remain uncertain (Bia & De Fronzo, 1981).

Adrenergic activity has important effects on K^+ distribution. D'Silva (1934) postulated that adrenaline and the autonomic nervous system might be involved in the acute extrarenal regulation of plasma K^+. Intravenous injection of adrenaline in the anaesthetized cat and dog causes an initial rise in plasma K^+ due to its release from

the liver, followed by a more prolonged fall with undershoot due to uptake by the liver and other tissues (Lim, Linton & Band, 1982; D'Silva, 1934; Ellis, 1956). Rosa, Silva and Young (1980) reported that epinephrine improves, and beta blockade impairs, K^+ tolerance in human subjects receiving a K^+ load. This effect of the sympathetic nervous system to regulate extrarenal K^+ balance has also been documented in nephrectomized rats (Silva & Spokes, 1981).

It is attractive to propose that alpha-adrenergic catecholamines have an opposite effect to that of beta-adrenergic agents in the modulation of K^+ distribution. In fact, in recent studies, it has been reported that the alpha agonist phenylephrine significantly augments and prolongs the rise in K^+ level produced by K^+ infusion (Williams, Rosa, Gervino, Silva, Brown & Epstein, 1984). Alpha blockade with phentolamine blocked this effect of phenylephrine. Moreover, phentolamine also decreased the rise in plasma K^+ when infused into healthy subjects performing physical exercise (Williams et al., 1984). Urinary potassium secretion, which was unchanged did not account for the effect of either the phenylephrine or phentolamine infusion. Thus it appears that alpha agonists or endogenous alpha stimulation by exercise, that is, the release of catecholamines, especially noradrenaline, impairs extrarenal K^+ uptake by cells, in contrast with the beta-adrenergic effect to enhance K^+ disposal. Sympatho-adrenal activity via β-adrenoceptors increase cellular K^+ uptake in concert with insulin and aldosterone to restore the plasma K^+ level to normal (Brown, 1984).

It should be emphasized that factors which must also be important in extrarenal K^+ metabolism in addition to those described above are not ruled out. Even when insulin, epinephrine and aldosterone secretions are blocked and blood pH and serum tonicity are controlled, approximately one-half the retained K^+ is still taken up by cells (Smith, Bia & De Fronzo, 1985). Whether this regulation represents the intrinsic ability of extrarenal tissues to respond to a rise in plasma K^+ concentration or is mediated by other as yet unidentified mechanisms is not yet established. Tonicity is the effect of ionic strength on the cell membrane, and as tonicity increases, an osmotic gradient is created that favours the shift of fluid out of cells.

1.11.3 CATECHOLAMINES ON Na^+ - K^+ TRANSPORT

The first experiments suggesting a direct stimulating effect of epinephrine on K^+ uptake in skeletal muscle were performed almost 50 years ago. In the hindlimbs of anaesthetized dogs, epinephrine was found to increase the arteriovenous difference for plasma K^+ (Marenzi & Gerschman, 1936; Stickney, 1941). In isolated perfused frog hindlimb, the net K^+ release induced by electrical stimulation was reduced by 40% by epinephrine (Stickney, 1941). In cats, adrenaline was reported to increase the K^+ content of skeletal and cardiac muscle (Lockwood & Lum, 1974). In human subjects, intra-arterial injections of adrenaline were found to increase the arterio-venous difference for K^+ (DelaLande, Manson & Parks *et al.*, 1961; Grob, John & Liljestrand, 1957).

Several *in vitro* studies have demonstrated that both epinephrine and nor-epinephrine increase the K^+ content as well as the K^+_i in rat soleus (Clausen & Flatman, 1977;

Flear et al., 1977). In guinea-pig soleus muscle, epinephrine was found to induce approximately the same increase in ^{42}K uptake as in rat soleus (Buur, Clausen, Holmberg, Johanson & Waldeck, 1982).

In an attempt to determine whether norepinephrine released from sympathetic nerve endings might influence $Na^+ - K^+$ transport, chemical sympathectomy was performed in mice and rats. However, neither ouabain-suppressible ^{42}K influx nor $Na^+ - K^+$ contents were influenced (Clausen, Hansen & Larsson, 1981).

Mechanism of Catecholamine Action

The effects of adrenaline and noradrenaline on $Na^+ - K^+$ contents, ^{42}K influx, ^{22}Na efflux, and transmembrane potential (Em) were all blocked by propranolol, a β-adrenoceptor blocker, (2 to 8.0 x 10^{-6} mmol/l in the bathing solution), but not by phentolamine, an α-adrenoceptor blocker. The β_1-selective antagonist metoprolol was at least 50 times less potent than propranolol. This together with the repeated observations that isoproterenol as well as the β_2-selective agonists salbutamol and terbutaline mimic all of the above effects show that they are elicited via β_2-adrenoceptors (Bray et al., 1976; Buur et al., 1982; Clausen et al., 1977; Kuba, 1970 and McArdle & D'Alanzo, 1981). The combination of theophyline and dibutyryl cAMP increased ^{42}K influx, ^{22}Na efflux, the intracellular K^+ to Na^+ concentration ratio and the Em, and all of these effects were blocked by ouabain (Clausen et al., 1977). Taken together, the evidence indicates that catecholamines accelerate the active electrogenic $Na^+ - K^+$ - transport by a β_2-adrenoceptor-mediated stimulation of adenyl cylase.

The mechanism of activation of the Na^+-K^+-ATPase by catecholamines is poorly understood but seems to involve cAMP-induced activation of a protein kinase (Scheid et al., 1979) which acts on the internal surface of the membrane to dephosphorylate a membrane associated protein that regulates channel activity (Shuster et al., 1985).

Alpha-adrenoceptor mediated inhibition of rat liver adenylate cyclase by epinephrine has been reported by Aggerbeck, Guellaen and Hanoune (1980). The α-adrenoceptor-mediated inhibition of the adenylate cyclase was clearly different from the α-adrenoceptor-mediated phosphorylase activation, which has been shown to involve α_1-adrenoceptors (Aggerbeck et al., 1980). Whereas the α_1-antagonist, prazosin, was about 3 orders of magnitude more potent than the α_2-antagonist, yohimbine, in blocking epinephrine-induced phosphorylase activation, a yohimbine > prazosin order of potency was obtained in the adenylate studies. α-Adrenergic agonists acting through α_2-adrenoceptors might indirectly participate in the regulation of liver glycogenolysis. Thus α-adrenergic agonists reduce the accumulation of cAMP induced by high concentration of glucagon or by β-adrenergic stimulation in liver cells from adrenalectomized rats (Chan, Blackmore, Steiner and Exton, 1979). Beta-adrenergic stimulators have been found to increase the cAMP synthesis in experimental animals in which KCl i.v. toxicity is descreased by the same stimulators, for example adrenaline and isoproterenol (Lockwood & Lum, 1974; Cattani, Costrini and Cerill et al., 1980).

By inference, alpha-adrenergic agonists which reduce the accumulation of cAMP should be expected to increase plasma K^+ concentration or KCl i.v. toxicity.

1.12 THE MEMBRANE THEORY AND POTASSIUM CONDUCTANCE

According to the theory it is the semipermeability of the membrane surrounding the cytoplasm which determines the composition of the latter in relation to that of the external medium (Boyle & Conway, 1941). The idea of charged permeability channels favouring, for example, the passage of an anion over a cation of similar size was developed by Hutter and Warner (1967).

The diffusion potential depends not only on either the concentration or activity of diffusible ions inside and outside the cell but also on the relative magnitude of the permeability coefficients P_K, P_{Na} and P_{Cl}. Where the membrane is permeable to only one of these ionic species to the exclusion of the others, the membrane potential is determined by the Nernst equation:

$$E_K^{oc} = \frac{RT}{zF} \ln \frac{a_k^o}{a_k^c} \quad \text{(see equations 4 and 5)}$$

where E_K^{oc} is the potential within the cytoplasm relative to external fluid, and a_k^o and a_k^c are the activities of K^+ in external fluid, and cytoplasm respectively.

OR Simply put: $E = \frac{RT}{zF} \ln \frac{a_o}{a_i}$

where o, i refer to ions extracellular and intracellular respectively.

In the instance where two solutions of dissimilar cations are in contact, for example NaCl and KCl:

$$E = \frac{RT}{F} \ln \frac{u[Na^+] + v[Cl^-]}{u[K^+] + v[Cl^-]} \quad \ldots\ldots\ldots\ldots\ldots (9)$$

where u, and v, are the mobilities of the positive and negative ions, respectively. The sarcolemma separates an interstitium rich in sodium from intracellular fluid rich in potassium. The passive rate of ion penetration through the membrane is in large part a function of the concentration gradient, the voltage gradient, and the permeability of the membrane to the ion under consideration, in this case K^+.

An ion is at equilibrium across a membrane if no net ion movement occurs. If a membrane is permeable to only potassium, the side of the lower concentration will be more positive than the other because of diffusion of K^+ down the concentration gradient. The membrane potential at which no net K^+ current will flow is given by the equation

$$E_{K^+} = \frac{RT}{F} \ln \frac{K^+_i}{K_o} \quad \ldots\ldots\ldots\ldots\ldots\ldots (10)$$

where E_{K^+} is the equilibrium potential of K^+, for example, for heart muscle, $E_{K^+} = -92.6$ mV and $E_{Na^+} = +84$ mV. Because K^+ is the most permeable cation the resting membrane potential is close to E_{K^+} and hence K^+ is the most important ion in maintaining the resting membrane potential (RMP or Em). Em is not exactly E_{K^+}, because other ions influence the resting potential according to the relation:

$$Em = \frac{RT}{F} \ln \frac{P_{Na^+}(Na^+)_i + P_{K^+}(K^+)_i + P_{Cl^-}(Cl^-)_o}{P_{Na^+}(Na^+)_o + P_{K^+}(K^+)_o + P_{Cl^-}(Cl^-)_i} \quad \ldots\ldots\ldots (11)$$

It is now well established that cardiac muscle and other excitable cells utilize an energy dependent ionic pump to expel any Na^+ which may increase and enter the cell and pump K^+ into the intracellular compartment (Besch & Schwartz, 1970; Bonting, Simon & Hawkins, 1961). The maintenance of the ionic concentration gradients by active Na^+-for-K^+ exchange results in the separation of charge and hence generates the transmembrane potential, which is a function of the net difference between the pumping and leakage rates. The Na^+-pump is sensitive to changes in Na_i^+ within the cell, and therefore has been considered to be under negative feedback control (Langer, 1972). (See Fig. 1.7).

The Na^+, K^+-pump also regulates cell volume and any reduction in its activity results in greater concentration of osmotically active particles intracellularly.

From Ohm's Law where R_i = resistance to ith ion,
I_i = current carried by ion i
and Em = membrane potential

$$R_i = \frac{Em}{I_i} \quad \ldots\ldots\ldots\ldots\ldots \quad (12)$$

Ion conductance $_i = \frac{1}{R_i}$ $\ldots\ldots\ldots\ldots\ldots$ (13)

and therefore $K^+ = I_{K^+}/Em - E_{K^+}$,

$$Na^+ = I_{Na^+}/Em - E_{Na^+}$$

and

$$Cl^- = I_{Cl^-}/Em - E_{Cl^-}$$

where $(Em - E_i)$ is the driving force "behind" each ion. At rest K^+ is high, and I_{K^+}, Na^+ and I_{Na^+} are low.

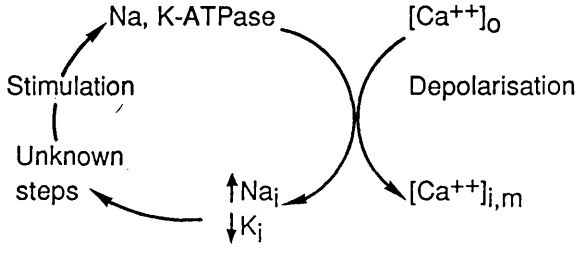


Fig. 1.7.
Net sodium influx and potassium efflux attend each depolarization cycle. These changes are reversed by Na^+, K^+- pumping energized by Na^+, K^+-ATPase. Calcium influx also occurs during the action potential in cardiac muscle.

The time- and voltage-dependence of ion conductances are explained by the opening of ion channels controlled by "gates". One possibility is that charged flexible polar end groups of membrane phospholipids alter their position, thus allowing changes in ion conductance. This hypothesis has led to the search for "gating currents", caused by the opening and closing of the gates. In the axon, such currents have been identified (Armstrong and Bezanilla, 1973). For ionic fluxes involved in depolarization and repolarization leading to cardiac contraction (for example) and their electrical consequences, see references, Kones (1976).

1.12.1 METABOLISM OF POTASSIUM IN THE NORMAL AND ISCHAEMIC HEART CELL

Potassium is an important electrolyte in heart cells and has the greatest membrane permeability in the unexcited state (Kones, 1976). The potassium gradient is often the main determinant of the potential difference between the inner and outer bulk solutions, while extracellular Na^+, Ca^{2+} and Mg^{2+} reduce the membrane potential by partially neutralizing fixed negative charges on the membrane surface (Burton, 1985).

Ventricular arrythmias may begin to show when the plasma potassium levels exceed 7.5 mmol/l. A reduction in extracellular K^+ below 3.0 mmol/l is positively inotropic and is associated with net Ca^{2+} uptake (Thomas Jr., 1960). K^+ movements within the cell are intimately dependent upon metabolic reactions; at the same time the activity of many metabolic pathways is affected by changes in K^+ concentration. K^+ is an essential activator in several enzymatic reactions (Ussing, 1960),

some leading directly to the synthesis of high energy phosphate compounds (Dixon & Webb, 1964).

K^+ in its role as a modifier of hormone interactions may also affect the heart indirectly. For instance, K^+ may interfere with some actions of the catecholamines, increased in concentration as part of the generalized metabolic response to acute myocardial infarction (Kones, 1976). The association between failing heart muscle and K^+ depletion is well known, and it is entirely possible that inefficient oxygen metabolism of K^+-depleted failing heart muscle is due to changed mitochondrial respiratory control (Harrison, Coleman & Zujko et al., 1970).

1.12.2 MYOCARDIAL K^+ DURING ISCHAEMIA

The anoxic or ischaemic myocardium cannot metabolize fatty acids, and depends upon inefficient anaerobic glycolysis for its energy production (Ballinger, 1962; Braasch, Gudbjarnason & Puri et al., 1968; Owen, Thomas & Young, 1970).

While the normal myocardium extracts from 20-40 per cent of the arterial lactate content, during hypoxia lactate is produced from glycogenolysis and anaerobic glucose metabolism, (Clark, Gaddie & Stewart, 1932; Gorlin, 1969; Parker, Chiong & West, 1969).

Na^+, K^+-pumping within the myocyte is an important energy dependent process, without which cell survival is impossible. Loss of energy supply to this pump may account for the early intracellular potassium loss from ischaemic/ hypoxic myocardium (Obeid, Smulyan & Gilbert, 1972).

After myocardial infarction, myocardial K^+ loss from the infarcted area may be delayed because of deficient coronary blood flow, but within 12 hours the K^+ in the infarcted area is equal to the K^+ in the extracellular fluid (Jennings, Crout & Smitters, 1957; Jennings, Sommers, Kattenbach, 1964). Therefore the K^+ content of myocardial necrotic tissue depends on the time that has elapsed since myocardial infarction but approaches 30 per cent of normal (Iseri, Alexander, McCaughey et al., 1952). (See 1.8 for a summary of events leading to myocyte death).

1.12.3 POTASSIUM ION LOSS AND THE ELECTROCARDIOGRAM

It has been found by Regan, Harman and Lehan et al. (1967) that the associated ventricular dysrhythmia after ligation of the coronary artery or production of a coronary thrombus is related to the rate of rise and level of extracellular K^+. A fall in coronary sinus K^+ after glucose and insulin infusion resulting in a diminution of ventricular premature beats has been reported by the same workers. Application of K^+ to the myocardium and an intracoronary injection of K^+ salts produce the injury current noted on the electrocardiogram, which is similar to the pattern observed after experimental ligation of a coronary artery (Nahum, Hamilton & Hoff, 1943; Wiggers, 1930; Wolferth, Bellet, Liveszy et al., 1945).

Recently it has been reported that S-T segment elevation during epicardial lead mapping is associated with sub-endocardial and epicardial ischaemia, as evidenced by lactate production and high energy phosphate depletion (Roselle, Crampton & Case, 1966).

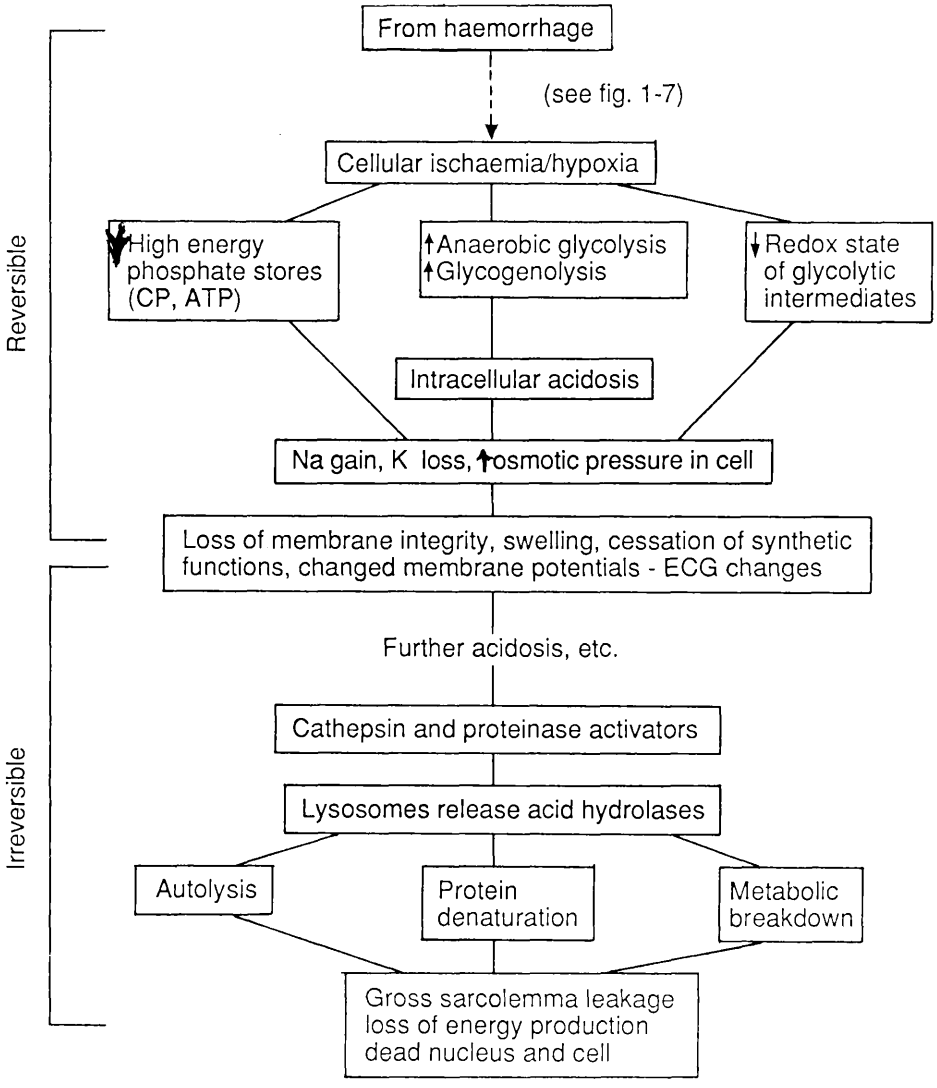


Fig. 1-8
The evidence leading to myocyte death connecting lines to not necessarily imply a cause and effect relationship.

Changes in action potential that affect the S-T segment and the T-wave have been attributed to either hypoxaemia, hyperkalaemia or both. Some evidence indicates that the S-T segment elevation is due to changes in action potential duration, while S-T displacement due to decreased resting membrane potential maintains the S-T elevation (Samson & Scher, 1960). Hypoxaemia causes changes in the ECG which may be confused with the effect of changes in plasma K^+ on the ECG. While hyperkalaemia may cause flattening or disappearance of the P-wave due to atrial standstill, widening of the QRS-complex, peaking of the T-wave and depression of the S-T segment, hypoxaemia causes peaking of the T-wave and elevation of the S-T segment without the flattening of the P-wave which is found in hyperkalaemia.

The pattern of effects of increasing plasma K^+ concentration on the ECG has been reported in the literature (Burgh, 1972; Ettinger et al., 1974) as follows:

i) At concentrations of plasma K^+ greater than 7 mmol/l, K^+ causes progressive depression of the excitability and conduction velocity. Peaking of the T-wave as described above occurs at 7 to 8 mmol/l of plasma K^+.

ii) Shortening and widening of the P-wave due to slow atrial conduction.

iii) Prolongation of the P-R interval as a result of decreased atrioventricular conduction.

iv) Eventual disappearance of the P-wave at 9 or 10 mmol/l of plasma K^+ concentration.

v) Widening of the QRS complex and irregular R-R intervals with patterns of complete bundle branch block, and sine wave type QRS complexes.

vi) Ventricular fibrillation may develop.

It has been reported that if isolated hearts are perfused with K^+-deficient solution (0.8 mmol/l of K^+) at 37^oC with a pH range between 7.3 and 7.5 (Surawicz et al., 1963), ventricular arrest can occur when the extracellular K^+ concentration is suddenly increased to a physiological concentration. This paradoxical phenomenon of K^+ on the heart was first reported by Zwaardemarker (1919-20) followed by Libbrecht (1921). It has therefore since been known as the Zwaardemarker-Libbrecht (Z-L) phenomenon. In order to throw more light on the mechanism of this Z-L phenomenon, Surawicz and Gettes (1963) made records of ECG, transmembrane potential of atria and ventricles before, during and after ventricular arrest, and revealed that the Z-L mechanism differed from the mechanism of cardiac arrest produced by an increase in external K^+ concentration above physiological level. The Z-L phenomenon is caused by an increase in the velocity of repolarization while the usual effects of high K^+ on the resting membrane potential are either very slight or absent. Conduction in the atria, ventricles and between the atria and the ventricles apparently is not disturbed during the Z-L phenomenon; therefore, the cardiac arrest is attributed to a selective inhibition of the pace-maker activity, which might be due to an inhibition of the

diastolic depolarization of the pacemaker fibres. A speculation was made by Surawicz et al. (1963) that the type of imbalance between the intra- and extracellular K^+ concentration in the myocardium which causes the Z-L effect in the isolated heart could occur in vivo and cause sudden death.

That changes in acid-base balance can affect plasma K^+ metabolism has been reported by Young, Sealy and Harris (1954). Disturbances in other electrolytes including hydrogen ion can change the ECG in such a way as to simulate the pattern of hypokalaemia (Burgh, 1972), while hypoxaemia as described above can simulate some features of the ECG produced by hyperkalaemia.

1.12.4 POTASSIUM ACTIVITIES IN REVERSIBLE ISCHAEMIA OF THE BRAIN

Maintenance of ionic homeostasis is a necessary pre-requisite for the normal functioning of the brain. Interruption of cerebral blood flow, even of short duration, causes severe disturbances of the ionic equilibrium because inhibition of the energy-dependent ion pumps leads to a breakdown of ionic gradients across cell membranes and consequently to cell depolarization. Shifts of electrolytes and fluid between blood and brain may result and as a consequence will lead to ischaemic brain swelling (Plum, Posner and Alvord, 1963).

It has been demonstrated that as a consequence of oxygen deficiency, K^+ is released from the brain into the blood or into the cerebrospinal fluid (Bito & Meyers, 1972; Selzer,

Myers and Holstein, 1972). Kirshner et al. (1975) have also shown that brain extracellular K^+ activity remains essentially constant until the arterial PO_2 decreases to 20 to 23 mm Hg provided that the MABP remains above 100 - 110 mm Hg. If the MABP is allowed to fall during hypoxia even to the 70 to 100 mm Hg range, the associated increase in K^+ activity is accentuated, often to levels more than 20 mmol/l. Elevations of the MABP with epinephrine injections reversed both the increases in K^+ activity and the electrocorticogram flattening. Kirshner et al. concluded that cerebral extracellular K^+ homeostasis during hypoxia appeared to depend on the maintenance of a normal arterial perfusion pressure in the brain.

Three stages in the disturbance of K^+ homeostasis produced by progressive ischaemia of the brain have been identified by Branston, Strong & Symon (1977). In the first stage, at flow levels similar to those sufficient to abolish the cortical evoked potential (12-16 ml/100 g/min), small self-limiting increases in K_e^+ (extracellular K^+) occur, probably reflecting K^+ efflux, into the extracellular space (ECS) with partial impairment of K^+ clearance from the ECS. The second stage occurs at distinctly lower levels of flow (8-11 ml/100 g/min), and is characterized by a massive (30-80 mM) increase in K_e^+ which they attribute to an increase in ionic permeability of cell membranes with further impairment or overloading of K^+ clearance mechanisms. In the third stage, at flows below about 6-8 ml/100g/min, their data indicated an inverse relationship between flow and K_e^+ with persisting high K_e^+ levels, suggesting complete

loss of potassium clearance.

On the other hand, Blank and Kirshner in 1977 observed two patterns of K_e^+ increase in the hypoxic and hypothermic cerebral cortex of the cat. A slow, linear rise occurred during hypoxia and hypothermia and was correlated with changes in MABP. A fast, complex exponential rise occurred during anoxia and was unassociated with MABP changes. The fall of K_e^+ after reversal of the insult-lowering of O_2 in inspired air to give P_aO_2 levels of 20-23 mm Hg (hypoxia), or removal completely (anoxia) - was described by a single exponential function with rate constants from 0.009 to 0.194 sec^{-1}. These workers suggested that the linear rise was primarily a result of sodium pump inhibition and that the exponential rise was due to a superimposed sudden increase in cell membrane permeability perhaps secondary to transmitter release. The kinetics of the fall of K_e^+ was consistent with the normalization of the sodium and K^+ gradients across the cell membranes secondary to Na^+-K^+-ATPase activity.

The same workers found no significant correlation between change in K_e^+ and duration of MABP less than 50 or 100 mm Hg, P_aO_2, pH, P_aCO_2 or duration of electrocorticogram isoelectricity. In general, however, they found that those hypoxic or anoxic insults which lasted the longest had the lowest fractional change in K_e^+.

In similar experiments to Blank and Kirshner (1977) where Cohen (1973) studied the biochemistry of cerebral anoxia, hypoxia and ischaemia, the K_e^+ fell in all animals which were successfully resuscitated, although to a level several mmols higher than the prior control K_e^+. In some

animals, the K_e^+ continued to fall very slowly, and in others, this new level was maintained. That the K_e^+ did not normalize immediately may indicate that some degree of damage had occurred to the ionic pumps. Repair of this damage could require new synthesis of RNA and proteins, and these mechanisms may also be damaged by hypoxia or anoxia. Irreversibility and brain death may be the end result.

1.12.5 POTASSIUM TRANSPORT IN SALIVARY GLANDS

It is possible to study K^+ movements in salivary glands of anaesthetized cats and dogs through the use of external K^+-selective microelectrodes (Poulsen & Bledsoe, 1978). It was found by this means that extracellular potassium concentration increased from 2.3 mmol/l to 15.2 mmol/l in dogs, and from 2.2 mmol/l to 18.7 mmol/l in cats following electrical stimulation of the parasympathetic chorda lingual nerve. However the concentration fell once more at the end of stimulation, probably because of active uptake by the gland cells. There appeared to be a parallel between the amount of K^+ lost from the cells and the rate of secretion by the gland. The link between the two processes has yet to be defined. It has been suggested that during K^+ release from the acinar cells an equivalent amount of sodium entered the cells and that this was the trigger for secretion. It is not clear how a net influx of sodium may be reconciled with the membrane hyperpolarization induced by stimulation.

Although the K^+ movement into the lumen of the salivary duct is generally considered to be passive, when its concentration in the primary saliva rises to 160 mmol/l after sympathetic stimulation it is difficult to be convinced

that active secretion is not taking place. K^+ movement into the lumen might on the other hand have been brought about by electrogenic pumping of sodium into the blood, at least in the rat where the transepithelial potential was as high as 70 mV and favoured passive K^+ flux (Poulsen et al., 1978).

1.12.6 POTASSIUM TRANSPORT IN LIVER

Intracellular concentrations of ions in dog liver based on extracellular volume measured by tritiated inulin were found to be as follows: $[K]_i$, 172 ± 13, $[Na]_i$, 22.1 ± 4.4, and $[Cl]_i$, 24.1 ± 2.9 mmol/l cell water. The diffusion potential based on the Goldman equation, where P_{Na}/P_K was taken to be 0.17, was found to be -37 mV, compared with -44.4 mV measured by microelectrodes (Lambotte, 1977). The dependence of K^+ influx and Na^+ efflux on active transport was shown by the fact that within minutes of adding 10^{-4}M ouabain to the medium, Em became less negative, suggesting inhibition of an electrogenic sodium pump, and this was also indicated by the Na:K coupling ratio of 3:2 (Claret, Claret & Mazet, 1973). On the other hand, partial depletion of ATP by hypoxia or by addition of 10^{-2}M antimycin or 2 x 10^{-2}M fructose produced membrane hyperpolarization accompanied by K^+ loss from the liver.

In isolated liver cells from rats (Beigelman & Thomas, 1972) 10^{-3}M cyanide reversibly depolarized the membrane by 35%, while ouabain even at a concentration of 10^{-2}M had no effect on membrane potential. The ouabain inhibited Na efflux and K influx by 58% and 72%, respectively.

Histochemical evidence of increased hepatic ATPase in haemorrhagic shock has been presented by De Palma et al. (1968). Not only did the ATPase activity progressively increase as the length and therefore the severity of shock increased, but also the increased activity returned towards normal following treatment of both early and late haemorrhagic shock. An increase in intracellular Na^+ as a consequence of an initially depressed activity of the sodium pump mechanism appears to be a probable explanation of the increased Na^+-K^+-ATPase activity. Increased intracellular Na^+ has been shown to stimulate Na^+-K^+-ATPase in erythrocyte ghosts (Glynn, 1962).

Whether the change in ATPase activity is a primary or a secondary response to shock is not certain yet.

K^+ efflux from the liver cells has also been found to be subject to acute regulation by catecholamines (D'Silva, 1935; Linton et al., 1984; Coats, 1985) and opioids (Chistyakov et al., 1980). Such regulatory mechanisms have been found to be via specific receptors, viz; α- and β-adrenergic receptors mediating the catecholamine-induced K^+ changes, and opiate-receptors (μ- and delta-receptors) mediating the opioid-induced K^+ changes.

1.12.7 POTASSIUM TRANSPORT IN SKELETAL MUSCLE

The skeletal muscles contain the largest single pool of K^+ in the body (75% of total). During maximal exercise in human subjects, K^+ may be released at a rate sufficient to increase the K^+ level in arterial blood plasma by 3 mM within 1 min. (Hermansen, Orheim & Sejersted, 1984), implying a net loss of muscle K^+ of at least 40 mmol/min. On the

other hand, measurement of the concentration of ^{3}H ouabain binding sites in human skeletal muscle indicates that the maximum capacity for active transport of K^+ into the total pool of muscle cells amount to 125 mmol/min (Norgaard, Kjeldsen & Clausen, 1984). Thus the muscles may either flood the plasma with K^+ or induce hypokalaemia by clearing a substantial fraction of extracellular K^+ within very short intervals of time - in either case with the risk of severe interference with excitability, contractile performance and metabolism. Therefore an effective control of the active Na^+-K^+ transport is essential for the maintenance of optimum muscle function. In skeletal muscle, the rate of active Na^+-K^+ transport is determined by the following factors:

(i) The basal activity of the Na^+-K^+-ATPase (the Na^+-K^+ pump) which primarily depends on cytoplasmic ATP and the concentration of Na^+ and K^+ on either side of the plasma membrane;

(ii) Long-term changes in the concentration of Na^+-K^+-ATPase which is mainly determined by cellular differentiation and age, muscle activity, thyroid status and the availability of K^+;

(iii) Short-term activation of the Na^+-K^+-ATPase by insulin, epinephrine, and norepinephrine, which are the main mediators of acute hormonal control.

From several studies performed over the last decade, the Na^+-K^+-ATPase has emerged as the common target for a variety of control mechanisms determining Na^+-K^+ distribution and membrane potential in skeletal muscle. The fundamental

relationships between active Na^+-K^+ transport and Na^+-K^+-ATPase as well as the influence of cations and ATP on enzymatic activity have been described in several recent reviews (Glynn & Ellory, 1985; Jorgensen, 1980; Sjodin, 1982).

In the intact organism, K^+ deficiency leads to a marked and reversible decrease in the concentration of Na^+-K^+-ATPase as well as the capacity for performing active Na^+-K^+ transport. The essence of both acute and long term regulation of the Na^+-K^+-ATPase for the maintance of muscle Na^+-K^+ homeostasis, optimum excitability and motor function is emphasized by the repeated observation of exercise-induced hyperkalaemia and the notion that this may be an important limiting factor for physical performance.

1.13 POTASSIUM AND BLOOD PRESSURE REGULATION

Extra K^+ intake has been found to be a factor affecting blood pressure for epidemiological and experimental reasons. People with hypertension have a small fall in MABP with added K^+ (Morgan, Teow & Myers, 1984). Morgan in 1982, showed that in people with sodium-sensitive hypertension, KCl prevented the rise in MABP produced by NaCl. The response in supine systolic and diastolic blood pressures was found to be critically dependent upon the Na^+ intake of an individual. In people on a low Na^+ intake, K^+ caused no significant fall in MABP in either Na^+-sensitive or Na^+-resistant subjects, an observation similar to that observed in rats by Louis *et al.* (1971).

While K^+ did not alter supine MABP, it did cause an alteration in the rise in MABP and pulse rate observed when suddenly changing from the supine to erect position. In subjects on a low Na^+ intake, the rise in pulse rate and rise in systolic (and possibly diastolic) BP was greater compared with those on a control diet (Morgan et al., 1984). Such an observation suggests an exaggerated responsiveness of the sympathetic nervous system in response to a postural stimulus; extra K^+ in the diet abolished this increased response to a postural stimulus. This effect of K^+ on the change in MABP and pulse rate due to posture was observed whether the subjects were on a normal or a reduced Na^+ intake, though it was greatest when subjects were on the low Na^+ diet.

The mechanism of action of K^+ in lowering MABP is obscure but the results of Morgan et al. (1984), when taken together with those of Dietz et al. (1984), suggest that one important effect is on the sympathetic nervous system. A small fall in plasma renin level and evidence of Na^+ loss were also found by Morgan (1982) which could explain in part the MABP fall. K^+ does appear to have a real but small effect on MABP in normotensive (Khaw and Thom, 1982) and hypertensive people (MacGregor et al., 1982; Morgan, 1982).

Saito et al. (1985) have found that red cell Na^+, and red cell Na^+ to K^+ ratio ($R\text{-}Na^+/K^+$) positively correlated with MABP, but red cell K^+ did not. The same workers found increased values of red cell Na^+ and $R\text{-}Na^+/K^+$ in young to middle aged hypertensives which were consistent with their previous reports (Saito et al., 1984) in which it was suggested

that the reduced net Na^+ efflux across the red cell membrane in essential hypertensives may contribute to their increased intracellular Na^+ content. Red cell Na^+ increases with age in normotensive subjects and such effects of age on red cell Na^+ may be caused by the alteration of membrane Na^+ transport activity with age (Beilin et al., 1966; Cumberbatch et al., 1981). However, this phenomenon cannot be explained by the age effect on red cell K^+ transport because K^+ influx decreases with age (Saito et al., 1984), thus making the mechanism of age effect on red cell K^+ unclear at the moment.

But whatever the effects of other variables which have known effects on MABP, Saito et al. (1985) concluded that increased values of red cell Na^+ and R-Na^+/K^+ are important factors in the mechanism of high blood pressure.

On the other hand, arterial pressure and heart rate have been found to increase reflexly by increasing the K^+ concentration of the gracilis muscle interstitium by intra-arterial injection of KCl (Rybicki, Kaufman, Kenyon and Mitchell, 1984).

An interesting point of note in the interstitial K^+-induced pressor-tachycardia effect was that the arterial pressure returned to control levels even though interstitial concentrations of K^+ remained elevated after injection of KCl into the gracilis artery. It may be that the muscle afferent endings have adapted to the increase in interstitial K^+ concentration, thereby terminating the reflex pressor response. Likewise, other changes such as decreased pH or increased osmolarity may alter afferent sensitivity to K^+ and consequently alter arterial pressure responses to increased K^+ levels.

In similar studies to Rybicki et al., Liu, Higgins and Hoff (1969) studied the mechanisms of intra-arterial induced cardiovascular and respiratory responses in dogs. Their findings supported their postulate that the hypertension and tachycardia induced by K^+ results from activation of the vasomotor centre and the release of norepinephrine and epinephrine at the nerve endings of sympathetic fibres. This was based on the fact that α- and β-adrenergic blocking agents diminished significantly the circulatory changes following an intra-arterial injection of KCl. However, Struthers et al. (1983) found that hypokalaemia was associated with raised systolic blood pressure during high dose infusion of adrenaline (0.06 μg/kg per min.). This effect of adrenaline was thought to be the result of stimulation of a β_2 adrenoceptor linked to Na^+-K^+-ATPase. Such adrenaline induced K^+ influx has been shown both in human erythrocytes and rat skeletal muscle (Clausen, 1980).

It seems possible that there is an association between MABP and extracellular K^+ levels, but there has been no one single hypothesis flexible enough to include all the cause-and-effect factors to give a plausible explanation binding them all.

1.14 PLASMA POTASSIUM TRANSIENTS DURING HAEMORRHAGE, CONTINUOUS MONITORING WITH ION SELECTIVE ELECTRODES

Until recently, the experimental and routine determination of plasma K^+ has been the orthodox method of intermittent withdrawal of blood samples for emission flame photometry. This method is cumbersome and difficult and the information

obtained is necessarily limited if only by the quantity of blood samples that can be withdrawn from a small experimental animal. Changes in plasma K^+ occur in seconds during haemorrhage (Treasure, 1977), and such intermittent sampling may miss out some intermediate changes in the transient levels of plasma K^+. Also, peripheral blood samples will give information only about the K^+ efflux from one region of the body unlike a catheter tip electrode in the vena cava or right atrium which gives an index of mixed venous K^+.

K^+ selective electrode catheters can be used as described in Section One to provide a measurement in blood intravascularly. The result is obtained more quickly and conveniently than is possible with a flame photometer. The electrode responds reversibly and, in the case of the PVC membrane used in this work, virtually instantaneously. It is therefore possible to measure continuously the K^+ concentration in flowing blood.

Early systems depended on taking blood past an electrode and discarding it. Sherwood and Jones *et al.* (1960) used an electrode of this type for intensive care work which could be used at a sampling rate of 0.1 ml blood per minute for steady state observations and the flow rate increased as necessary. The blood sample was pretreated by dilution with neutralized saline in proportions 1:10. Band and Semple (1966, 1967) used whole blood in a flow-through model and also discarded the blood in their experiments on human subjects. However, these were short experimental runs and flow was fast enough to record breath by breath arterial oscillations in pH. In the cat the electrode was placed in a carotid

arterial loop and blood was returned to the animal (Band, Cameron & Semple, 1969). Although inserting a catheter tip electrode may create some difficulties it has more appeal than continuously bleeding a patient past an extracorporeal electrode system. Liquid membranes have been used in the venous blood to observe K^+ efflux from muscle (Hnik *et al.*, 1973, 1974) and the brain. MacKinley *et al.* (1981) has continuously monitored interstitial fluid potassium during haemorrhagic shock in dogs using ion-sensitive field effect transistor (ISFET) devices.

Treasure and Band (1977), using the catheter-tip ion-selective electrode for continuous measurement of K^+ changes after a number of physiological and pharmacological stimuli observed marked hyperkalaemic effects of haemorrhage in cats and dogs. While it is known that the liver releases K^+ under a variety of circumstances, the speed at which the blood level changed in their preliminary experiments had not been reported in the literature on haemorrhage before then. They also noted that an animal which failed to show hyperkalaemia at the beginning of an experiment might later produce a marked rise in response to quite a small bleed. Changes in plasma K^+ differed significantly in different parts of the cardiovascular system at the same time after adrenaline injection. For example, the plasma level in the thoracic (high) inferior vena cava was higher than that in the abdominal (low) inferior vena cava. Level of plasma K^+ in the aorta was always intermediate between the thoracic and abdominal inferior vena cava plasma levels, while that

in the superior vena cava was lowest of the four. They suspected the source of the K^+ therefore to be in the tissues drained by the inferior vena cava. The difference in the K^+ levels in the thoracic and abdominal parts of the inferior vena cava and the nature of the oscillations on the HIVC K^+ traces made the hepatic vein the likely source, they concluded.

Recording of high plasma K^+ in the hepatic vein has been made previously (Anderson & Shoemaker, 1967) and evidence gained from haemorrhage experiments where the major hepatic vessels were cannulated (Shoemaker et al., 1959) confirmed that it is the liver rather than the portal system that is the source of the K^+ efflux. Docherty and MacKenna (personal communication) also examined the effects of haemorrhage on the plasma K^+ levels using ion-selective electrode catheters and observed significant changes in the high inferior vena cava but little or no changes in the low inferior vena cava K^+. They observed a range of 8 mmol/l to 10 mmol/l in the high inferior vena cava depending on the severity of haemorrhage.

Treasure and Band (1977) considered three possible explanations for the sudden K^+ release from the liver, viz: i) A reduction in liver blood flow

ii) A response to an increase in the levels of circulating catecholamines.

iii) A result of acidosis.

However, in their overall conclusion they observed that as a result of a variety of metabolic and physiological stimuli there occurred a redistribution or translocation of K^+ in the body. The level of plasma K^+ in the superior vena

cava changed least during their experiments. In medical practice, blood is usually drawn from an upper limb so the rapid changes observed in the inferior vena cava and the arterial circulation would not necessarily be noticed. Furthermore, just because the venous level remains about the same in the low inferior vena cava, it does not mean that there has been no change in the distribution of K^+. The occurrence of oscillations from breath to breath in the inferior vena cava indicating the different K^+ content of hepatic venous and abdominal vena cava blood, is evidence of the unsteady nature of the body's K^+ balance. The work of Shoemaker on movements of K^+ in and out of the liver (Shoemaker & Elwyn, 1969); Barcroft (1964) on the release of K^+ from exercising muscle; Clancy & Brown (1963) on acute movements of K^+ into bones, and Chappins et al. (1975) on K^+ movements into brain, all suggest the changing nature of the body's K^+ balance. The simultaneous recordings of K^+ in several sites confirm this mobility and illustrate redistribution in the cat under a variety of circumstances.

In cats undergoing haemorrhage or catecholamine infusions, although K^+ was released from the liver it was unusual to produce a significant rise in the K^+ level in blood returning from the periphery. The K^+ released by the liver appears to be taken up almost completely by tissues elsewhere, in one circulation. The acute changes in K^+ in patients under stress conditions must also be due to redistribution and when significant changes in the venous level occur this may indicate failure of compensation on that occasion rather than an abnormal K^+ shift (Treasure, 1977).

It was generally difficult for Treasure to produce significant changes in K^+ by altering ventilation. The H^+/K^+ exchanges seemed variable and unpredictable in both direction and magnitude. The association of hyperkalaemia with acidosis as is usually taught was not observed by Treasure (1977).

However, there is abundant evidence that prolonged exposure to metabolic and respiratory acidosis from any source including haemorrhagic shock is associated with a rise in plasma K^+. Recent experimental results on acid/base associations with plasma K^+ changes are in the main conflicting. Lim, Linton and Band (1982) observed an initial fall in plasma K^+ after the onset of hypercapnia. Orringer (1977) reported an acute reversible lactic acidosis without hyperkalaemia, while an early increase of plasma K^+ in hyperventilation was reported by Blesa, Gonzalez and Cingolani (1965), and Hickam, Nilson and Frayser (1956) observed an early elevation of serum potassium during respiratory alkalosis.

The present study was therefore primarily undertaken to investigate the transient changes in plasma K^+ continuously monitored with ion-selective electrode catheters during haemorrhagic hypotension with a view to throwing more light on these phenomena in order to try to establish the cause of the increase in plasma K^+. Electrocardiographic changes before, during and after haemorrhage up to the irreversible stage of shock, were also recorded to investigate any possible role of K^+ in the cause of irreversibility in haemorrhagic shock.

1.15 CONSTRUCTION AND EVALUATION OF POTASSIUM ION-SELECTIVE ELECTRODE CATHETERS (K-ISE)

1.15.1 METHODS AND MATERIALS

Preparation of Solutions

Weighings were made using a Gallenkamp electronic single pan balance. Solutions were made up in volumetric flasks and stored either in these flasks or in polythene bottles. The required stock solutions made were:

140 mmol/l Sodium Chloride (NaCl: 8.1816 g/l in distilled water)

100 mmol/l Potassium Chloride (KCl: 7.456 g/l in distilled water)

4 mmol/l Potassium Chloride (KCl: 0.29824 g/l in distilled water)

KCl standards were made up volumetrically from KCl 100 mmol/l stock solution in 140 mmol/l sodium chloride solution to provide 1, 2, 4, 6, 8, 10, 15 and 20 mmol/l KCl standards, using "A" grade pipettes and volumetric flasks. These were stored in the volumetric flasks.

1.15.2 THE CONSTRUCTION OF K-ISE's

All the K-ISE's used included Valinomycin in a polyvinyl chloride (PVC) matrix. The actual composition of the membrane is as shown in Table 1.1.

Ionophores

Valinomycin M.W. 1111 (Sigma Chemical Co.)

Potassium tetraphenylborate $K^{+}B(C_5H_5)_4^{-}$, M.W. 358 (prepared in this laboratory from sodium tetraphenylborate (Phase Separations Ltd.))

Other Membrane Constituents

Bis-2-Ethylhexyl Adipate (or dioethyl adipate DOA) (Phase Separations Ltd.)

Nitrobenzene (Analar Grade) (B.D.H. Chemicals Ltd.)

Tetrahydrofuran (Analar grade) (B.D.H. Chemicals Ltd.)

Polyvinyl Chloride (PVC) (High Molecular Weight, 200,000) (B.D.H. Chemicals Ltd.)

The potassium tetraphenylborate was prepared by a modification of the method described by Vogel (1951) for quantitative inorganic analysis.

(a) Sodium tetraphenylborate, 1.2 g was weighed and dissolved in 200 ml distilled, de-ionized water. After mixing for 20 minutes this solution was filtered and the filtrate saved.

(b) 80 ml of this sodium tetraphenylborate reagent was added to 100 ml of 100 mmol/l KCl, stirred and stood for 30 minutes. This produced a white floccular precipitate.

(c) This suspension was filtered through sintered glass.

(d) The precipitate thus prepared was rinsed with 100 mmol/l KCl solution and then with distilled de-ionized water.

(e) The precipitate of potassium tetraphenylborate was dried for one hour at 150°C. The potassium tetraphenylborate was stored in a desiccator.

High Selectivity and Conductivity of the K-ISE

Valinomycin is an electrically neutral compound, acquiring a charge of +1 when carrying a potassium ion, which may be the reason why thick films of oil that contain valinomycin do not conduct sufficiently well to operate as satisfactory electrodes. A combination of valinomycin and potassium tetraphenylborate, with valinomycin in molar excess, combines high selectivity

at the interface with conductivity in the bulk of the oil (Ebden, 1975). However, such membranes still rapidly become contaminated on contact with plasma unless the surface is renewed continuously.

A PVC membrane was found to be a considerable advance on the liquid membranes by Moody et al. (1970). Valinomycin is soluble in bis-2-ethylhexyl adipate, which acts as a plasticizer for PVC. The membrane used for the present study has in addition potassium tetraphenyl borate dissolved in nitrobenzene, which also acts as a plasticizer. This has the effect of lowering the electrical resistance and improving the anion rejection (Band & Kratochvil,1974; Morf et al., 1974).

1.15.3 PREPARATION OF THE K-ISE MEMBRANE

The valinomycin, potassium tetraphenylborate and PVC were weighed into the same glass specimen vial; the nitrobenzene, dioctyl adipate and finally the tetrahydrofuran were added. The mixture was stirred with a magnetic stirrer for 2 hr before it was covered loosely and left to evaporate slowly over 24 hr to allow the polymer to form.

Table 1.1: Composition of the PVC Potassium-Ion Selective Membrane

Constituent	Amount
Potassium Tetraphenylborate	0.000050 g
Valinomycin	0.0030 g
Bis-2-ethylhexyl adipate (dioctyl adipate)	0.30 g
Nitrobenzene	0.10 g
High Molecular Weight PVC	0.150 g
Tetrahydrofuran	6 ml

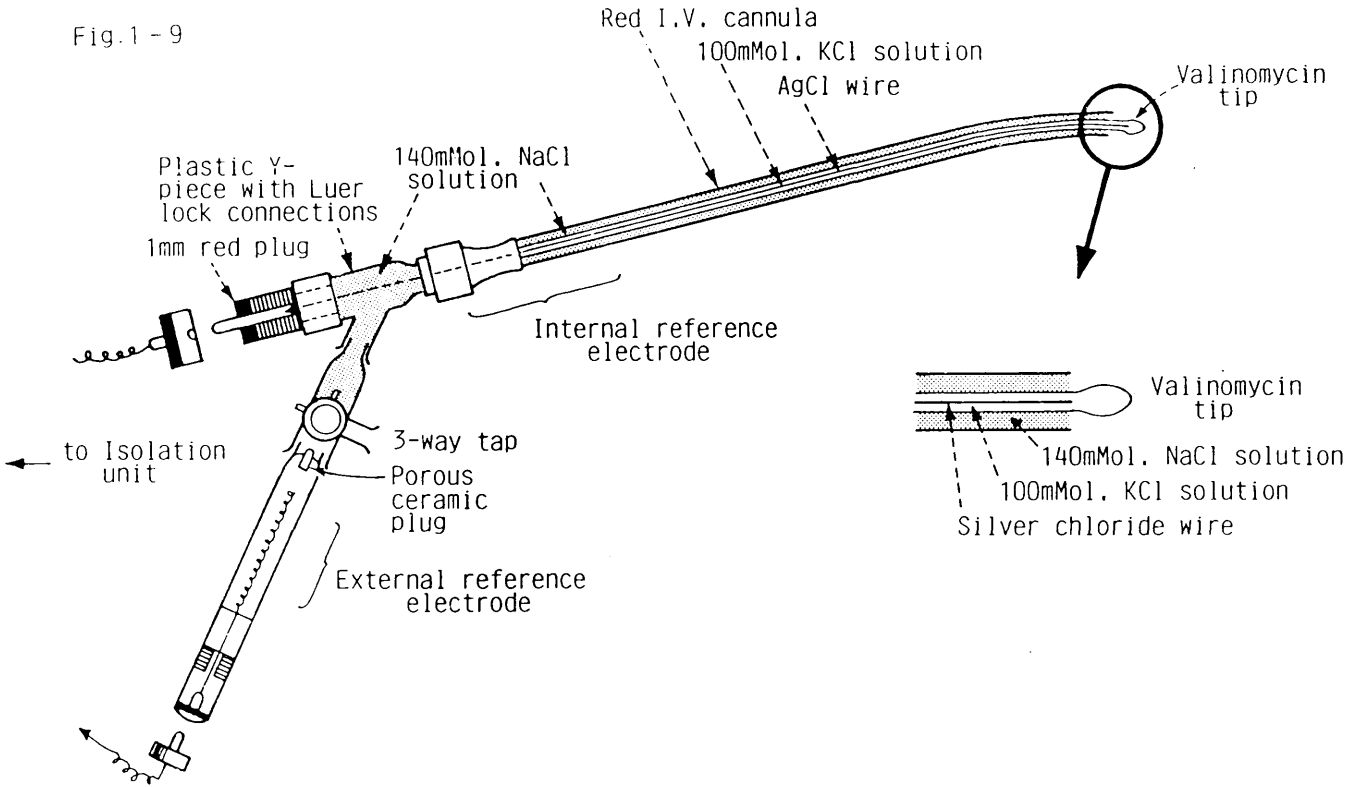
1.15.4 REFERENCE ELECTRODES

Each K^+-selective electrode catheter consisted of an internal reference, and an external reference component made up of silver/silver chloride electrodes (See Fig. 1.9).

The internal reference electrode comprises a length of PVC tubing (35 cm long with an external diameter of 0.9 mm), which is plugged at its distal end with a porous ceramic plug. The PVC membrane prepared as above, incorporating valinomycin, was dip-cast on the plugged end by dipping it in the membrane redissolved in a minimum of tetrahydrofuran, and allowing it to dry.

A triamel-coated silver wire (35 cm long) was stripped of its coating for 2 cm lengths at both ends. The distal end was chloridized using 1.5V and a current density of 10 mA/cm^2 for 20 min. The PVC tubing was filled with 100 mmol/l KCl solution and the Ag/AgCl wire threaded down the inside of the tubing. The wire was soldered at its proximal end to a plug as shown (Fig. 1.9) and the proximal end of the tubing was sealed with silicone rubber and fixed to the plug. The

Fig.1 - 9



The Potassium-selective electrode catheter incorporating the internal and external reference electrodes.

internal reference electrode was then threaded through a Vygon Y-piece and down the inside of a polythene cannula (Portex) of 1.6 mm external diameter. The space between the inner PVC tubing and the cannula was filled with 140 mmol/l NaCl solution. The NaCl served as a salt bridge connecting the blood to the external reference electrode and so completing the electrical circuit between the two reference electrodes.

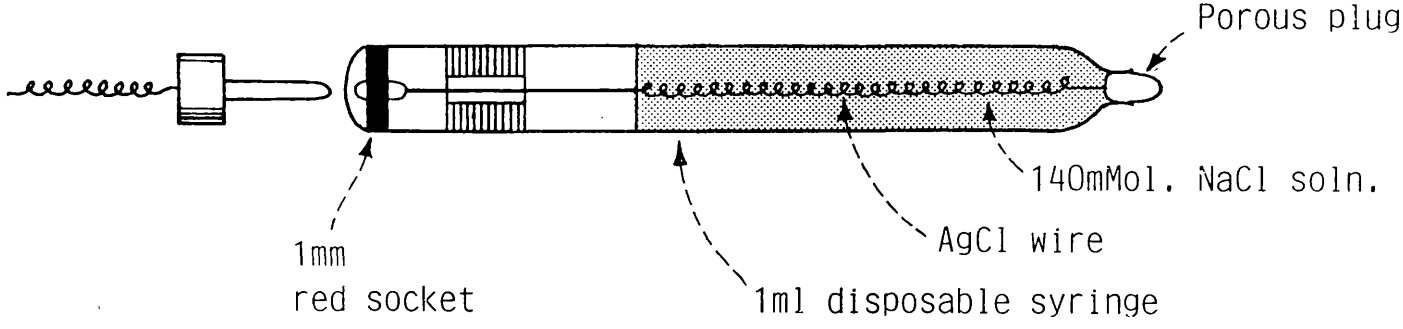
The external reference electrode had its proximal 2 cm stripped of its triamel coating and was chlorided as above and then bathed in 140 mmol/l NaCl solution inside a modified piece of 1 ml syringe tubing. The proximal end of the wire was then soldered to a socket as shown in Figure 1.10 and the proximal end of the syringe tubing sealed with silicone rubber and fixed to the socket. The distal end of the syringe contained a porous ceramic plug.

Before and after each experiment, all electrode catheters were rinsed through with 140 mmol/l NaCl solution via a 3-way tap to ensure that there were no air bubbles between the internal reference electrode and the cannula. The Nernstian equation as applied to these K^+-selective electrodes in the present study is $E = 61.4 \log_{10} \frac{K^+}{100}$ plasma at 37°C, where 100 is the concentration of KCl in the internal reference electrode tubing.

1.15.5 CALIBRATION OF ELECTRODES

Each of the K^+-selective electrode catheters was calibrated in standard 1,2,4,6,8,10 and 15 mmol/l KCl in 140 mmol/l NaCl solution at 37°C. The calibrations were done before and after each experiment. Calibration readings were recorded on the

Fig.1 - 10 The external reference electrode



polygraph trace (see Fig. 1.11) and from these a plot of log K^+ concentration against polygraph readings (heights in mm) on the trace was drawn for each electrode. All K^+ values recorded in the experiment were measured from these standard calibration line-graphs. A typical example of a calibration curve for one electrode is shown in Figure 1.12 and an example of the calibration records from the polygraph trace is as shown in Figure 1.11.

1.15.6 EVALUATION OF ELECTRODES

Before calibrating the electrodes for each experiment, a high-input impedance electrometer, the Vibron electrometer 33B (Electronic Instrument Ltd.) was used in assessing two factors:

(1) the voltage response across the K^+-selective membrane in varying standards of KCl solution increasing in magnitude from 1 to 20 mmol/l in 140 mmol/l NaCl.

(2) the linearity of the voltage response compared with the logarithm of the concentration.

The internal reference electrode was connected by screened cable to the high impedance input of the Vibron. The temperature of the standards was maintained at 37°C in a water bath.

Voltage Response in Standards

The voltage recorded by the electrometer for each electrode per decade difference in concentration of the standards (for example, between 1 and 10 mmol/l, and between 2 and 20 mmol/l KCl in 140 mmol/l NaCl) was compared with the theoretical Nernst response:

Percentage Nernstian response

$$= \frac{\text{measured response}}{\text{theoretical response}} \times 100$$

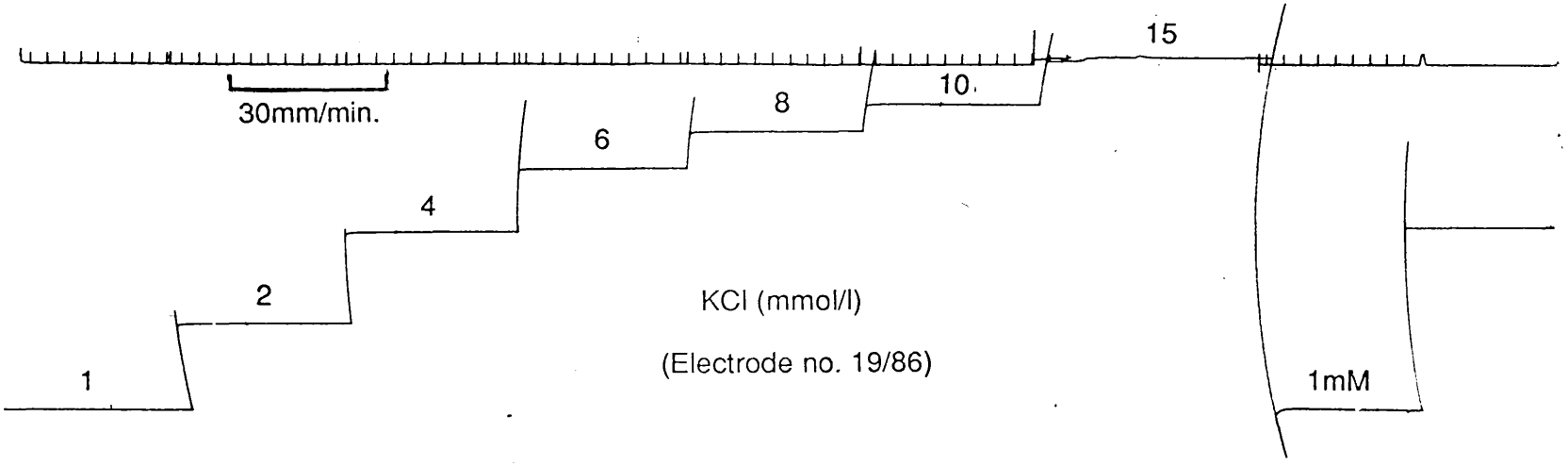


Fig.1-11. A typical calibration of the valinomycin-based potassium-selective electrode catheter (number 19/86) in standard KCl solutions made up in 140 mmol/l NaCl. Range of the standards: 1 to 15 mmol/l.

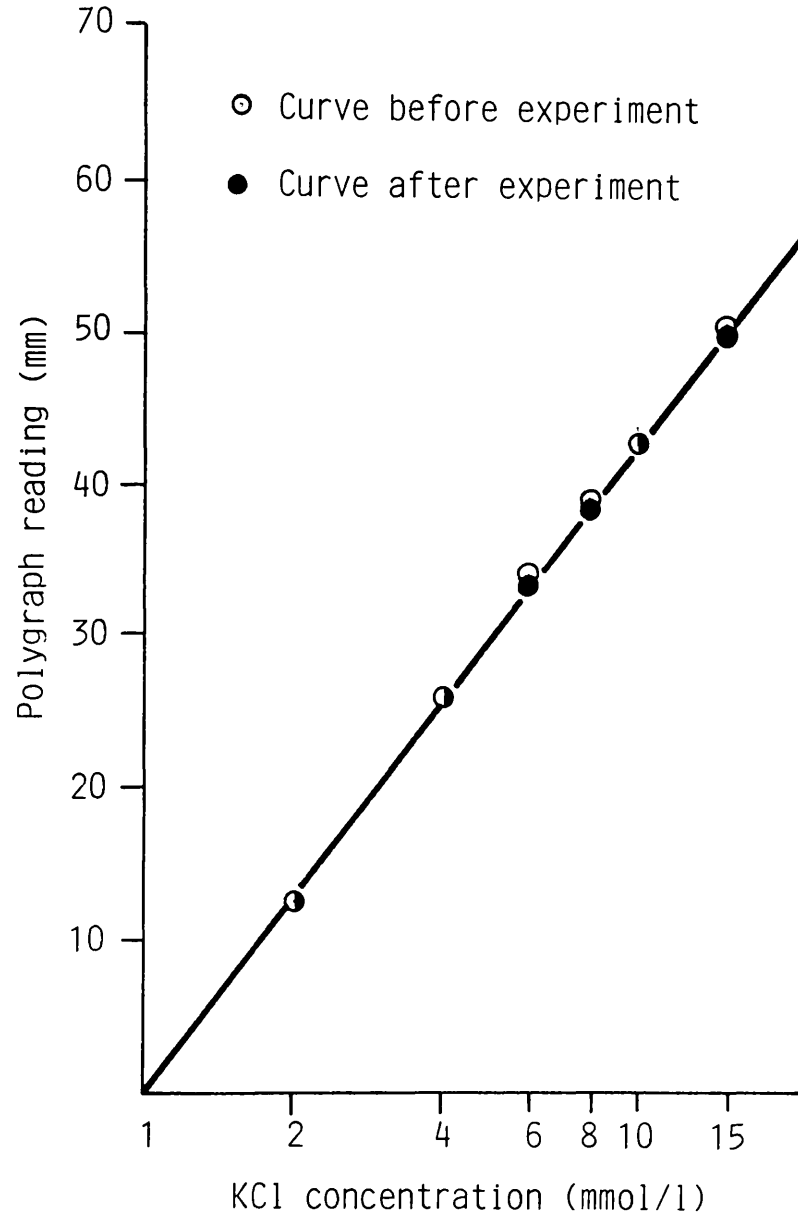


Fig. 1-12:
A graph of polygraph readings against standard KCl solutions in 140mmol/l NaCl for a typical K^+-selective electrode catheter.

All the K^+-selective electrodes were checked in this way and their performance recorded. Before calibration for every experiment, testing of this type was performed for the electrodes in use to assess their continuing accuracy.

Linearity of the Response

The voltages recorded in (i) above were plotted against the log of K^+ concentration in the standards. In a highly selective K^+ electrode of the type required for biological use such as the present study, this technique of assessment should produce a straight line down to 1 mmol/l KCl in NaCl where the ratio is 1:140 K^+:Na^+.

1.15.7 RESULTS AND DISCUSSION

Standards

The standards were used routinely to test the electrodes and to calibrate them. A typical example of response curve in per cent of the standard Nernstian response exhibited by one of the K^+-selective electrodes is shown in Figure 1.13. Table 1.2 also shows the slope constants in millivolts per decade change in the concentration of the standards for 12 electrodes used in the present study.

These results show a linear relationship between the potential measured and the logarithm of the K^+ concentration. The standards with a NaCl solution background have the advantage of bringing all the solutions to a similar ionic strength so that the activity coefficient is effectively identical (0.74) (Bates and Alfenaur, 1969). They approximate the ionic strength of plasma and check on the development

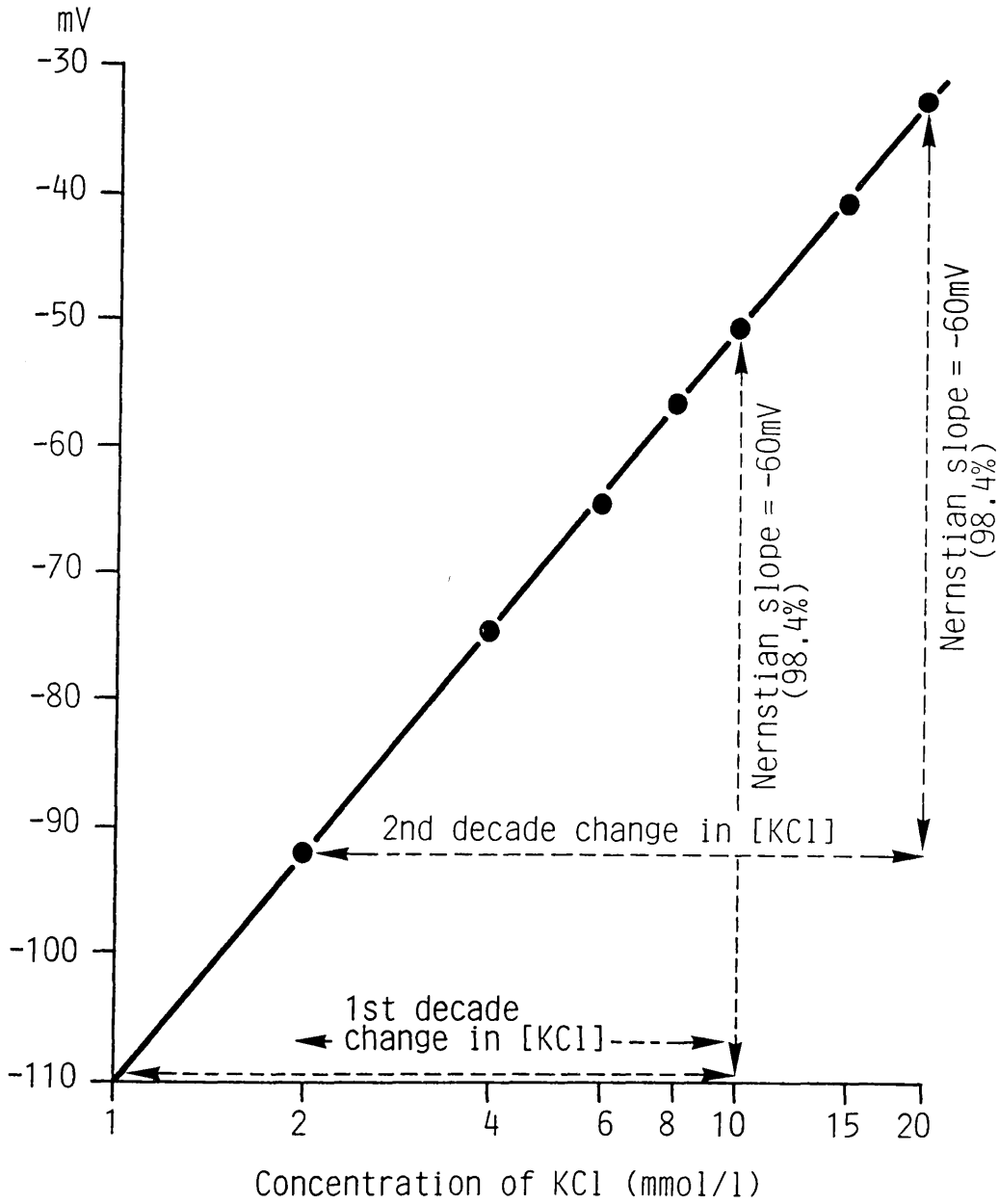


Fig. 1-15:
A Typical Straight Line Graph of Voltage against Standard KCl Solutions in 140mmol/l NaCl for the Evaluation of a K^+-selective Electrode Catheter.

of cross-sensitivity with other ions in the plasma. They also minimize the residual liquid junction potential.

NERNST RESPONSE

Most of the freshly prepared K^+-selective electrodes showed more than 90% of the theoretical Nernst response to K^+ in the presence of 140 mmol/l NaCl. Such responses were still found to be present in some electrodes after their wet storage in 100 mmol/l KCl for about 6 months in this laboratory.

Electrode Number	Slope Constant (mV/decade change)	% Nernst Response
8/85	58	95.1
9/85	59	96.7
15/85	59.5	97.5
1/86	59.5	97.5
2/86	60	98.4
4/86	59	96.7
7/86	60	98.4
17/86	60	98.4
18/86	60	98.4
19/86	59	97.5
10/87	60	98.4
12/87	60	98.4

Table 1.2: % Nernstian response for twelve potassium selective electrodes of same composition.
(Dipped Electrodes of composition
Valinomycin: KTPB = 30.1)

The slope constant ($2.3 \frac{RT}{F}$) at $37^{o}C$ is 61.4 mV/decade change.

1.15.8 SUMMARY

K^+ -selective electrode catheters have been constructed using PVC membranes incorporating valinomycin, a K^+ ionophore. They produced stable and reproducible Nernstian responses, and are therefore suitable for biological measurements *in vivo* because of their high selectivity for K^+ over Na^+. A means of testing or checking them has been evaluated.

1.16 HAEMORRHAGE EXPERIMENTS

MATERIALS AND METHOD

Cats, male and female weighing 2.35 to 4.56 kg were deeply anaesthetized with pentobarbitone sodium (Sagatal) (May and Baker Ltd.), 45 mg/kg intraperitoneally, and anaesthesia maintained with top-up doses intravenously as necessary. A tracheal cannula with a side-arm was inserted. The side arm was connected by a narrow bore tube to a carbon dioxide analyser 901-MK_2 (P.K. Morgan Ltd.) for continuously sampling inspired and expired gas and recording CO_2%. End tidal CO_2% (the CO_2% in percentage at the end of each expiration) was thus continuously monitored as well as the respiratory frequency. This was to obtain a continuous indication of alveolar and hence of arterial PCO_2 and its changes from which the time course of arterial pH changes could be related to those in arterial K^+ concentration. The right common carotid artery was cannulated and the cannula was connected to a blood pressure transducer EM 751 (Elcomatic Ltd.), for monitoring arterial blood pressure. A catheter was inserted into the right external jugular vein for the infusion of intravenous fluids and drugs. The left femoral artery and vein were exposed and prepared for the insertion of the aortic and high inferior vena cava (HIVC) electrode catheters. These electrode catheters were marked in 5 cm. divisions along their length prior to insertion to

determine how much of the length is being advanced intravascularly to enable the tip to be positioned at the desired site.

The HIVC electrode tip was advanced until it was about 0.5 cm above the diaphragm in the thorax. The tip was thus positioned in the inferior vena cava (IVC) above the point where the hepatic vein joins the IVC so that if the K^+ content of the hepatic venous blood changes, the HIVC K^+ content will also change and this change will be recorded by the HIVC electrode. The aorta electrode catheter was placed in the abdominal aorta. These positions were confirmed at the end of the experiment by post mortem examination. Animals were maintained at 37 $\pm$ 0.5°C body temperature by placing them on a blanket thermostatically controlled (Homoeothermic blanket control and thermometer CFP 8185, Palmer Bioscience). The right femoral artery and vein were also cannulated to allow the removal of blood samples for pH and gas analyses (see Fig 1-13(a) for set-up). The venous sample was necessary to relate acid-base changes to plasma K^+ changes in the HIVC which is of great interest in the present study. In some experiments in which the comparative effects of infusions of shed blood, normal saline and dextran 110 were investigated, needle electrodes were inserted subcutaneously in the right fore limb and the left hindlimb, and Lead II electrocardiographic (ECG) recordings were taken. Heparin 500 i.u./kg was given intravenously (i.v.) as an anticoagulant and the animal was allowed to stabilize for 60 minutes before control values were taken.

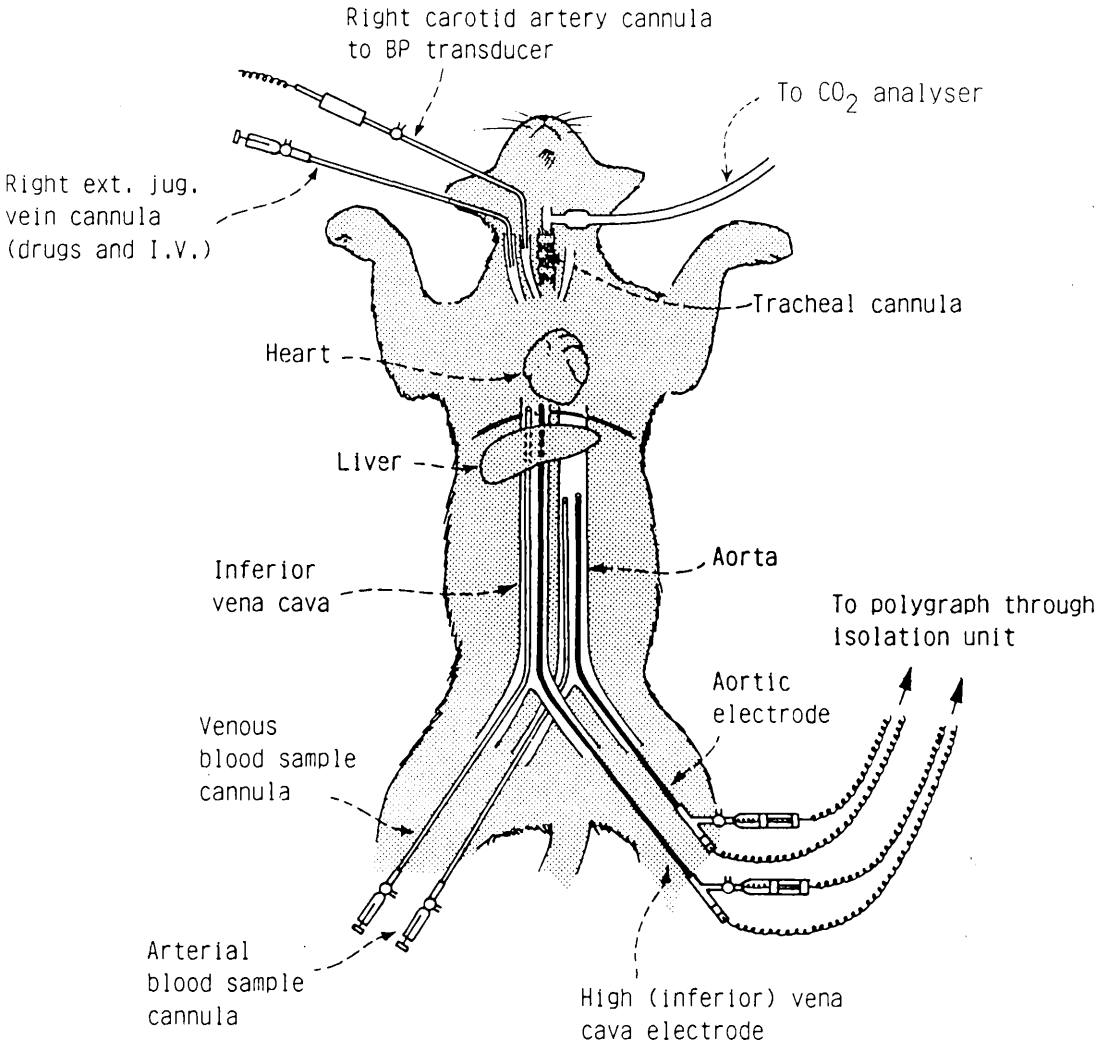


Fig.1-13(a).

Set-up for the continuous monitoring of the HIVC and the aortic plasma K^+ in the cat.

The outputs from the electrode catheters were taken to an electrometer in which the input was isolated with a photo-electric isolation unit and the output from the isolation unit was then recorded with a Grass Polygraph, Model 7D. The isolation unit was incorporated in the recording apparatus so that it could be adopted for use in humans. It ensures that no current can be passed from the electrical equipment to the source and hence animal's safety during measurements is assured.

At the end of each experiment, the electrode catheters were recalibrated in mixed standards of KCl/NaCl solutions as described earlier in the evaluation of K^+-ISE's.

1.16.1 PROCEDURES

Injections of Adrenaline and Noradrenaline: Intravenous injections of both adrenaline and noradrenaline, 2 µg/kg, were administered and each response was observed for at least 5 minutes. In some experiments, about 20 to 25 minutes were allowed between injections for the plasma K^+ levels to return to control values from below the control.

1.16.2 WITHDRAWAL OF BLOOD

Percentages of the total blood volume (estimated at 72 ml/kg body weight, MacKenna, 1984, personal communication) were drawn into 50 ml syringes and stored in a water bath thermostated at 37°C before reinfusion. A range of between 5 and 35% of total blood volume was withdrawn arterially and the responses of the blood pressure and plasma K^+ were recorded during, and following each episode of haemorrhage, over a 5 min. period of observation.

1.16.3 35% HAEMORRHAGE AND BLOOD PH/GAS ANALYSIS

A single withdrawal of 35% of total blood volume was chosen to induce significant changes in mean arterial pressure and both aortic and HIVC plasma K^+, as observed from preliminary results with different haemorrhages. The withdrawn blood was stored in a thermostatically controlled water bath at 37°C as above and reinfused after recording the responses over 5 minutes following each withdrawal. Arterial and venous samples of blood for pH/gas analysis were removed at the first peak of potassium rise in the aorta and HIVC, respectively.

1.16.4 INDUCTION OF HAEMORRHAGIC SHOCK AT MABP OF 40 mm Hg for 120 MINUTES

Haemorrhagic shock was induced by withdrawing arterial blood until the MABP fell to 40 mm Hg. Small quantities of blood were subsequently reinfused or withdrawn as appropriate to maintain the MABP at 40 mm Hg. When the initial sudden withdrawal of blood was completed (we call this time zero) the first samples of arterial and venous blood were taken for pH/gas analysis. Further samples were withdrawn at intervals of 30 min. for 120 mins. Each sample withdrawn measured 0.6 - 0.8 ml and hence the total volume of blood withdrawn for gas analysis at the end of each experiment was less than 10 ml (less than 5% of total blood volume). Inspired and expired CO_2 levels were recorded simultaneously, as well as a lead II ECG.

1.16.5 MAINTENANCE OF HYPOTENSION AT 80 mm Hg for 180 MINUTES

This procedure was similar to the previous procedure except that the MABP was maintained at 80 mm Hg. This allowed a comparison of the associated changes in acid-base with alterations in plasma K^+ in different sustained hypotensive states. The level of 80 mm Hg was chosen because the baroreceptors taking part in the maintenance of MABP are said to be no longer effectively responsive ("unloaded") at 70 mm Hg although effective above 70 mm Hg (Guyton et al., 1961).

1.16.6 COMPARISON OF THE EFFECTS ON PLASMA K^+ AND MABP OF REINFUSION OF SHED BLOOD, NORMAL SALINE AND DEXTRAN "110", AFTER HAEMORRHAGIC SHOCK

The procedure was similar to that of maintaining the MABP at 40 mm Hg. In this series of experiments, instead of the reinfusion of shed blood, normal saline or Dextran 110 (a plasma expander) was infused to restore the blood volume in three separate sets of experiments using four cats each. Blood samples for pH/gas analysis were withdrawn before haemorrhage, after haemorrhage, and after infusion with the blood substitutes.

1.16.7 HYPERVENTILATION STUDIES

The constant and repeated observations that hyperventilation preceded the rise in the levels of plasma K^+ as a result of haemorrhage prompted this investigation. Hyperventilation was induced by mechanically ventilating the animal with 125 ml of room air per breath at the rate of 34 breaths/minute for 5 minutes. This volume and rate of

hyperventilation were found to produce percentages of end-tidal carbon dioxide similar to that obtained by withdrawing 30 to 35% total blood volume in the cats.

Blood samples for pH/gas analysis were withdrawn between the 4th and 5th minutes of artificial hyperventilation. End-tidal CO_2 and MABP were continuously monitored along with aortic and HIVC plasma K^+. Only HIVC K^+ changes are reported here as the aortic and lower I.V.C. K^+ levels showed no significant changes.

Similar studies were made after pre-treatment of the animals with 0.2 mg/kg iv of either propranolol, prazosin or naloxone to investigate whether or not catecholamines and/or opioids are released during hyperventilation. Propranolol and prazosin are β-adrenergic and α_1-adrenergic blockers respectively, while naloxone is a specific opioid receptor blocker. The receptor agonists, catecholamines and opioids have been reported to produce transient rises and uptake of plasma K^+, and the plasma levels of catecholamines and opioids have been reported to increase during mechanical hyperventilation or haemorrhage. It is therefore expected that propranolol, prazosin or naloxone should block the release of any K^+ during mechanical hyperventilation.

1.16.8 DRUGS AND INSTRUMENTS

DRUGS

The drugs and equipment used in the present study are given below with the manufacturers' names in parenthesis.

Drugs: Naloxone hydrochloride (Dupont (UK) Ltd.) Prazosin hydrochloride (Pfizer Ltd.), DL-Propranolol hydrochloride (Sigma Chemical Ltd.), Phentolamine mesylate (Rogitine) (Ciba Laboratories), Adrenaline (epinephrine bitartrate) and Nor-Adrenaline (norepinephrine bitartrate) (Sigma Chemical Co.), Morphine hydrochloride (MacFarlan Smith Ltd.), Valinomycin (Sigma Chemical Co.), Sodium tetraphenyl borate, and Bis-2-ethylhexyladipate (dioctyl adipate) (Phase Separations Ltd.), Nitrobenzene (Analar grade), Tetrahydrofuran (Analar grade), and Polyvinyl chloride (high molecular weight 200,000) all from (B.D.H. Chemicals Ltd.), Heparin sodium (5,000 units per ml) (Evans Medical Ltd.), Pentobarbitone Sodium (Sagatal, 60 mg per ml) (May and Baker Ltd.), and Dextran 110 (Fisons).

Instruments:

Polygraph - Model 7 (Grass Instruments), CO_2 Analyser (Type 901-MK2) (P.K. Morgan Ltd.), Blood Gas Analyser (Model 1302) (Instrumentation Laboratory (U.K.) Ltd.), Blood Pressure Transducer (Elcomatic, Type EM 751) (Elcomatic Ltd.), Isolation Units (Physiology Department, Glasgow University), Thermostatic Water Bath (Grant Instruments), Continuous Injector Apparatus (C.F. Palmer), Respiration Pump (C.F. Palmer), Single Pan Electronic Weighing Balance (Gallenkamp), Vibron Electrometer (Electronic Instruments Ltd.), Homoeot' mic Blanket Control (CFP 8185) and Thermometer (Palmer Bioscience).

1.16.9 STATISTICS

Mean values were derived from the data obtained from the number of animals used as indicated for each section of the study. Both standard deviations and standard errors were derived from the sample means and results expressed as means ± standard errors of the mean. Student paired t-tests were applied between control and experimental values. The table of t was referred to for test of significance, and P values of 0.05 and less were considered significant.

1.17 RESULTS

1.17.1 EFFECTS OF INJECTION OF ADRENALINE AND NORADRENALINE

The effects of injections of 2 µg/kg body weight of adrenaline and noradrenaline intravenously are shown in Figures 1-14 (a-c). Adrenaline increased the aortic plasma potassium from a control level of 2.92 ± 0.2 mmol/l to a value of 5.31 ± 0.15 mmol/l within 35 secs. The value then gradually fell to 2.90 ± 0.4 mmol/l, which is below control value, within 3 min. Mean arterial blood pressure (MABP) increased from 147 ± 6.0 to 170 ± 6.0 mm Hg and then fell to control level. Noradrenaline produced similar responses causing an aortic potassium rise from 2.90 ± 0.13 to 5.91 ± 0.64 mmol/l.

The increases in the high inferior vena cava (HIVC) plasma K^+ levels were greater than in the aortic levels. Adrenaline produced a rise to a peak of 7.73 ± 0.56 mmol/l from 3.00 ± 0.14 mmol/l whereas noradrenaline produced a rise to a peak of 5.54 ± 0.18 mmol/l from 3.02 ± 0.09 mmol/l. The rise to the peak levels of potassium occurred within a shorter period of 24 seconds, in the HIVC than in the aorta, 35 seconds.

Fig.1-14a. Histograms showing the effects of adrenaline (2ug/kg) i.v. on mean arterial blood pressure.

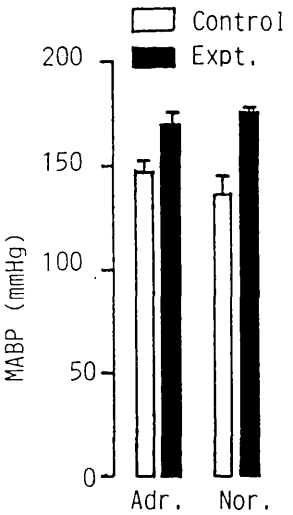


Fig.1-14b.

Fig.1-14c.

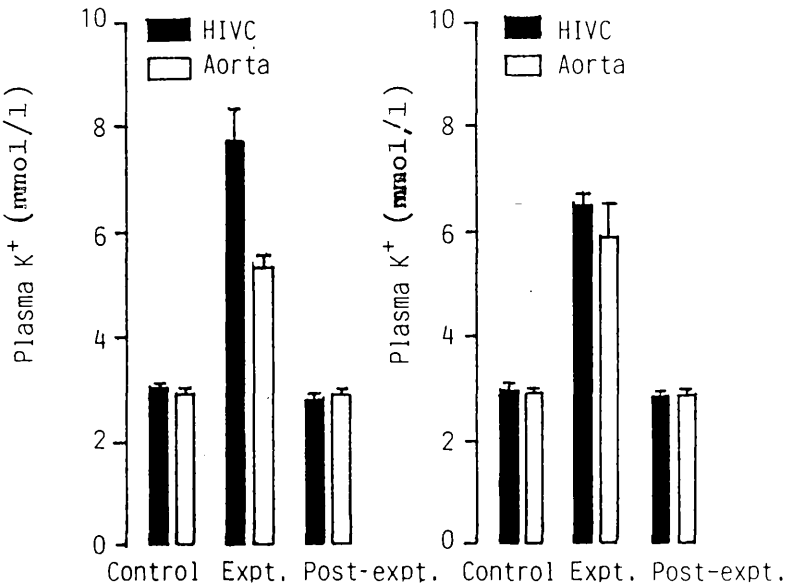


Fig.1-14 (b & c): Effects of (b) adrenaline and (c) noradrenaline on Aorta and HIVC plasma K+. Means±SEM. n=8.

Potassium levels returned to control levels from below baseline within 25 ± 5.0 minutes.

1.17.2 EFFECTS OF WITHDRAWAL OF VARIOUS PERCENTAGES OF THE TOTAL BLOOD VOLUME ON PLASMA K^+ AND MABP

Figures 1.15 (a & b) show the effects of withdrawing various percentages of total blood volume (5-35%) on MABP and plasma K^+.

Withdrawal of between 5% and 10% of blood showed no significant effects on the MABP, or plasma K^+.

Significant changes ($p < 0.05$) began to appear at 15% haemorrhage in both MABP and HIVC plasma K^+ but not in the aortic plasma K^+. 20% to 25% haemorrhage caused significant changes in aortic ($p < 0.02$) and in HIVC ($p < 0.01$) K^+ levels accompanied by a significant fall in MABP ($p < 0.02$). Changes due to 30 to 35% haemorrhage reduced the MABP to zero before recovering to 19 ± 3.0 mm Hg and to 7 ± 3.0 mm Hg, respectively. Aortic and HIVC potassium levels rose to 5.65 ± 0.23 mmol/l and 7.94 ± 1.50 mmol/l respectively. In some cats, it was found that if reinfusion of the shed blood was not instituted within 5 minutes, a second peak in the levels of potassium in both the aorta and the HIVC ensued to 9.10 ± 2.0 mmol/l and 14.8 ± 1.5 mmol/l respectively, at which stage all resuscitatory manoeuvres did not revive the animals.

T[illegible]e were thus repeatedly observed two peaks of plasma K^+ that formed plateaux in each of the blood vessels whose plasma K^+ was recorded. The time taken for the first plateau to form in each vessel depended on the severity of the

Fig 1-15a:

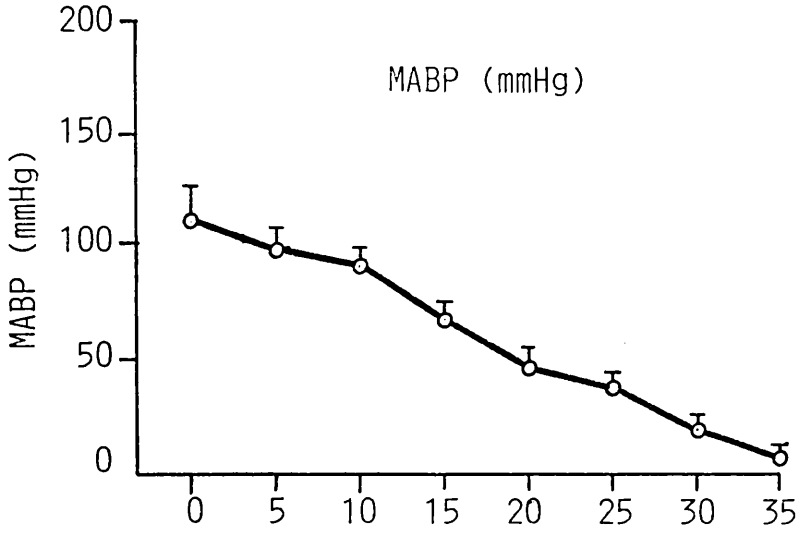
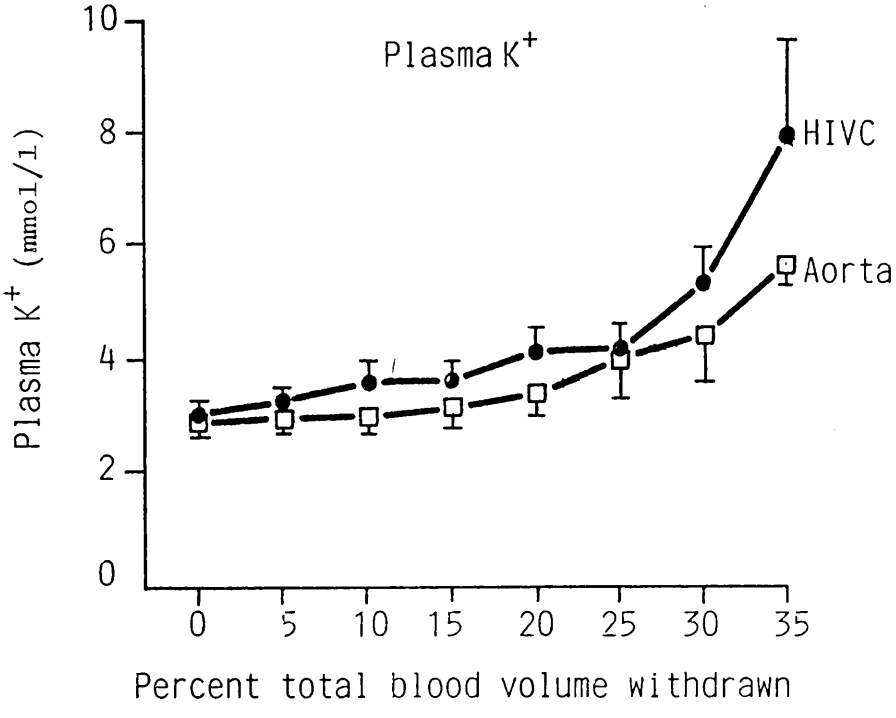


Fig 1-15b:



Figs 1-15: (a & b) Effects of Withdrawal of Various Percentages of Blood Volume on (a) Mean Arterial Blood Pressure (MABP), and (b) Plasma K^+. Means $\pm$ SEM. n=8. HIVC=High Inferior Vena Cava.

haemorrhage and the speed of blood withdrawal, while the time for the appearance of the second plateau depended more on the severity of haemorrhage than the speed of blood withdrawal. For example, withdrawal of 25% of total blood volume within 2 min. produced the first peak of HIVC plasma K^+ within 5 min, and the second peak after the 60th min. from time zero, whereas withdrawal of 30% or 35% within 2 min. produced the first peak within 1.5 min, and the second peak, within 10 min. from time zero.

1.17.3 EFFECTS OF MECHANICAL HYPERVENTILATION WITH A TIDAL VOLUME OF 125 ml AT 34 BREATHS/MINUTE FOR 5 MINUTES BEFORE AND AFTER DRUG PRETREATMENT

The results are shown in Figures 1.16 (a-g). There was a marked fall in end-tidal CO_2 percent, from 5.73 $\pm$ 0.25% to 0.50 $\pm$ 0.18% ($p < 0.001$) and a significant increase in arterial pH from 7.36 $\pm$ 0.02 to 7.75 $\pm$ 0.04 ($p < 0.02$). Changes in MABP and HIVC plasma K^+ also occurred. MABP fell from 146 $\pm$ 6.6 to 50.8 $\pm$ 2.80 mm Hg ($p < 0.01$). There was a rise in HIVC K^+ from 3.04 $\pm$ 0.06 to 5.42 $\pm$ 0.48 mm/l (ΔK^+ = 2.53 $\pm$ 0.39 mmol/l). Pretreatment with 0.2 mg/kg i.v. of either prazosin, naloxone or propranolol before hyperventilation produced similar effects on MABP but different effect on plasma K^+. The effects on blood pH and end-tidal CO_2 were also similar (see Fig. 1.16a). Hyperventilation after prazosin injection lowered the MABP from 146 $\pm$ 6.6 to 37.5 $\pm$ 4.98 mm Hg with an accompanying fall in end-tidal CO_2 from 5.73 $\pm$ 0.25 to 0.48 $\pm$ 0.19%. The pH rose from 7.36 $\pm$ 0.02 to 7.64 $\pm$ 0.02, while the HIVC K^+ increased to 5.13 $\pm$ 0.25 mmol/l from 3.04 $\pm$ 0.06 mmol/l. Prazosin

Fig. 1-16a:

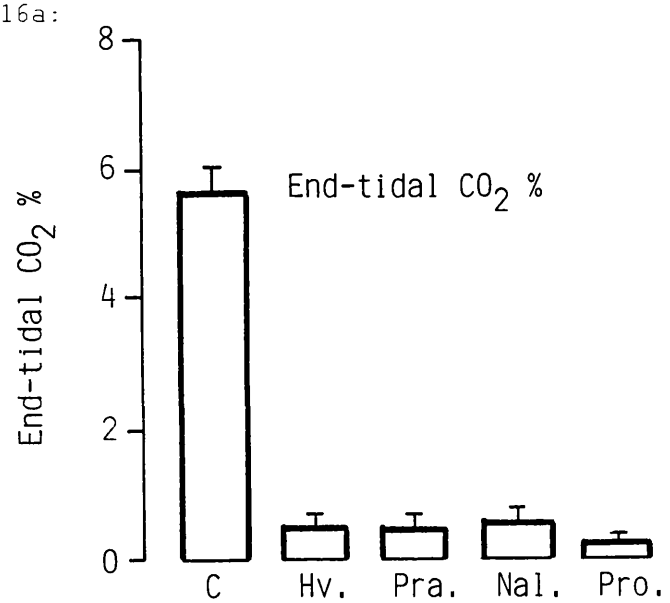
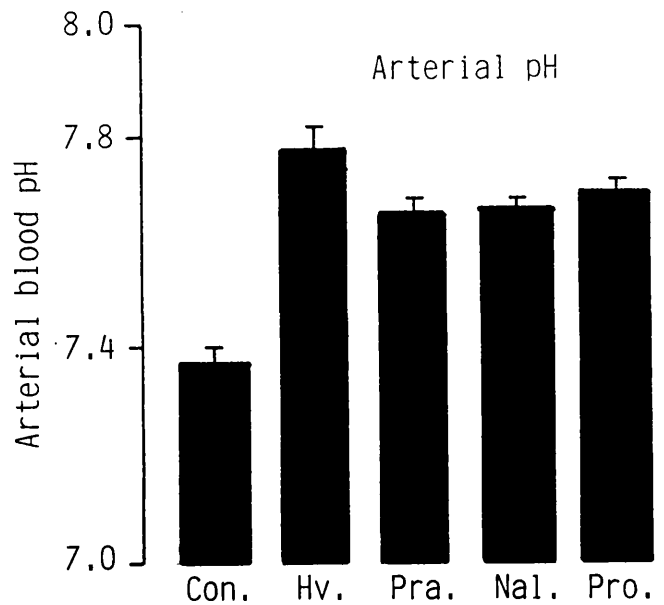


Fig. 1-16b:



Figs. 1-16 (a & b) Effects of Hyperventilation on (a) End-tidal CO_2 and (b) Arterial Blood pH in the presence of Adrenoceptor and Opioid Receptor Blockers. Means ± SEM. n=6.

Con.(control), Hv(Hyperventilation)
Pra(Hv. in the presence of Prazosin)
Nal(Hv. in the presence of Naloxone)
Pro.(Hv. in the presence of Propranolol)

Fig. 1-16c

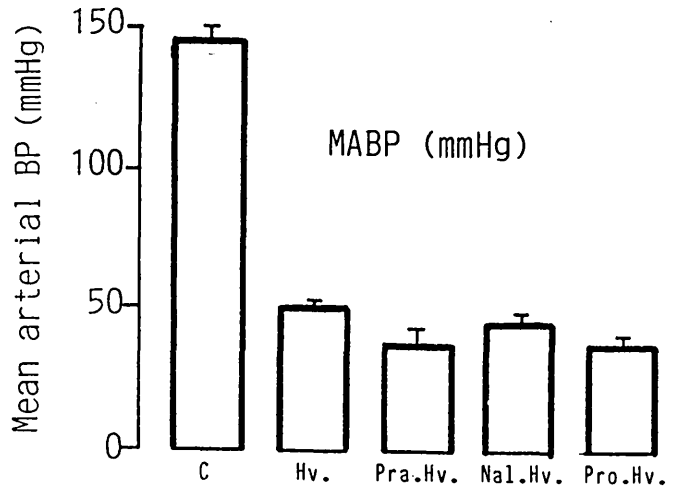
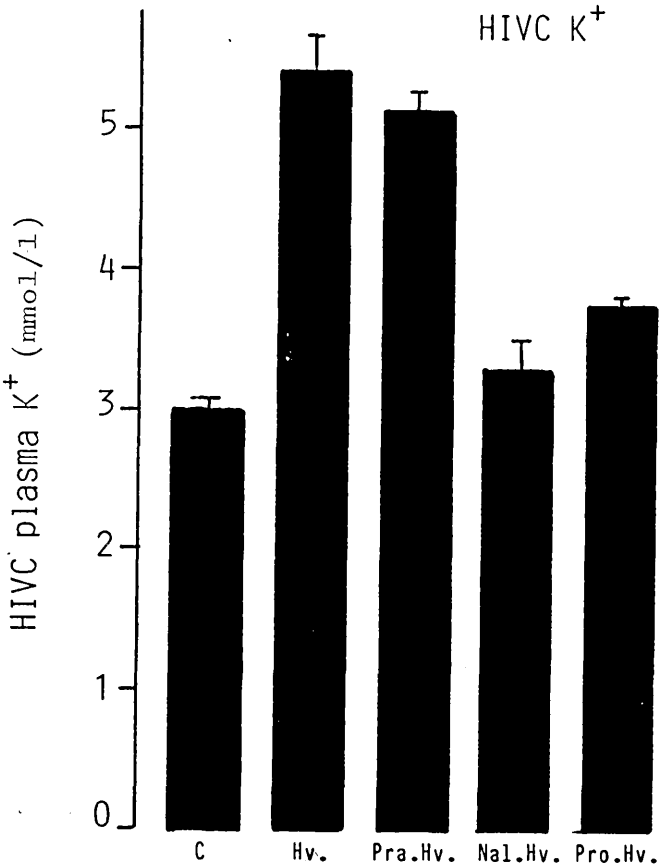


Fig. 1-16d



Figs.1-16(c&d):

Histograms showing the effects of hyperventilation (Hv.) on (c) MABP and (d) HIVC plasma K^+ before (C) and after pretreatment with 0.2mg/kg either of Prazosin (Pra-Hv), Naloxone (Nal-Hv), or Propranolol (Pro-Hv). Only the maximum responses within 5 mins are shown. Means$\pm$SEM. n=6.

Note the fall in MABP in Fig. 1.16c due to pooling effect in the thoracic cavity thus reducing cardiac output following the mechanical hyperventilation.

did not significantly alter the change in plasma K^+ produced by mechanical hyperventilation: before prazosin injection, $\Delta K^+ = 2.53 \pm 0.39$ mmol/l and after prazosin, $\Delta K^+ = 2.24 \pm 0.14$ mmol/l. (ΔK^+ is the difference between control plasma K^+ and that after hyperventilation). After pretreatment with naloxone, or propranolol, changes in HIVC K^+ were significant following artificial hyperventilation, with no significant differences from control plasma K^+ levels.

In the presence of naloxone, hyperventilation lowered the MABP to 46.2 ± 3.26 mm Hg from a control of 146 ± 6.6 mm Hg, and the end-tidal CO_2 to $0.58 \pm 0.12\%$ from the control of $5.73 \pm 0.25\%$. The pH rose to 7.65 ± 0.01 and plasma K^+ rose only to 3.26 ± 0.38 ($\Delta K^+ = 0.77 \pm 0.34$) mmol/l, from 3.04 ± 0.06 mmol/l. In the presence of propranolol, hyperventilation lowered MABP to 38.7 ± 2.56 mm Hg and decreased end-tidal CO_2 to 0.2 + 0.02% from their respective controls, while pH rose to 7.67 ± 0.02 and plasma K^+ rose only to 3.75 ± 0.08 ($\Delta K^+ = 1.35 \pm 0.24$) mmol/l from controls. Surprisingly, after a second dose of naloxone, artificial hyperventilation raised the HIVC plasma K^+ to 7.25 ± 0.44 mmol/l from 3.04 ± 0.06 mmol/l. Second doses of prazosin or propranolol did not cause such a change. The effects of artificial hyperventilation over a period of 5 min. and the time course of the changes in CO_2, MABP, and HIVC K^+ are shown in Figures 1.16 (e,f & g).

Fig. 1-16e:

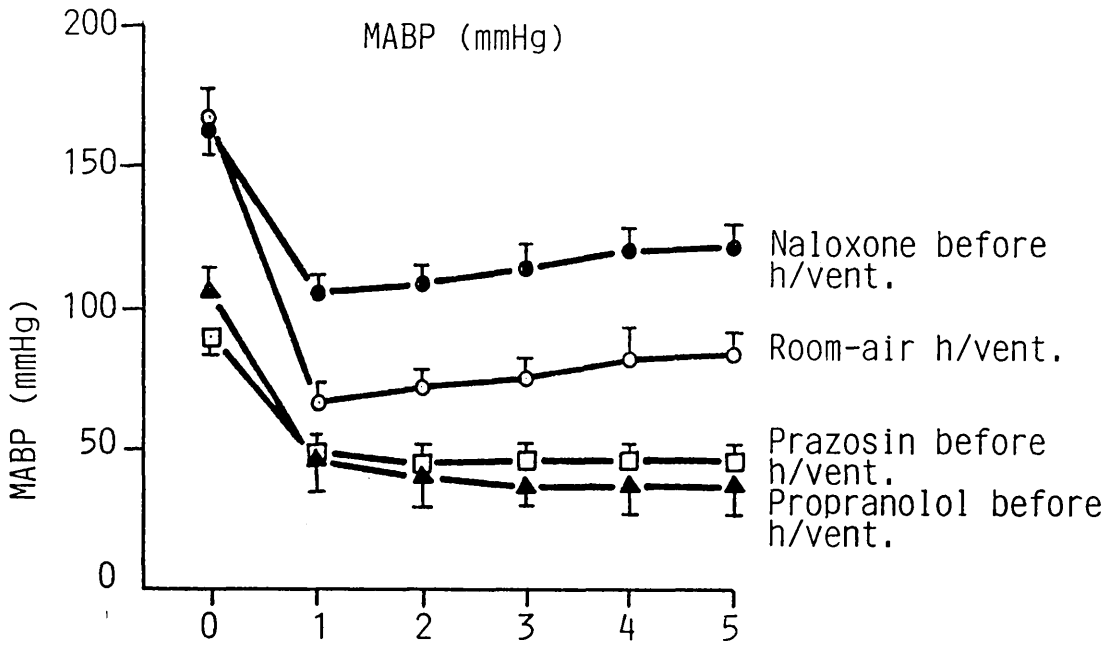
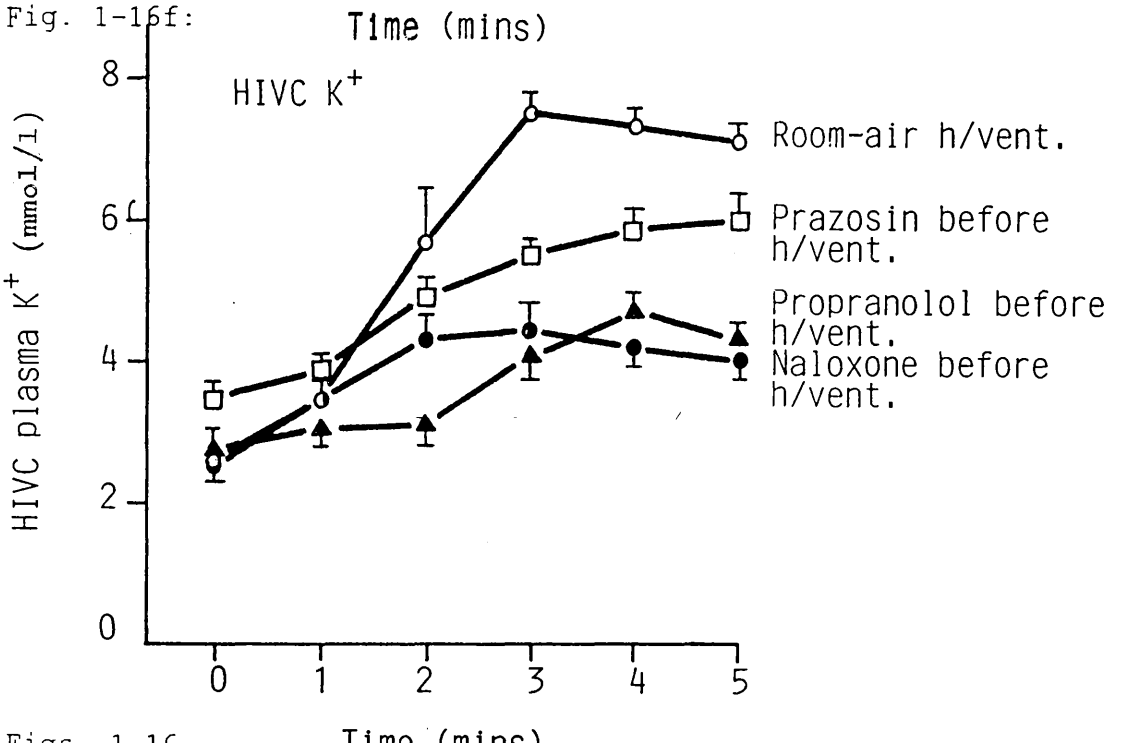


Fig. 1-16f:



Figs. 1-16 (e & f)

Effects of 5-minute Hyperventilation with Room-air on MABP and HIVC plasma K^+ before and after Pretreatment with 0.2mg/kg i.v. either of Prazosin, Naloxone or Propranolol. Means $\pm$ SEM. n=6. Continuous Recording of Results for 5mins are shown.

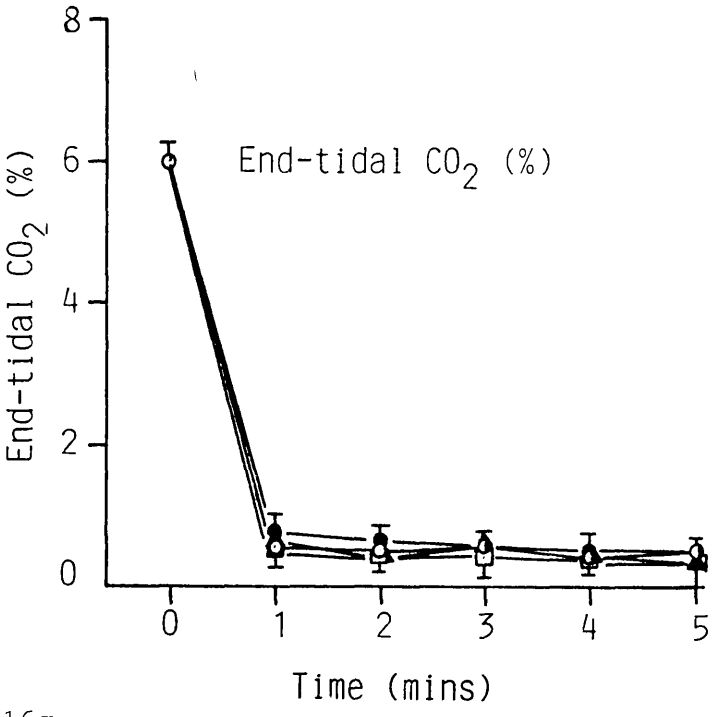


Fig. 1-16g:

Effects of 5 minutes of Hyperventilation with room-air on End-Tidal Co_2 before (o) and after Pretreatment with 0.2mg/kg, i.v. each, of Prazosin (●), Naloxone (▲), or propranolol (□). Mean $\pm$ SEM N=6.

There were no significant differences in the changes in End-Tidal CO_2 produced by Hyperventilation after pretreatment with the various drugs.

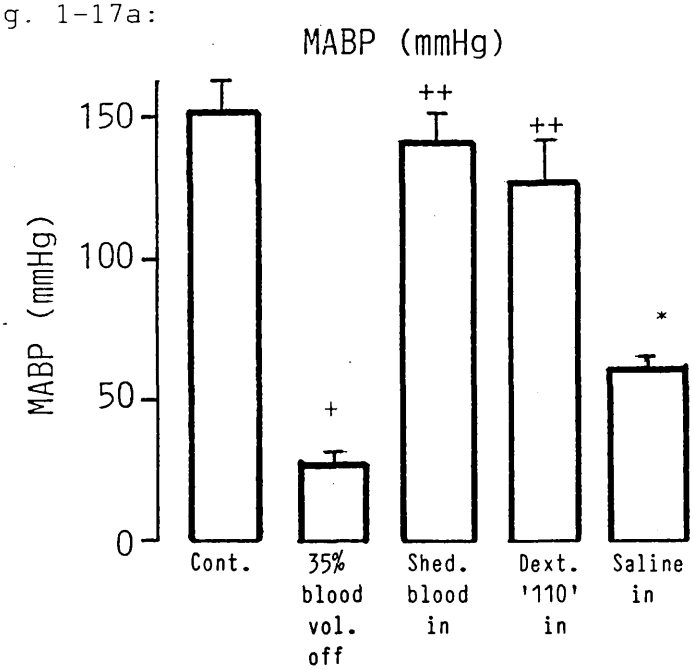
1.17.4 EFFECTS OF SUDDEN WITHDRAWAL OF SOME 35% TOTAL BLOOD VOLUME AND COMPARISON OF THE EFFECTS OF VOLUME REPLACEMENT WITH SHED BLOOD, NORMAL SALINE AND DEXTRAN 110 ON MABP AND PLASMA K^+

The effects of a sudden withdrawal of some 35% of total blood volume and the restoration of blood volume by the infusion of the shed blood, normal saline or dextran 110 on MABP and plasma K^+ are shown in Figures 1.17 (a-h). 35 per cent haemorrhage caused a fall in MABP from 152 $\pm$ 11 mm Hg to 0 mm Hg before rising to 27 $\pm$ 5.0 mm Hg. These MABP changes were accompanied by plasma K^+ changes from 3.76 $\pm$ 0.31 to 10.28 $\pm$ 1.21 mmol/l in the aorta, and from 4.78 $\pm$ 0.44 to 12.55 $\pm$ 0.73 mmol/l in the HIVC. The attending acid/base changes as shown in Figure 1.17 (e) indicate transient alkalosis in the arterial blood.

The pH of the blood in the aorta changed from 7.37 $\pm$ 0.01 to 7.49 $\pm$ 0.02 ($p < 0.05$) while there was no significant change in the HIVC pH. Changes in aortic pCO_2, PO_2, base excess and standard bicarbonate were also quite significant to reflect the significant change in pH towards respiratory alkalosis (see Figures 1.17 (a-d) for such changes).

Reinfusion of the shed blood raised the MABP from 27 $\pm$ 5.00 mm Hg to 142 $\pm$ 9.0 mm Hg, and caused the factors determining pH to return to near control values (see Figures 1.17(e & f). Blood pH/gas analysis performed on samples withdrawn immediately after reinfusion of the shed blood consistently showed readings indicating acidosis before returning towards control values. These changes were not statistically significant at the early stages of the experiment but were significant later when the animal had been under

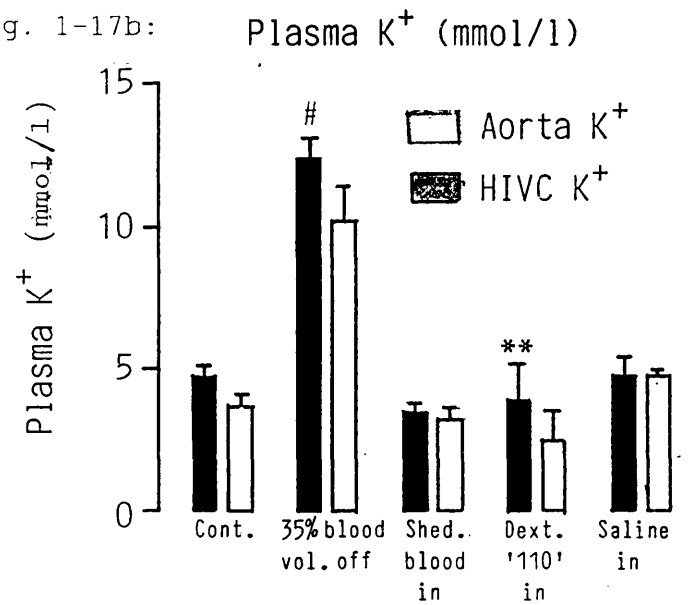
Fig. 1-17a:



+ $p < 0.001$
++ $p < 0.01$
* $p < 0.02$

compared with 35% blood vol. off

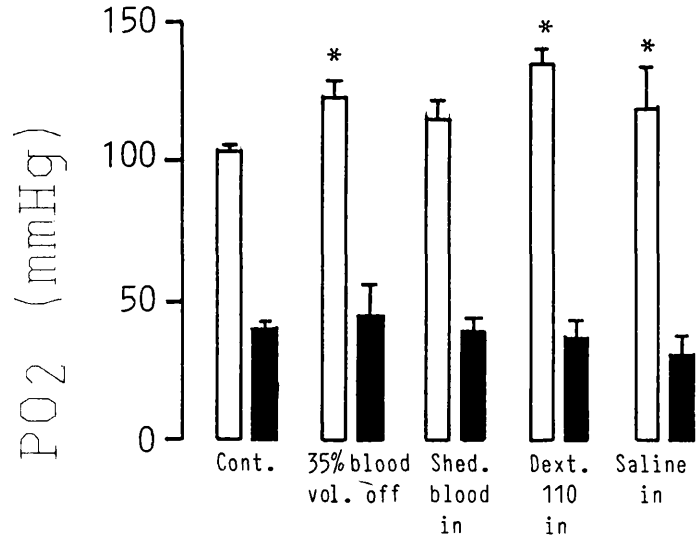
Fig. 1-17b:



# $p < 0.01$
** $p < 0.01$

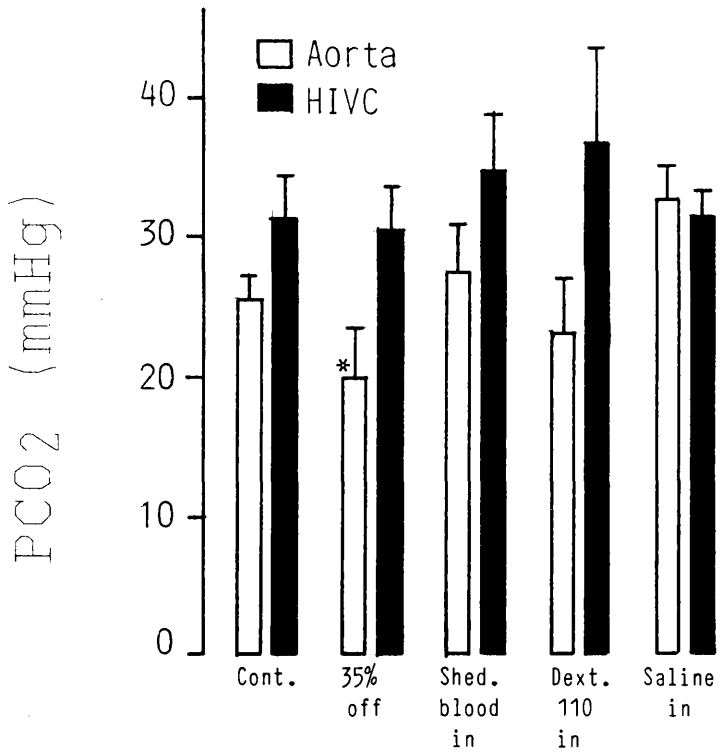
Figs. 1-17 (a & b) Histograms showing the Comparative Effects of Reinfusion of Shed-Blood, Normal Saline and Dextran 110 after haemorrhagic shock on (a) MABP and (b) plasma K^+. Post haemorrhage blood sample was taken immediately after time zero (end of haemorrhage) when the HIVC K^+ got to its first maximum level. Post infusion samples were obtained after MABP and HIVC K^+ were stable about their control levels. Mean ± SEM n=4 per experiment. P values < 0.05 are significant.

Fig. 1-17c:



*P<0.02 compared to control value.

Fig. 1-17d:



*P<0.02 compared to control value.

Figs. 1-17 (c & d) Effects of Reinfusion of Shed Blood, Normal Saline and Dextran 110 on (c) PO_2 and (d) PCO_2 after Withdrawal of 35% Total Blood Volume. Mean ± SEM. n=6.

Fig. 1-17e:

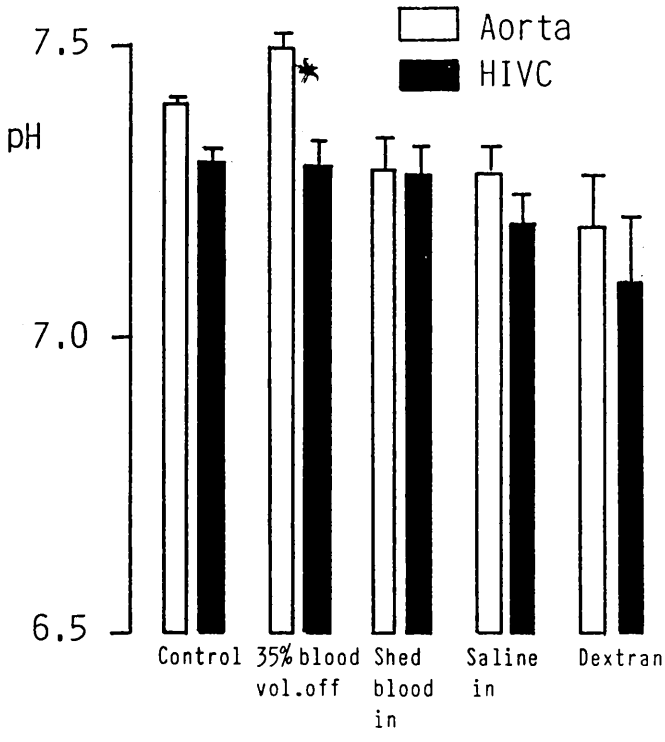
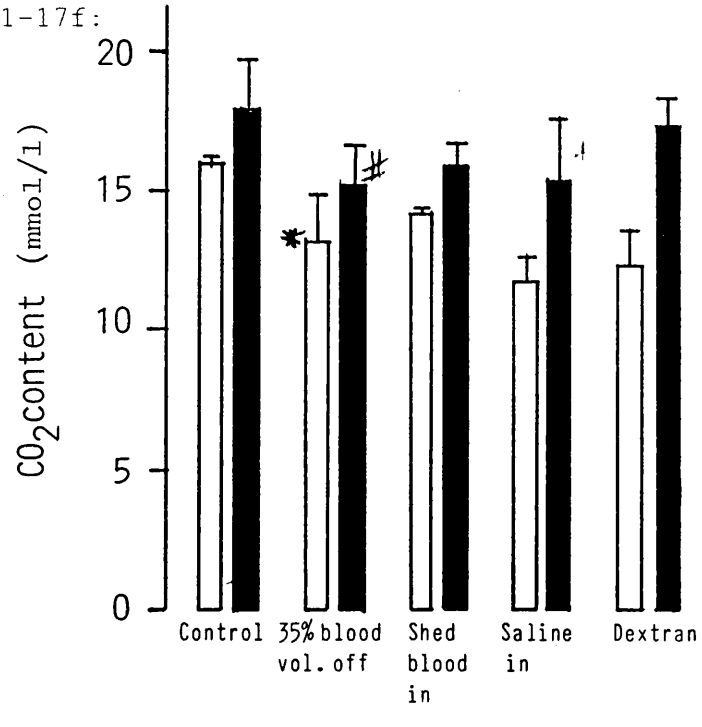


Fig. 1-17f:



Figs. 1-17 (e & f) Effects of Reinfusion of Shed Blood, Normal Saline and Dextran 110, after withdrawal of 35% total Blood Volume on (e) pH and (f) Carbondioxide Content. Mean $\pm$ SEM. n=6.

P values < 0.05 are significant.

Fig. 1-17g:

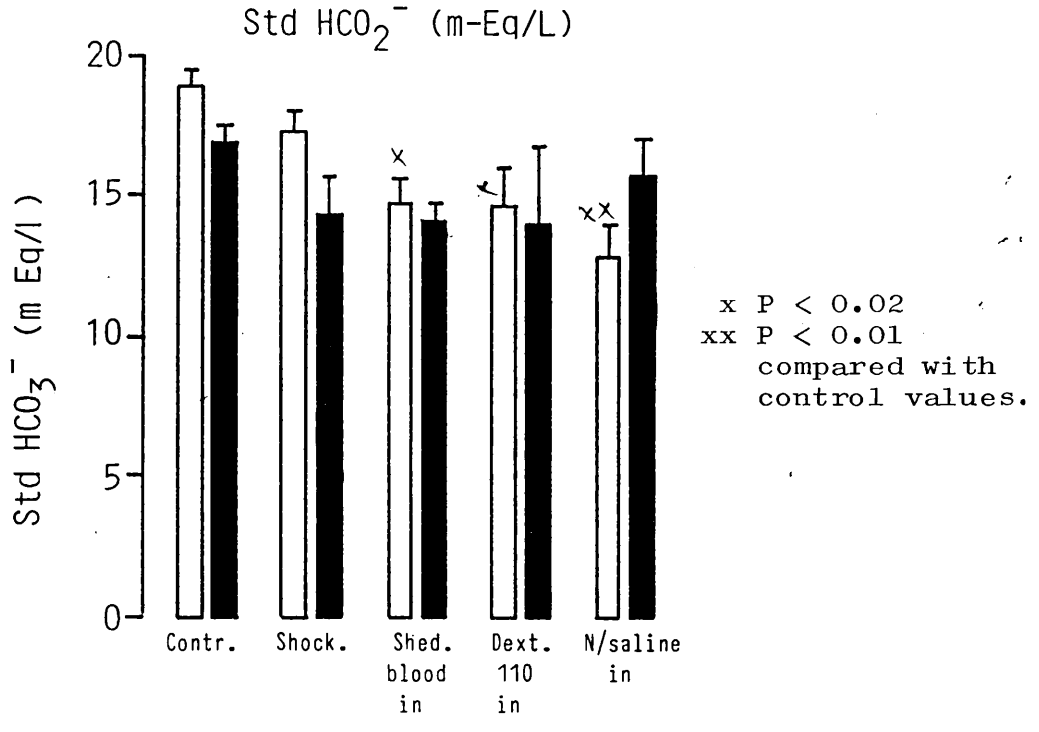
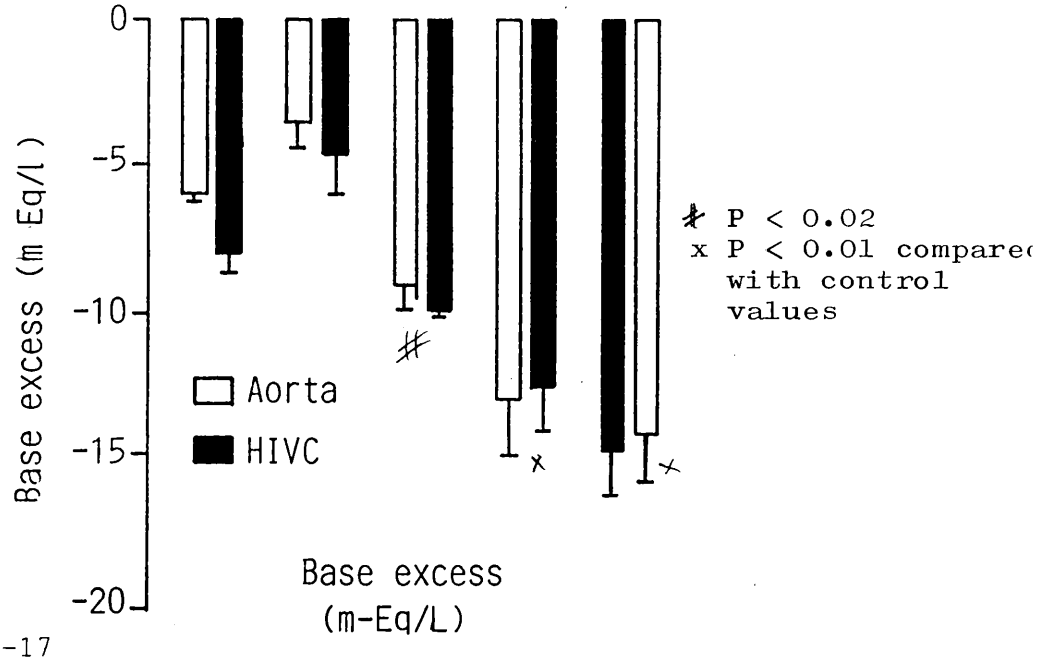


Fig. 1-17h:



Figs. 1-17 (g & h) Effects of Withdrawal of 35% total blood volume, and volume replacement with the shed blood (shed blood in), dextran 110 (Dext. 110 in) and normal saline (N/Saline in) on (g) standard bicarbonate (HCO_3^-) and (h) Base Excess (BE) Mean ± SEM n=6.

P values < 0.05 are significant.

anaesthesia for 2.5 ± 0.5 hrs. The resulting transient acidosis was not due to the reinfusion of acidotic blood because blood withdrawn and reinfused immediately without storing still showed the same transient acidotic changes. At a later stage "reinfusion acidosis" was followed by increasing acidosis with accompanying further rises in plasma K^+, instead of a fall after reinfusion of the shed blood as in the earlier stages.

Two separate groups of 4 cats were used for the experiments using normal saline, and dextran 110 as volume replacement fluids after haemorrhage.

As seen in Figures 1.17 (e-g) the results show that though both Dextran 110 and normal saline infusions returned the elevated plasma K^+ levels following haemorrhage to the control values as did reinfusion of shed blood, the rise in MABP accompanying normal saline infusion was significantly less ($\rho < 0.02$) than that accompanying shed blood or dextran infusion. Whole blood reinfusion, and Dextran 110 infusion, raised the MABP from 27 ± 5.00 mm Hg to 142 ± 9.0 mm Hg, and 128 ± 13.0 mm Hg, respectively; however, normal saline infusion raised the MABP to only 61 ± 4.0 mm Hg. The "reinfusion acidosis" was more marked with Dextran 110 and normal saline, than with whole blood reinfusion. Significant base deficits ($\rho<0.01$) and decreases in standard bicarbonate levels were observed with Dextran 110 and normal saline infusions ($\rho<0.02$) (See Figure 1.17 (g & h). Arterial oxygen tension (PaO_2) was slightly higher after infusion of Dextran 110 than after blood or saline infusions (Figs. 1.17 (c & d). A difference between shed blood reinfusion

and dextran 110 infusion was the relative increase in pulse pressure after dextran 110 (from 36 ± 5.5 mm Hg to 52 ± 4.0 mm Hg) than after shed blood (from 36 ± 5.5 to 38 ± 6.0 mm Hg). During haemorrhage, Lead II ECGs often showed an increase in heart rate from 120 ± 8 beats/min. to 172 ± 12 beats/min and this was followed by a fall in rate. Sinus rhythm was present and in some cases before volume replacement there was a decrease in the amplitude of the QRS-complex and the P-wave, and prolongation of the P-R interval. (See Fig. 1.18 (a)). Post-infusion cardiac arrhythmias were often observed after volume replacement with any of the three fluids studied (See Fig. 1.18(b)) before recovery to normal rhythm occurred. If volume replacement with whole blood, normal saline or dextran 110 was instituted after the first sustained maximum rise (first plateau) in plasma K^+ following haemorrhage, the resulting return of MABP towards control values was usually accompanied by a corresponding fall in plasma K^+. However if reinfusion was performed at the end of the first plateau, or the beginning of a further rise in plasma K^+ (the second maximum rise or the second plateau), then the resulting rise in MABP was only transient with a continuous rise, instead of a fall in plasma K^+, to irreversible levels. The accompanying lead II ECGs are shown in Figures 1.18 (c & d). Prolonged P-R and Q-T intervals were common at this stage with elevated S-T segments, peaked P-waves, and marked Q-waves in some cases. Resuscitatory measures to sustain the animal's life at such stages almost always proved unsuccessful,

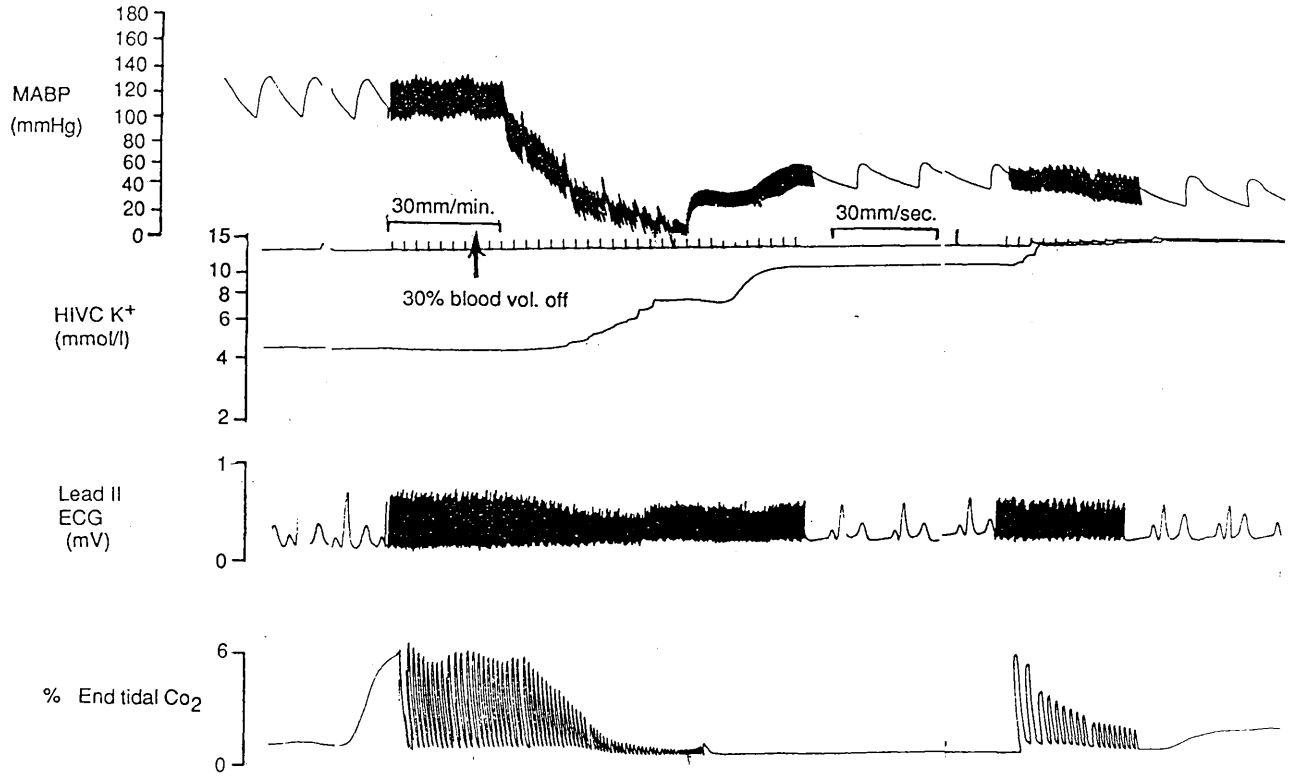


Fig.1-18a:

The effects of acute withdrawal of 30% total blood volume on the MABP, HIVC plasma K+, ECG and the end-tidal CO2. Note the respective responses, viz; hypotension, hyperkalaemia, tachycardia followed by bradycardia, and hyperventilation followed by an end-inspiratory apnoea. Note also that on resumption of breathing, the plasma K+ increased further resulting in the peaking of the T-waves which were hitherto relatively low in the initial severe hyperkalemic state.

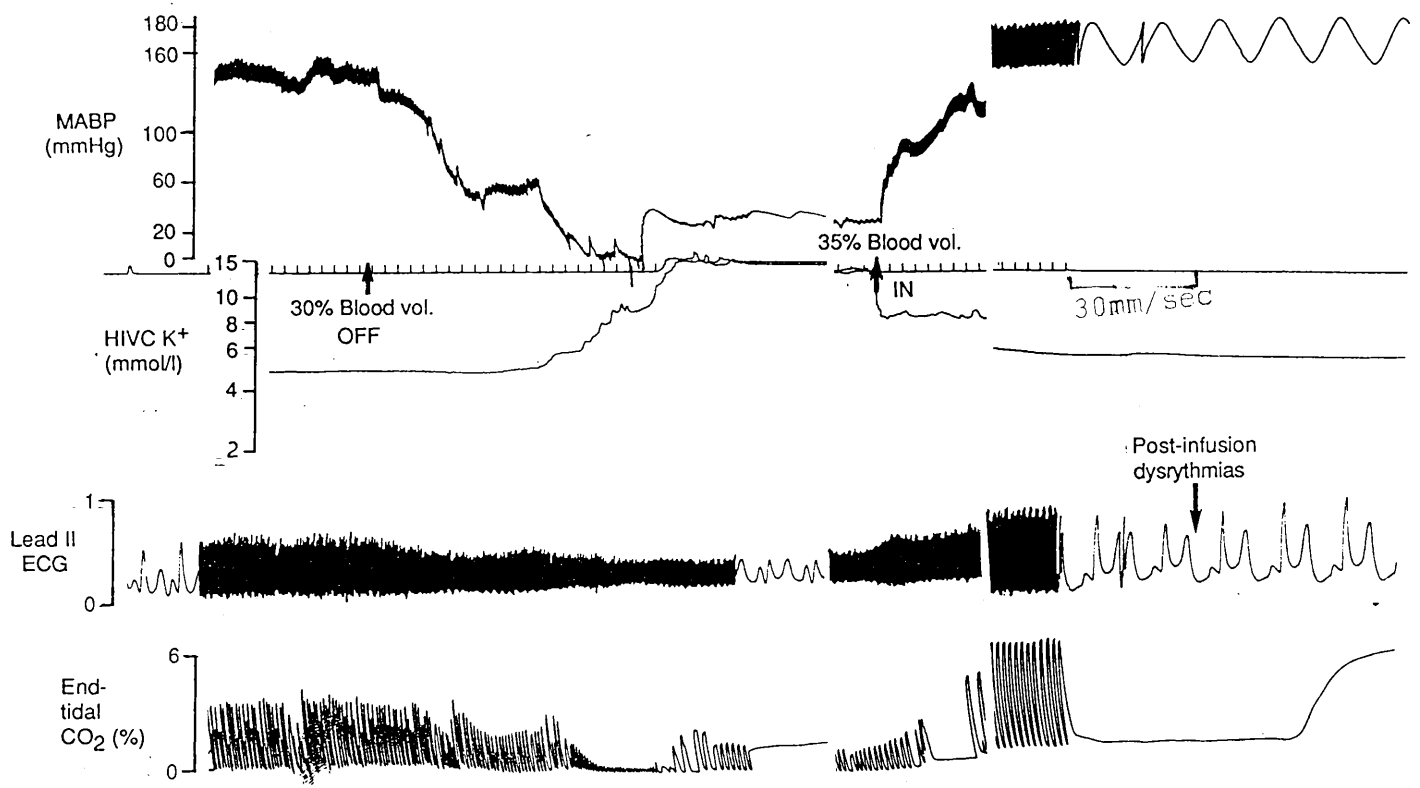


Fig.1-18b:

Post-infusion cardiac arrhythmias following volume replacement with the shed blood.

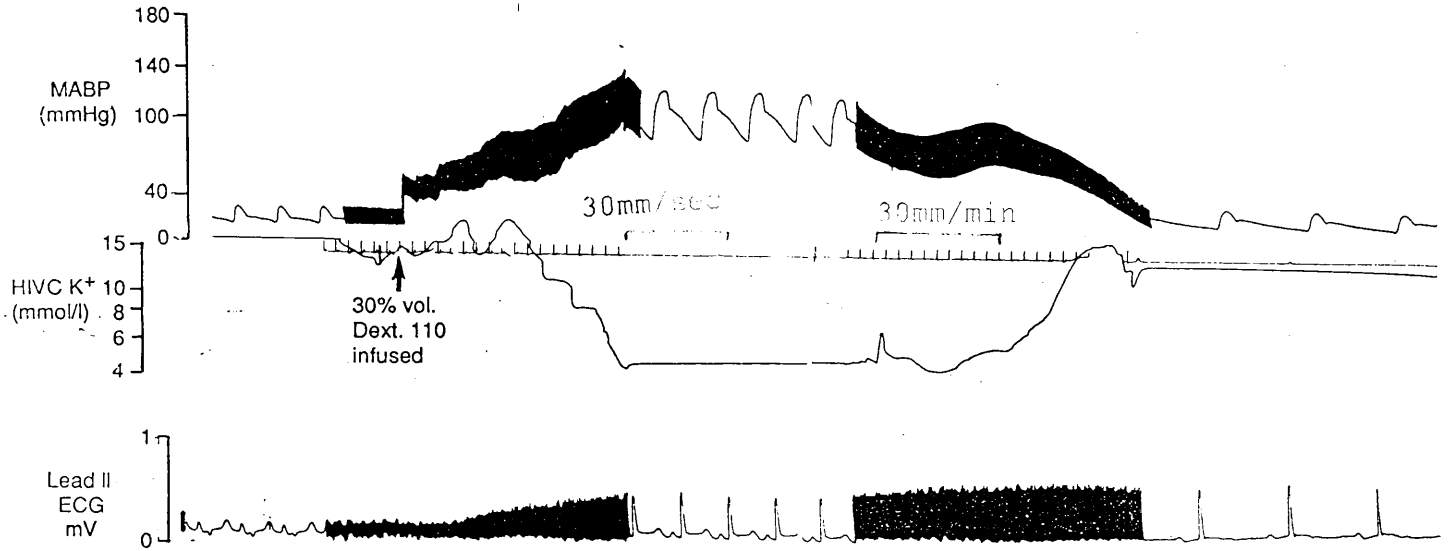


Fig.1-18c:

Failure of volume replacement with dextran 110 to restore the MABP and the HIVC plasma K+ to the control levels after prolonged haemorrhagic hypotension. Note the transient rise in the MABP and the fall in the plasma K+ with a transient recovery in the ECG also, followed by prolongation of the P-R and Q-T intervals as the MABP drops progressively with accompanying severe hyperkalemia.

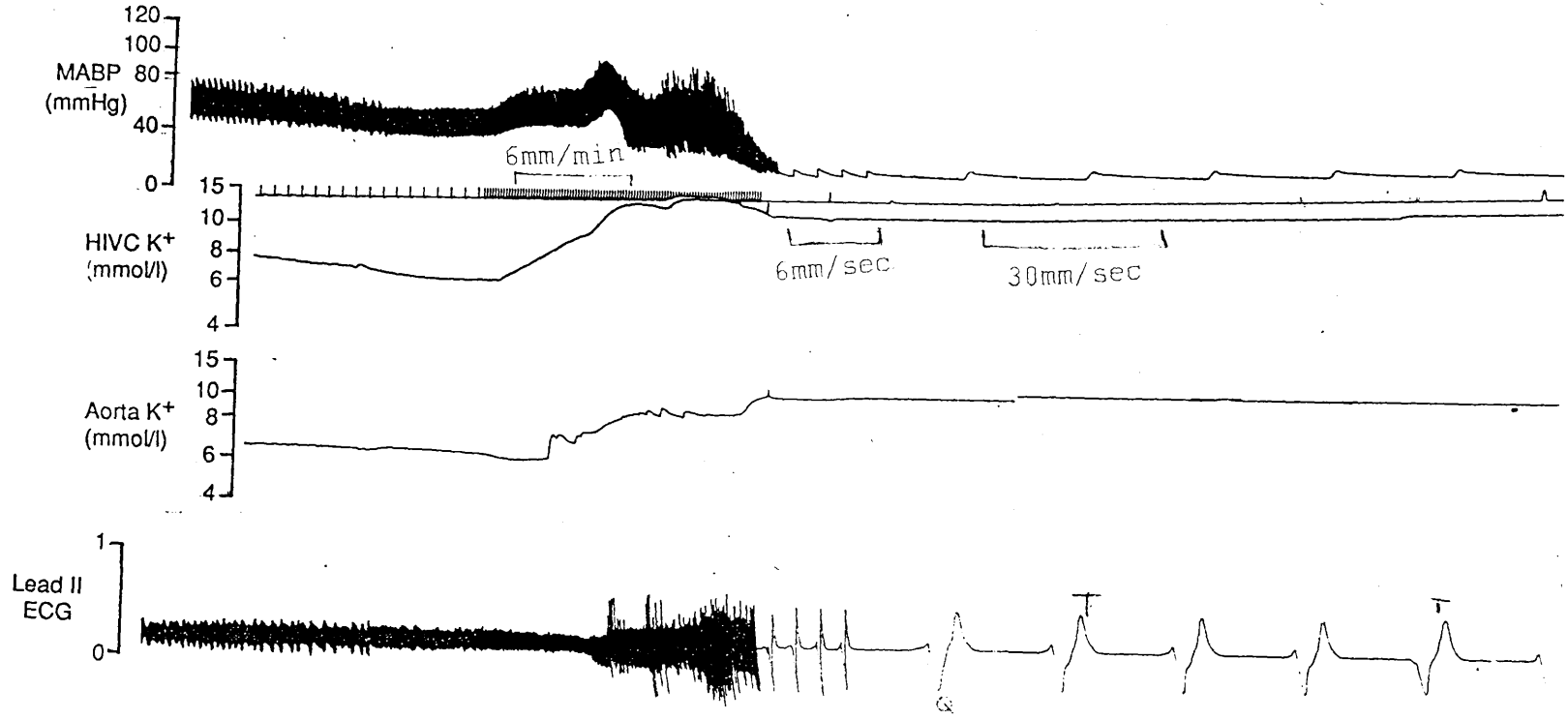


Fig.1-18d: Severe hyperkalaemia with marked Q-waves and peaked T-waves in the ECG in the terminal stages of a prolonged haemorrhagic hypotension without volume replacement.

and with external cardiac massage and mechanically assisted ventilation, further rises in plasma K^+ occurred.

The survival period of the experimental animal was found to be longest (over 8 hrs) when the shed blood (30-35 per cent of total blood volume) was replaced with half dextran 110 and half shed blood.

1.17.5 EFFECTS OF BLOOD WITHDRAWAL TO PRODUCE A MABP OF 40 MM HG FOR 120 MINS

The effects of blood withdrawal to produce a MABP of 40 mm Hg maintained for 120 minutes are shown in Figures 1.18 (e-h).

Withdrawal of arterial blood acutely reduced MABP from 159 $\pm$ 15 to 40 $\pm$ 2 mm Hg within 75.0 $\pm$ 15 secs with an accompanying rise in aortic and HIVC K^+. At the end of haemorrhage, (time zero), aortic K^+ had risen from 2.56 $\pm$ 0.08 to 4.75 $\pm$ 0.02 mmol/l while HIVC K^+, rose from 3.60 $\pm$ 0.04 to 6.44 $\pm$ 0.05 mmol/l. Blood samples obtained for pH/gas analysis at the end of haemorrhage showed the following changes in the parameters affecting pH. Aortic PCO_2 decreased from 29.6 $\pm$ 0.80 mm Hg to 20.6 $\pm$ 0.65 mm Hg, total CO_2 content fell from 17.9 $\pm$ 0.11 mmol/l to 13.8 $\pm$ 0.62 mmol/l, but aortic base excess rose from -5.96 $\pm$ 0.12 m.Eq/l to -2.20 $\pm$ 0.40 mEq/l; PaO_2 rose from 101 $\pm$ 3.0 to 121 $\pm$ 2.50 mm Hg, standard bicarbonate increased from 20.03 $\pm$ 0.28 mEq/l to 23.0 $\pm$ 0.48 mEq/l and the aortic pH rose from 7.37 $\pm$ 0.03 to 7.47 $\pm$ 0.05.

Fig.1-18e:

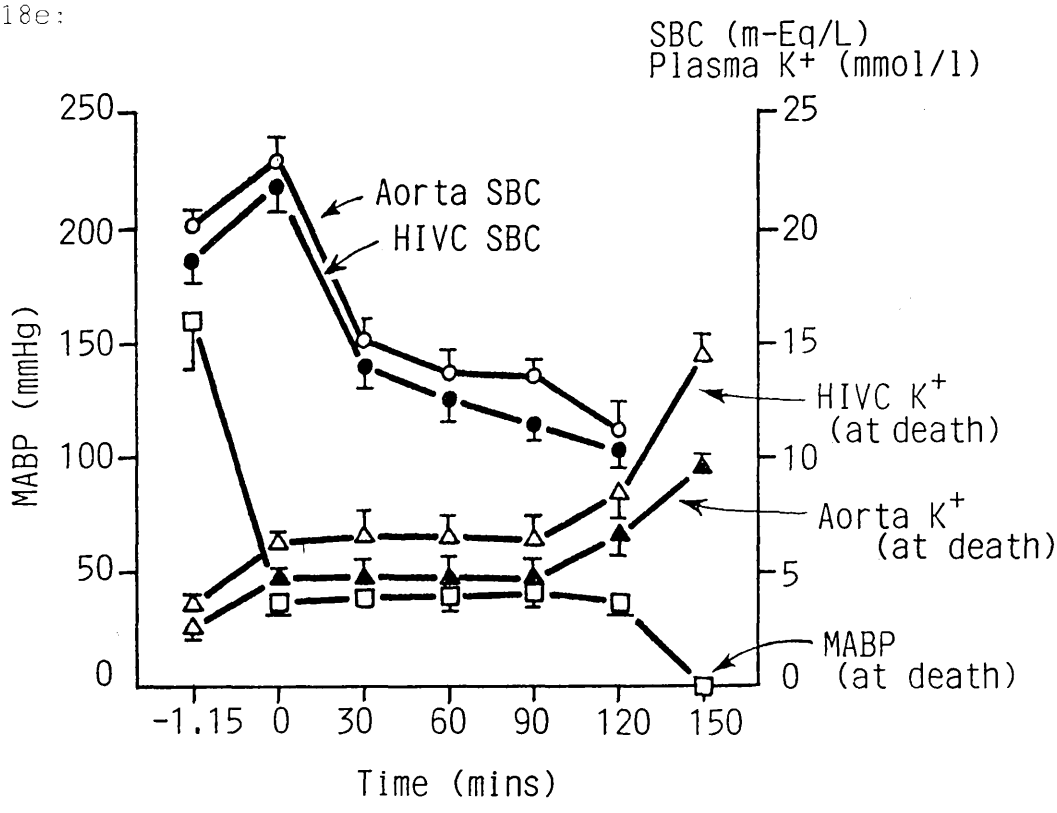


Fig.1-18f:

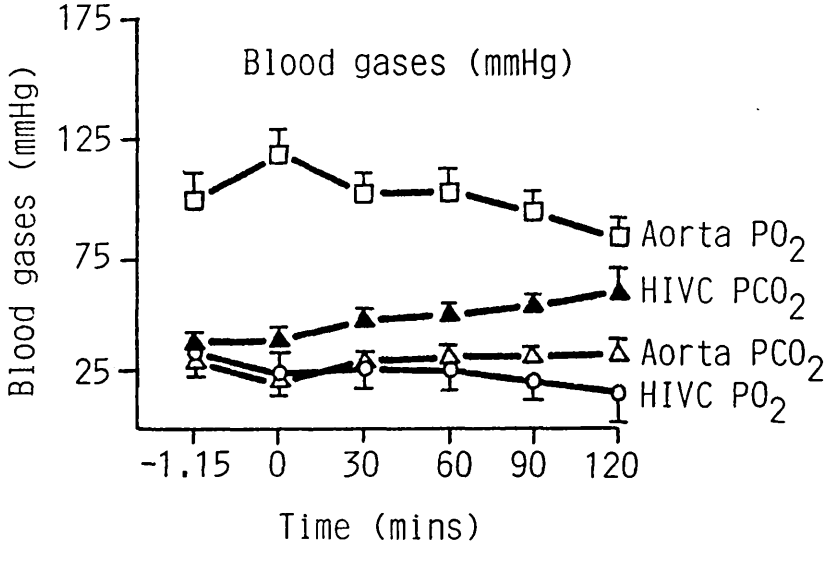


Fig.1-18e & f:

Effects of Blood Withdrawal to a MABP of 40mmHg on (a) Plasma K^+ and standard Bicarbonate (SBC), and (b) Blood Gases. Mean± SEM n=6.

Fig.1-18g:

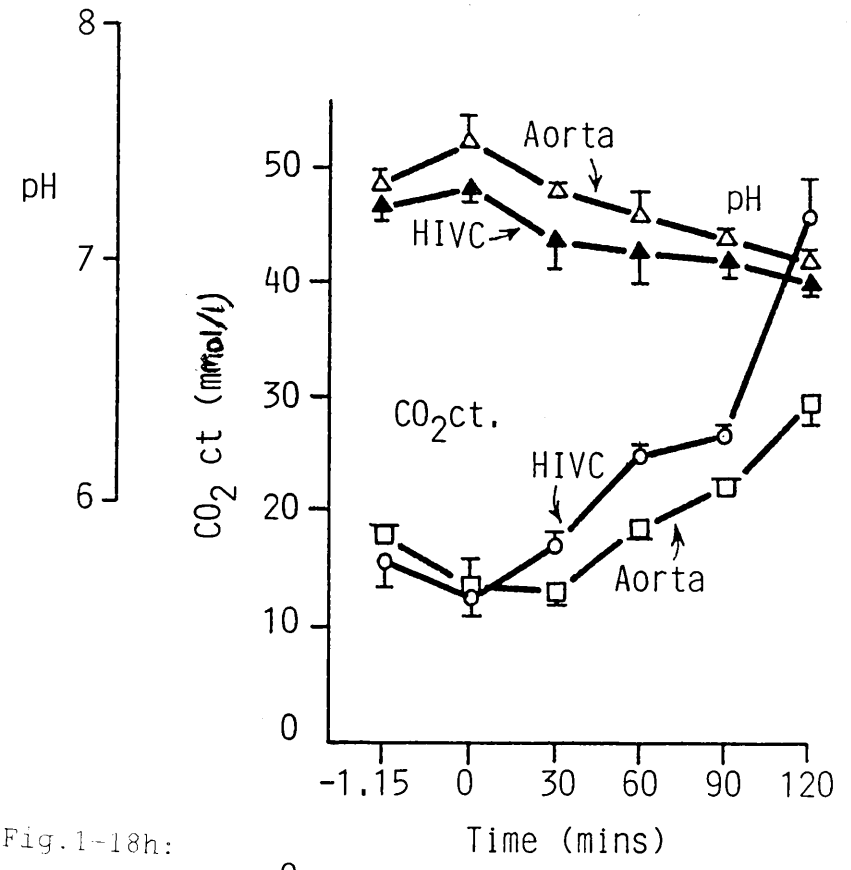


Fig.1-18h:

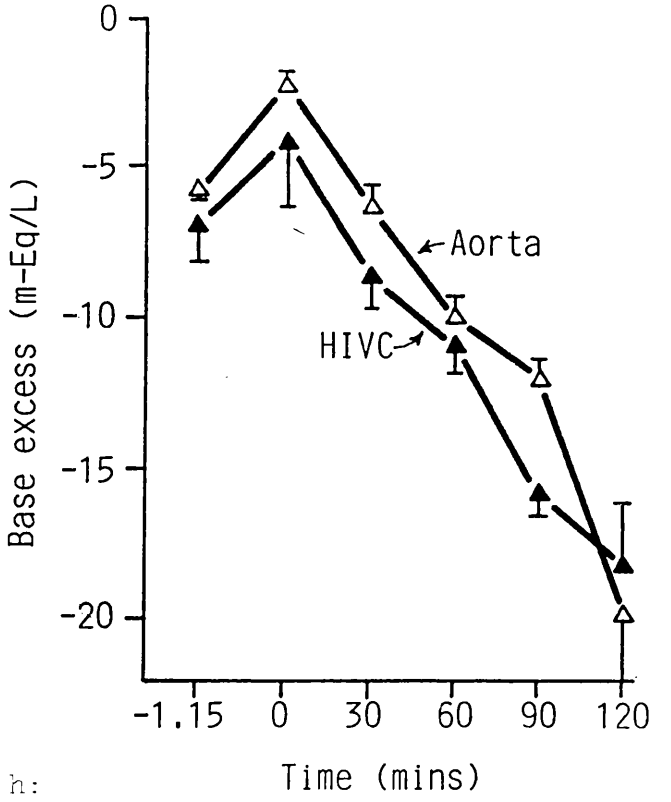


Fig.1-18g & h:

Effects of Blood Withdrawal to a Mean Arterial Blood Pressure of 40mmHg on (c) Total Carbon-dioxide content (CO_2ct) and pH, and (d) Base Excess. Mean± SEM. n=6.

In the HIVC, the increase in plasma K^+ shown above was not accompanied by a significant change in pH. Changes in PO_2, PCO_2 and base excess showed a picture of metabolic acidosis in the HIVC, viz: PO_2 decreasing from 33 $\pm$ 1.50 mm Hg to 24 $\pm$ 2.40 mm Hg, PCO_2 increasing from 38.4 $\pm$ 0.60 to 39.9 $\pm$ 0.12 mm Hg and base excess increasing from -7.00 $\pm$ 0.90 m.Eq/l to -4.20 $\pm$ 1.80 m.Eq/l, and standard bicarbonate increasing from 18.53 $\pm$ 0.63 m.Eq/l to 21.9 $\pm$ 0.68 m.Eq/l.

End-tidal CO_2 per cent which was monitored continuously decreased from 5.98 $\pm$ 0.4 to 0.4 $\pm$ 0.15%, and was followed by a transient end-inspiratory apnoea. After haemorrhage the plasma levels of K^+ remained at the raised value until between the 60th and 90th minute. The arterial pH value decreased from 7.33 $\pm$ 0.02 in the 30th min. to 7.18 $\pm$ 0.2 in the 90th minute. Values of the other measured parameters which can affect pH, all changed towards an acidotic picture by the 90th minute (see Figs. 1.18 (e-h)). Beyond the 90th minute and before the 120 minute there occurred consistently a transient rise in the MABP which was followed by a precipitous fall to zero. This fall was accompanied by continuous rising levels of plasma K^+ from 6.68 $\pm$ 0.48 to 9.60 $\pm$ 0.20 mmol/l in the aorta, and from 8.46 $\pm$ 0.60 to 14.50 $\pm$ 0.40 mmol/l in the HIVC.

Aortic pH had decreased to 7.02 $\pm$ 0.04 and the HIVC pH to 6.6 $\pm$ 0.01 by the 120th minute. Observation of the animal revealed slow and deep breathing. The end-tidal CO_2 increased to more than 6% and this preceded an arrest of breathing. Lead II ECG recordings showed increased P-R and Q-T intervals, reduced QRS-complexes with peaked T-waves, and occasional

Q-waves with ventricular fibrillation.

Resuscitatory measures including i.v. injection of 5 μg/kg adrenaline, mechanically assisted ventilation with a respiration pump and external cardiac massage, all proved unsuccessful, but instead, the recorded plasma K^+ continued to rise (Fig. 1.18 (i)). Animals died by the 120th ± 15 minutes from the beginning of haemorrhage in all experiments but Lead II ECG continued with marked Q-waves and ventricular ectopic beats at very slow rates for up to 25 to 30 minutes thereafter.

1.17.6 EFFECTS OF HAEMORRHAGIC HYPOTENSION AT MABP OF 80 MM HG

The effects of withdrawal of blood to produce a MABP of 80 mm Hg for 180 mins are shown in Figures 1.19 (a-d).

The initial transient changes in plasma K^+ and blood pH/gas parameters due to acute lowering of the MABP from 151 ± 4.0 mm Hg to 80 ± 2.0 mm Hg were qualitatively similar to those caused by lowering the MABP to 40 mm Hg by haemorrhage. The main differences were in the size and in the time course of the changes in plasma K^+ and blood gases. At MABP of 80 mm Hg, the initial transient changes in pH and plasma K^+ were 7.398 ± 0.2 to 7.49 ± 0.04 in the aorta, 7.395 ± 0.01 to 7.44 ± 0.05 in the HIVC, and 2.90 ± 0.32 mmol/l to 4.80 ± 0.48 mmol/l in the aorta, and 3.15 ± 0.46 mmol/l to 6.80 ± 0.48 mmol/l in the HIVC, respectively. The transient changes in the blood gases and other factors affecting pH like BE and SBC were all in the direction of alkalosis, for

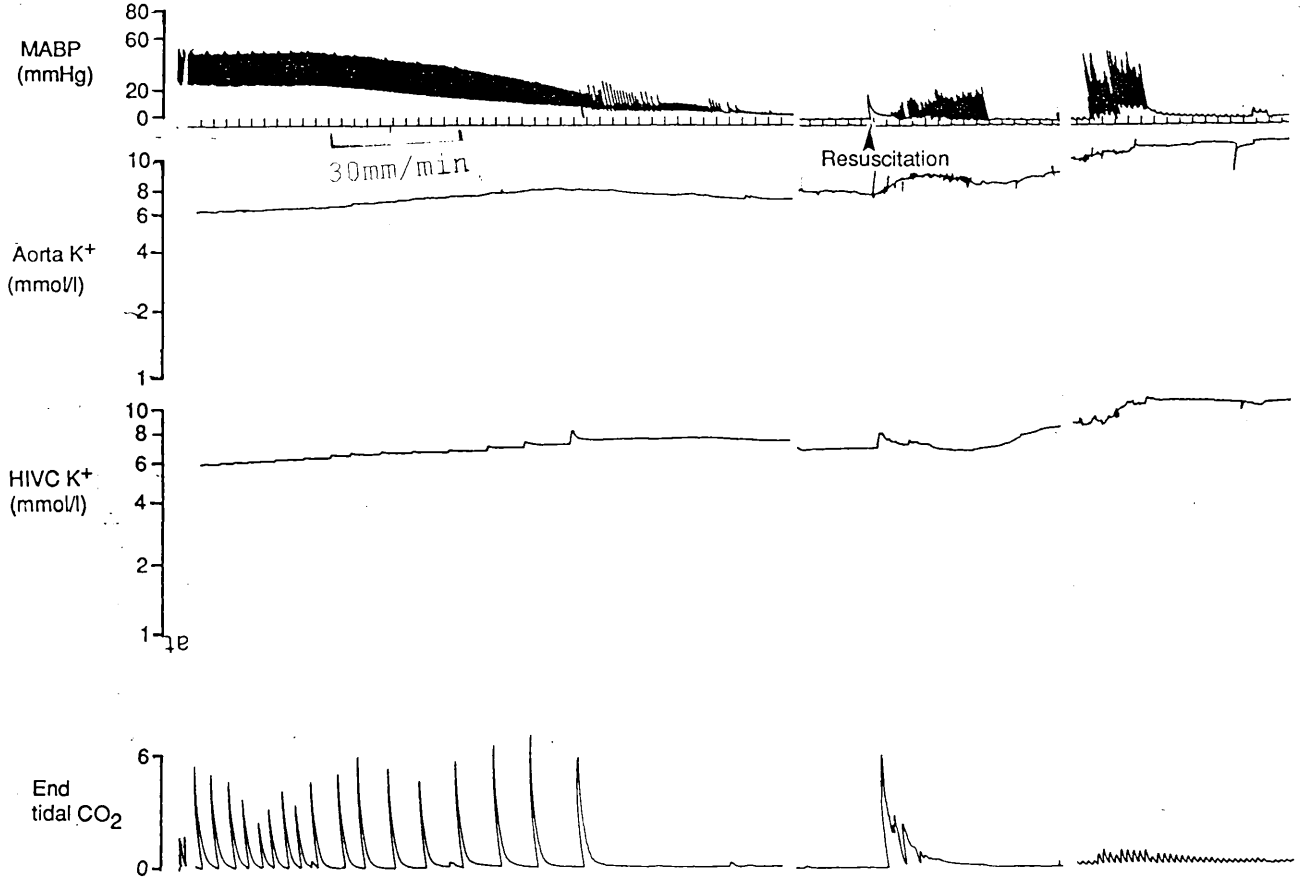


Fig.1-18i: Terminal stages of haemorrhagic shock showing the progressive fall in MABP, the slowing rate of breathing and the increasing hyperkalaemia. Arrow shows the beginning of resuscitatory measures which resulted in sudden further increase in the plasma K+ (compare with Fig.1-22, the level of plasma K+ without resuscitatory measures at the terminal stagges.

Fig. 1-19a:

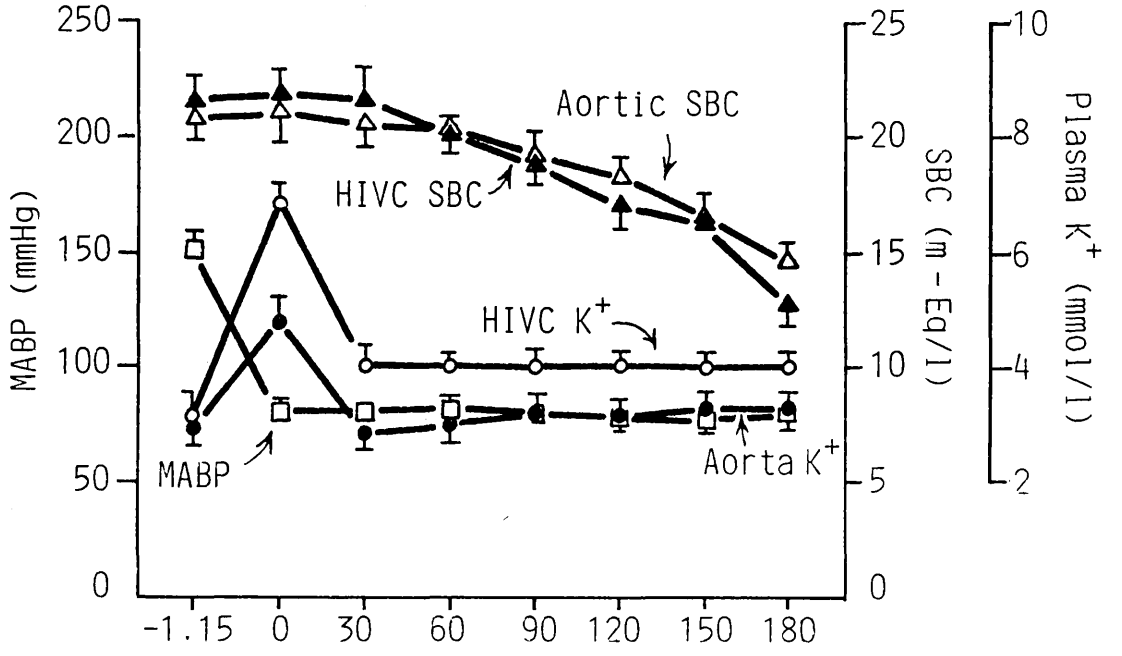
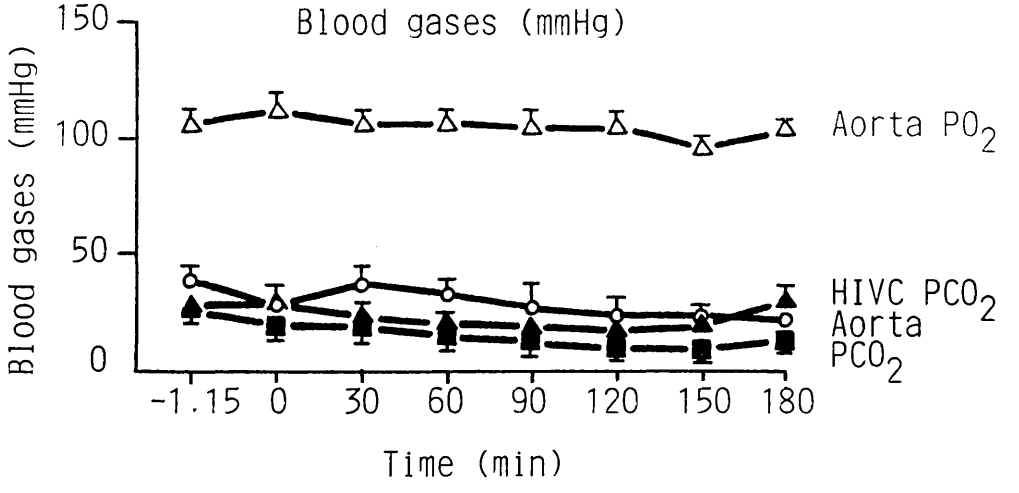


Fig. 1-19b:



Figs. 1-19 (a & b) Effects of Blood Withdrawal to a MABP of 80mmHg on (a) Plasma K^+ and Standard Bicarbonate (SBC) and (b) Blood Gases. Mean $\pm$ SEM n=6.

example, arterial $PaCO_2$ changed from 26.0 ± 1.50 mm Hg to 19.7 ± 2.20 mm Hg; PaO_2, from 106 ± 1.58 to 112 ± 4.00 mm Hg; standard bicarbonate (SBC), from 20.7 ± 0.58 to 21.0 ± 0.82 m.Eq/l; base excess from -5.40 ± 0.64 to -5.1 ± 0.02 m.Eq/l; and carbon dioxide content, from 18.3 ± 0.78 mmol/l to 15.7 ± 1.44 mmol/l. With the MABP maintained at 80 ± 2.0 mm Hg, the pH remained at the slightly raised level of 7.48 ± 0.04 until the 150th min. while the blood gases and the other factors determining pH varied. This was in marked contrast to the changes when the MABP was maintained at 40 mm Hg. In that case the pH changed towards acidosis from before the 30th minute from the onset of haemorrhage and the MABP fell precipitously to zero by the 120th minute.

In the experiments where the MABP was maintained at 40 mm Hg, the raised plasma K^+ levels remained elevated as long as the MABP remained at 40 mm Hg. In the present experiment however, the aortic plasma K^+ fell from its raised level to below the control level i.e. to 2.85 ± 0.33 mmol/l before returning by the 30th minute to about the control level of 3.00 ± 0.38 mmol/l. The aortic plasma K^+ level was maintained about this level until the 180th min. The HIVC plasma K^+ also fell from 6.80 ± 0.48 mmol/l to 4.00 ± 0.49 mmol/l but not to the control value of 3.15 ± 0.46 mmol/l. This level, which is slightly above the control was maintained until the 180th minute. Though the pH and plasma K^+ remained about the control values until the 180th minute, (Fig. 1.19(a)) it is interesting to note in Figures

Fig. 1-19c:

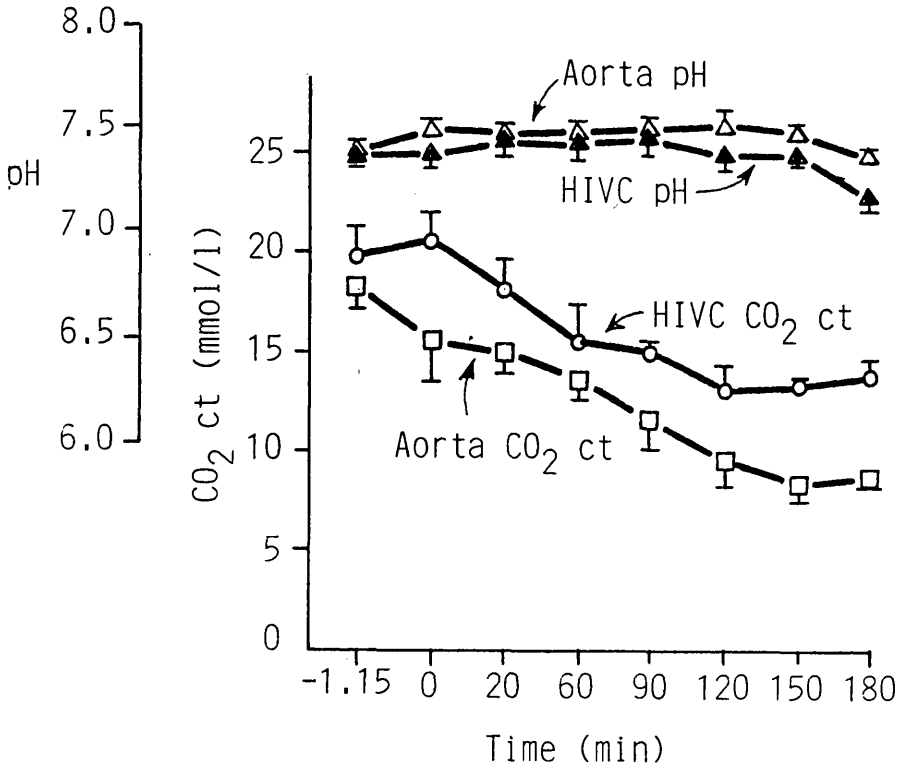
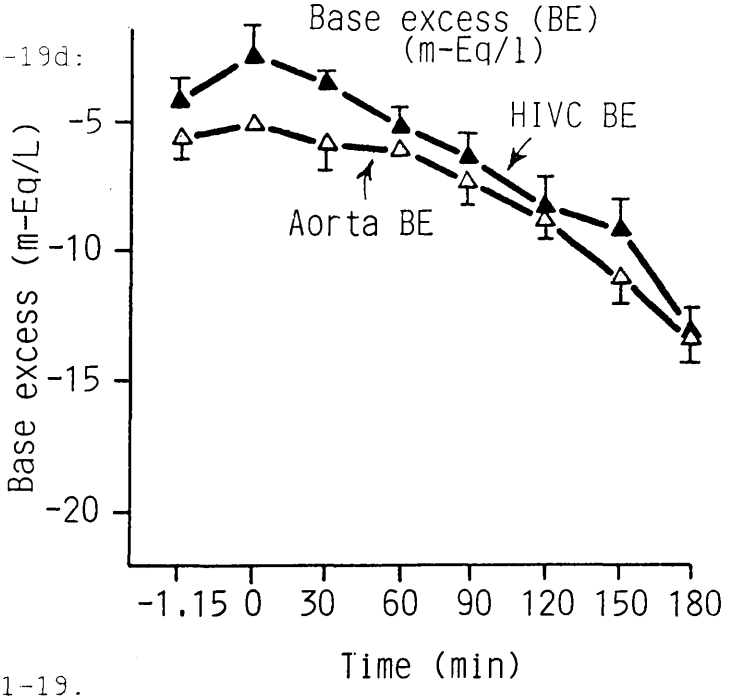


Fig. 1-19d:



Figs. 1-19. (c&d)

Effects of Blood Withdrawal to a MABP 80mmHg on (c) Total CO_2 content (CO_2ct) and pH, and (d) Base Excess (BE). Mean± SEM. n=6.

1.19(b-d) the significant changes which occur in the blood pH/gas analyses from the 90th minute, to keep the pH near the control. Beyond the 180th minute (not shown in the figures) the MABP fell gradually and needed greater top-up volumes of blood to maintain the pressure at 80 mm Hg and the pH started to fall with an accompanying rise in plasma K^+. Reinfusion of the whole shed-blood at this stage caused all measured values to gradually return approximately to the control values.

Experiments involving this procedure were all terminated at the 180th minute beyond which more top-up blood was needed, in order to compare more accurately the time-course of events with the procedure involving cats whose MABPs were maintained at 40 mm Hg for 120 min.

1.18 DISCUSSION

1.18.1 ADRENALINE AND NORADRENALINE INJECTIONS

These experiments showed that the catecholamines, adrenaline and noradrenaline produced a rise both in plasma K^+ and in MABP. After the rise, the MABP returned to control levels, but after the plasma K^+ rise in the HIVC and the aorta the levels fell to below control values before returning to the control.

The maximum increases in plasma K^+ produced by adrenaline and noradrenaline were not significantly different from each other, but the rise in K^+ in the HIVC was significantly higher than that in the aorta ($p < 0.01$). The higher values of plasma K^+ in the HIVC observed in this study are in agreement

with the findings of Da'Silva (1935) who attributed the higher K^+ produced by i.v. injection of adrenaline to the release of K^+ by the liver. Similar injections by Treasure (1977), and Treasure and Band (1978) which produced a higher rise in the level of plasma K^+ in the HIVC gave support to Da'Silva's suggestion. It is reasonable to speculate from the present study that the lower level of aortic plasma K^+ may be due either to an uptake by the heart or the lungs, or dilution with blood from the superior vena cava and the vena azygos. Other workers, van Der Meer, Valkenburg and Snijders (1986) suggested that the splanchnic organs were responsible for the extra K^+ increase in the HIVC because K^+ electrodes indwelling in the hepatic portal vein and the hepatic vein always showed higher levels of K^+ in the portal vein than in the hepatic, thus concluding that if anything, the liver actually took up K^+ in haemorrhagic shock. These authors suggested that plasma K^+ released during haemorrhage was a result of the stimulation of the sympatho-adrenal system to release catecholamines. Ross and Kurrasch (1969) reported that after β-adrenergic blockade by i.v. propranolol, vasodilatation produced by isoproterenol was abolished and vasoconstriction by epinephrine and norepine-phrine was intensified in the hepatic arterial bed. This indicated that β-adrenergic receptors are present in the hepatic arterial bed and that the changes in portal vein flow induced by catecholamines depended more on changes in the flow of blood through intestines and spleen than on vascular changes within liver. The recent works by Coat. (1983, 1985)

further demonstrated that the release of K^+ by the liver was mediated by α_1-adrenoceptors because this response was blocked by phentolamine, an α-adrenoceptor blocker, while the undershooting (uptake) aspect of the response was mediated by β_2-adrenoceptors as this was also blocked by propranolol, a β-adrenoceptor blocking agent.

The results of further investigations in which α- and β-adrenoceptor blocking agents are given before adrenaline injection or blood withdrawal in the present study is discussed in Section Two of this thesis.

1.18.2 INCREASING PERCENTAGE OF HAEMORRHAGE

The results showed that removal of 5 to 10 per cent of total blood volume had no significant effect on either MABP or plasma K^+ levels. Significant changes occurred in these parameters with removal of more than 10% of the total blood volume. If 25% of the total blood volume was removed, MABP was reduced to about 40 mm Hg and marked changes in plasma K^+ appeared with a haemorrhage of 25 to 35% of total blood volume.

Haemorrhages of 35% and over caused the MABP to fall to zero before recovering to 27 ± 2.0 mm Hg. The plasma K^+ responses in big cats (over 4.0 kg) agree with observations made by Treasure (1977).

The decrease in arterial pressure caused by blood loss initiates powerful sympathetic reflexes to produce vaso-constriction throughout the body. The accompanying rise in plasma K^+ level with increasing severity of haemorrhage in the present study might reflect the intensity of the sympathetic stimulation either on blood vessels, splanchnic structures, or the release of other substances that affect the release of K^+ in the body following haemorrhage. By studying the effects of increasing the volume of blood removed over a prolonged time and making frequent measurements, it was possible to observe the sequence of events which led to death, and to obtain information about the changes in the observed parameters which led up to this and relate these to the developing picture of irreversible haemorrhagic shock. Walker and associates reported in 1959 that removal of as little as 10 to 20 per cent of the blood volume increased the plasma levels of the catecholamines, adrenaline and noradrenaline.

Shoemaker and his colleagues (1961) found in addition to the above an increase in hepatic blood flow. As more blood was progressively withdrawn, the ability of the catecholamines to redistribute the cardiac output was reduced by the limited effective circulating volume; the hepatic blood flow then settled down to a rate which was less than the initial or control blood flow rate.

In interpreting the results of the present study, one is struck by the remarkable similarity between the effects of graded haemorrhage on the anaesthetized cat and the response of the liver to adrenaline and noradrenaline. It is therefore

tempting to speculate that the effects of haemorrhage including increased hepatic blood flow (Shoemaker, Walker and Van Itallie, 1959) and increased K^+ output are largely due to an increased liberation of adrenaline and noradrenaline from the adrenal glands and the sympathetic nerves. The direct relationship between MABP and plasma K^+ in response to the catecholamines, and the inverse relationship in response to haemorrhage, led me to further investigate the role of MABP in plasma K^+ changes during and after haemorrhage. The catecholamines increased MABP and there were corresponding increases in plasma K^+, while haemorrhage produced a fall in MABP with an accompanying rise in plasma K^+.

After haemorrhage of above 30% of total blood volume, plasma levels of K^+ never returned to control values, instead, increases in K^+ occurred to irreversible levels unless the shed blood was reinfused. However if the catecholamines are injected as shown above, they often cause an uptake of the transiently released K^+ to below control levels before returning to the control. If the catecholamines were responsible for the effects of haemorrhage on plasma K^+, then such differences are inconsistent.

There is no clear explanation for such differences, but recent studies by Mason, Medbak and Rees (1987) revealed that catecholamines as well as circulating plasma levels of metenkephalins increased during acute hypotension in anaesthetized greyhounds. Increase in plasma opioid levels may therefore play a role in K^+ changes, as well as reduced blood flow and tissue hypoxia.

The sustained high levels of plasma K^+ after 35% haemorrhage may be due either to excessive release of K^+ from the liver as a result of haemorrhage-induced metabolic acidosis or it could be due to developing insensitivity in the face of increasing acidosis, of the tissue β_2-adrenoceptors to catecholamines responsible for K^+ uptake. As regards the body tissue responses to catecholamines, Burget and Visscher (1927) have shown that as the pH of blood is decreased, the vascular response to injected epinephrine decreases during haemorrhagic shock.

The role of endogenously released opioids in plasma K^+ changes during haemorrhage is investigated and discussed in Sections Two and Three of this thesis.

1.18.3 EFFECTS OF SUDDEN WITHDRAWAL OF 35% TOTAL BLOOD VOLUME AND COMPARISON OF THE EFFECTS OF VOLUME REPLACEMENT WITH SHED BLOOD, NORMAL SALINE AND DEXTRAN 110 ON MABP AND PLASMA K^+

From the results of preliminary studies into the effects of increasing the percentage of total blood withdrawn on MABP and plasma K^+, it was found that a sudden withdrawal of 35% of the total blood volume produced quite a significant fall in MABP with an accompanying rise in plasma K^+. Reversal to control values was possible with reinfusion of the shed blood after 5 min of observation. This degree of haemorrhage was therefore chosen to allow frequent measurements of other parameters such as blood pH and gases, and lead II ECG, follow the sequence of events, that accompanied the changes in MABP and plasma K^+ leading to irreversibility, before and

after fluid replacement.

These studies showed that the plasma K^+ levels which were increased following acute lowering of the MABP by withdrawal of 35% total blood volume could be effectively restored to normal by infusion of normal saline, Dextran 110 as well as the infusion of the shed blood.

Using a separate group of animals for the study of the effects of each of the three different fluids showed that the survival period was longest (over 8 hr) after reinfusion of 50% of the shed blood by volume and completing the volume replacement with 50% by volume of Dextran 110.

The better improvement in pulse pressure after Dextran 110 infusion than after volume replacement with shed blood or normal saline may be due to the increase in total oxygen transport capacity which accompanies haemodilution with plasma expanders (Hartsfield, 1985). Optimal O_2 transport capacity has been found to occur at a haematocrit of 25 to 30 per cent. The recorded higher increase in PaO_2 after Dextran 110 infusion than after shed blood reinfusion in the present study is in agreement with the findings of Hartsfield (1985). Improvement in tissue blood flow by Dextran has been reported by Lehtola *et al.* (1986), who showed that dextran increased liver flow by 7 per cent in piglets while normal saline decreased it by 58 per cent. The same workers also reported increases in blood flow to the ileum, kidney and spleen. Increase in tissue perfusion pressure therefore appears to facilitate plasma K^+ uptake after dextran 110 infusion in the present study.

The rise in MABP ($p < 0.01$) after Dextran 110 infusion, the falling plasma K^+ and the decreasing plasma levels of HCO_3^- and base excess are not qualitatively different from those produced by whole blood or normal saline infusion. The inverse relationship between plasma K^+ and HCO_3^- levels is well documented (See Coleman & Young, 1981). The direct relationship between these two ions observed in the present study after volume replacement could be explained by the fact that extracellular fluid volume expansion is usually attended by suppression of reabsorption of fluid in the proximal tubule of the kidney and the delivery of an increased tubular fluid to the distal convoluted tubule where the HCO_3^-/Cl^- ratio and the K^+/H^+ ratio decrease in the reabsorbate (Kunau et al., 1968; Cohen, 1967 and Galla et al., 1977). The mechanism has not yet been established. However, it appears that in the present study, and in agreement with the reported decrease in the ratios referred to above, H^+ and Cl^- concentrations increase while K^+ and HCO_3^- concentration decrease in the reabsorbate following volume expansion. This may have contributed to the fall in plasma K^+ with accompanying fall in HCO_3^-.

Reinfusion acidosis was observed in the present study after volume replacement. The same phenomenon has also been reported for blood reinfusion after haemorrhage by Takacs, Szanto and Vandor (1976). Such reinfusion acidosis cannot be attributed to acidosis developed in the shed blood because even when shed blood was reinfused immediately after withdrawal without storage, the reinfusion acidosis was consistently

reproducible. The short time that elapsed in such cases (75 ± 15 secs) between withdrawal and reinfusion under careful anaerobic conditions was not enough to allow development of acidosis in the syringe. A likely explanation is that it is due to a washout of tissue metabolites by the infused fluid following the severe acute haemorrhage.

The onset of ventricular arrhythmias following withdrawal of blood and those occurring immediately after volume replacement appear to be produced by different mechanisms. While haemorrhage was associated with prolongation of the duration and a decrease in the amplitude of the QRS complex and a reduction in the peak of the T-wave, in contrast, after reinfusion, there appears to be an instantaneous onset of ventricular arrhythmias. The sudden appearance of these reperfusion arrythmias has been attributed by Sewel, Koth and Huggins (1955), and Corbalan, Vernier and Lown (1976) to a condition they describe as "supply-demand mismatch" occasioned by the reinfusion. This mismatch of blood supply to cardiac tissue with zones of different degrees of ischaemia after haemorrhage has been explained by Sewel *et al.* (1955) to result from inhomogeneity in the recovery processes in the different zones of the heart with varying degrees of ischaemia after haemorrhage.

The arrhythmias following haemorrhage or coronary artery ligation have been attributed by Scherlag *et al.* (1970, 1974) and Kaplinsky *et al.* (1978) to marked disturbances of local myocardial activity in the ischaemic zone due to severe delay and fragmentation of activation spreading into diastole.

ECG in the cat during haemorrhagic shock is fully discussed later in this section of the thesis.

It is concluded that Dextran 110 and normal saline can both effectively return the haemorrhage-induced hyperkalaemia and hypotension to normal as can whole blood. Such effects by the Dextran 110 and saline are found to be associated with the enhancement of O_2-carrying capacity of the blood resulting from haemodilution as indicated by the increase in PaO_2. Increase in renal tubular flow rate after extracellular fluid expansion may also contribute to changes in electrolyte resulting in the return of plasma K^+ to normal.

The transient reinfusion acidosis in prolonged hypotension may play a significant role in the cause of irreversibility resulting from sustained hyperkalaemia refractory to reinfusion.

1.18.4 EFFECTS OF BLOOD WITHDRAWAL TO PRODUCE A MABP OF 40 MM HG FOR 120 MIN, AND A MABP OF 80 MM HG FOR 180 MIN

Following the study of the effects of sudden withdrawal of 35% total blood volume and volume replacement on MABP and plasma K^+ for 5 min, it became necessary to investigate the effect of prolonged hypotension to simulate haemorrhagic shock without volume replacement. This made possible the study of the time-course of acid-base changes accompanying alterations in plasma K^+ during haemorrhagic shock up to the stage of irreversibility.

The doubling of the MABP from 40 mm Hg (shock) to 80 mm Hg (above 70 mm Hg) was performed in order to compare the prolonged effects of removing 25-30 per cent, and 5-15 per cent

of the total blood volume, respectively. It has been reported that the baroreceptors mainly concerned with the maintenance of arterial blood pressure are no longer effectively sensitive to changes in MABP - "unloaded" - below 70 mm Hg (Guyton and Crowell, 1961), and that other neuronal, chemical and volume receptors are brought into play to maintain the MABP in order that body functions may continue at this lower level.

The present results indicate that the drop in MABP from 159 $\pm$ 15 mm Hg to 40 mm Hg ($\rho < 0.01$) was accompanied by a hyperventilation which led to a transient respiratory alkalosis in the arterial blood with a venous blood picture that looked like a mixture of respiratory alkalosis and metabolic acidosis within the first two minutes from the end of blood withdrawal. The hyperventilation always preceded and then accompanied a rise in plasma K^+, before generalized acidosis followed in about the 60th min, before which hyperventilation had ceased.

The study indicates that the changes in plasma K^+ and pH are not closely related. This lack of close relationship is demonstrated by the findings that the changes in plasma K^+ reached a plateau level before the 30th min whereas the pH continued to fall throughout the rest of the period after haemorrhage.

The relationship of extracellular pH to plasma K^+ under conditions of haemorrhage and ischaemia is not known with certainty (Poole-Wilson, 1978). The diffusion of carbon dioxide, which rises very quickly during ischaemia across cell membranes should tend to equalize pH in the intracellular

and extracellular compartments (Case, Felix & Castellana, 1979; Poole-Wilson & Cameron, 1975), but differences in buffering capacities may cause differences in pH and base excess (See BE and SBE on Table 8) despite similar values of PCO_2. The time of onset of extracellular acidosis following ischaemia has been found to be as early as 15 to 20 secs after ischaemia (Cobbe & Poole-Wilson, 1980). The fall in extracellular pH stimulates chemoreceptor areas and produces increased respiratory ventilation (Tung, Bettice, Wang and Brown, 1976). The accompanying fall in $PaCO_2$ and end-tidal CO_2, with rise in pH but fall in HCO_3^- in arterial blood represents the well-known arterial blood picture of partially compensated metabolic acidosis. The earliest changes in pH before 75 ± 15 secs, i.e. during blood withdrawal in this study are not known. Be that as it may, the pH changes due to the blood withdrawal during this time seemed to be inadequate to cause the liver, muscle or any organs responsible for the release of K^+ to do so, but seemed adequate coupled with the hypoxia resulting from haemorrhage to trigger a hyperventilatory response. Indeed Fenn and Asano (1955) have reported that there is a threshold CO_2 concentration usually the inhalation of between 10 and 20 per cent in pure O_2 which can induce the liver to release K^+. Below this threshold concentration of CO_2 there is in fact a small fall in plasma K^+ and above which the liver releases K^+. The initial rise in plasma K^+ in the present study during haemorrhagic shock seemed therefore not to be due to a fall

in pH from metabolic acidosis, but accompanied by the hyperventilation-induced alkalosis following haemorrhage.

In this study, mixed venous blood (HIVC) showed an increase in PCO_2, a small reduction in pH and slight increase in standard bicarbonate (SBC) and base excess, which is a respiratory acidosis apparently superimposed on a metabolic acidosis. The contrast between the picture in arterial and venous blood reflects the fact that arterial blood is altered by the response of the respiratory system and the composition of alveolar air, whereas mixed venous blood represents the metabolic changes in peripheral tissues. The data on blood-gas analysis reported here are similar to those earlier reported by Brow *et al.* (1967) and Kircheim and Baubkus (1967).

As time went on after haemorrhage (about the 60th minute), the combination of low HCO_3^-, low pH, and increased PCO_2 in both the arterial and mixed venous blood reflected elevated alveolar PCO_2 and inadequate pulmonary ventilation as well as inadequate tissue perfusion with the resulting low PO_2.

Along with the rise in plasma K^+ during the hyperventilatory phase there was a significant increase ($p < 0.01$) in base excess.

Though there were increases in both aortic and HIVC plasma K^+, the rise in the HIVC was consistently greater than the former. In two experiments in which the K^+ electrodes were inadvertently pushed up through the HIVC into the superior vena cava (SVC) (discovered after post mortem), the K^+ levels there were lower than those either in the

aorta or the low inferior vena cava. The extra K^+ in the HIVC seemed to be coming from the region drained by the hepatic vein above which the tip of the HIVC K^+-electrode was situated, and not from the periphery. It has been reported that K^+ is taken up by skeletal muscle (Treasure, 1977; Treasure and Band, 1978 and Coats, 1985) by the liver (Treasure, 1977; Coats 1983, 1985) and by the heart muscle in alkalaemia (Spurr and Liu, 1966).

The oscillatory excursion of the HIVC plasma K^+ traces (see Fig. 1.20) with no similar oscillations in the aortic plasma K^+ traces may give a clue to the source of the extra HIVC K^+, and rules out artefacts from arterial blood pressure and flow as causes. The oscillations in the HIVC K^+ traces appear to correspond with the oscillations as recorded by the end-tidal CO_2 trace. When the change in plasma K^+ reversed and the K^+ recently released began to be taken up from the circulation, the oscillations inverted with respect to ventilation, that is the phase changed by 180^o (see Fig. 3.8). These findings are in agreement with those observed by Treasure (1977). These oscillations became more marked as the ventilation became faster or slower as seen in the studies with morphine injections (see Fig. 3.8). Venous return into the thoracic cavity is influenced by ventilation with the negative pressure on inspiration encouraging flow into the thoracic vena cava. The only oscillatory excursion in the aortic plasma K^+ recorded in the present study is shown in Figure 3.8(a). In this case, the tip of the aortic electrode catheter which was inserted through the right common carotid artery was found

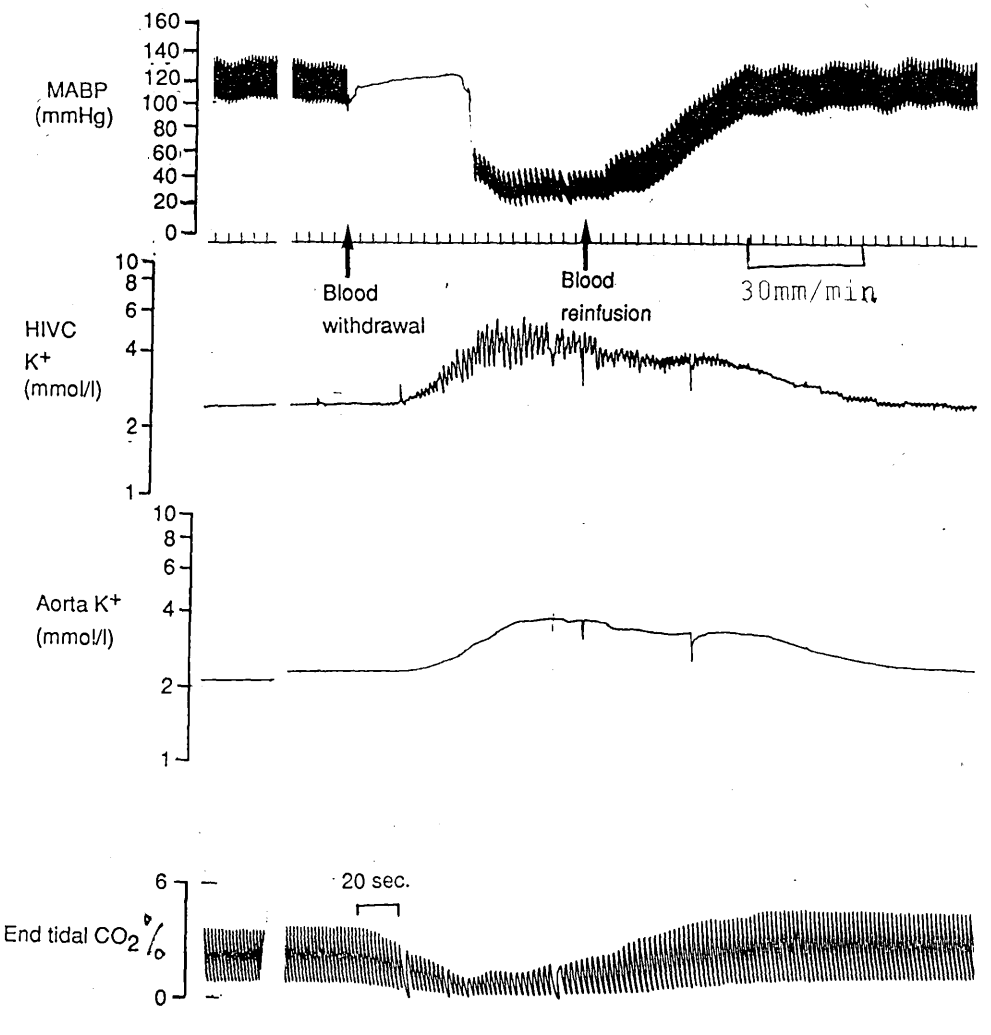


Fig.1-20. Effect of withdrawal of 25% total blood volume on the HIVC and aortic plasma K+, and on the end-tidal CO2, illustrating the occurrence of hyperventilation of about 20 secs before the rise in plasma K+ following haemorrhage.

to be lying right in the left ventricle at post mortem examination. As reported above, blood of different K^+ concentrations from the SVC, HIVC passes through the heart and eventually enters the inferior vena cava, and if they are incompletely mixed, a fast responding sensor (as the K^+ electrodes used here are) will respond to different K^+ levels as they go past the electrode tip. Also, artefacts resulting presumably from the rubbing of the aortic electrode catheter tip against the wall of the left ventricle may contribute to the oscillatory excursion in the aortic plasma K^+ trace. The descent of the diaphragm has a pinchcock effect on the inferior vena cava, reducing return from the abdominal vena cava (Franklin & Ranker, 1934), but the increase in abdominal pressure increases flow above the diaphragm from the splanchnic and hepatic venous beds (Alexander, 1951) via the hepatic vein which enters the inferior vena cava. On inspiration therefore, the HIVC tends to fill from the hepatic vein, and on expiration a greater contribution comes from the abdominal IVC. If the phasic nature of this venous return and the incomplete mixing in the large veins are considered, a K^+ sensor in the IVC between the diaphragm and the heart (i.e. the HIVC here) might well be exposed alternately to blood from the hepatic vein and from the abdominal IVC (Treasure, 1977). This is the probable explanation for the oscillations seen in the HIVC. Because the source of K^+ is endogenous rather than from an injected bolus the oscillations were seen at their greatest when the maximum change in K^+ was occurring (see Fig. 3.4(a)),

for example after the injection of adrenaline, or just at the end of haemorrhage (Fig. 1.20). The source of the extra increase in HIVC plasma K^+ is therefore suggested to be from the liver and the area drained by the portal vein. This study does not provide any data to show whether the splanchnic region is the source of the extra K^+ which is passed through the portal vein into the liver and then passed on into the hepatic vein, or whether the liver itself actually releases more K^+ into the hepatic vein than it receives from the portal vein.

Cause of K^+ release

There is abundant evidence that adrenaline and other catecholamines can cause a transient elevation in plasma K^+ concentration in cats (Da Silva, 1936) and in man (Muirhead, Goth and Jones, 1954), the source being the liver. However, the present findings do indicate that the plasma K^+ elevation during the alkalotic phase early in haemorrhagic shock may not all be mediated by epinephrine or norepinephrine. Though increases in plasma levels of catecholamines during haemorrhage (Darby & Watts, 1964) and during hyperventilation (Mason & Medbak, 1983) have been documented, the K^+ concentration elevated by i.v. catecholamines subsequently falls below control levels (Da'Silva, 1935; Treasure, 1977; Treasure and Band, 1978; Coats, 1983, 1985). No such fall in plasma K^+ below control was observed after acute withdrawal of blood. Evidence that raised level of plasma K^+ can be produced in subjects without a significant rise in plasma levels of catecholamines was provided by the studies of Luft and von Suler (1953) who found that subjects with postural

hypotension excrete much less epinephrine and norepinephrine in response to hyperventilation producing alkalosis than do normal subjects, yet they show a rise in plasma K^+. This evidence indicates that potassium elevation occurs in normal subjects during respiratory alkalosis without the collateral effects, such as causing the plasma K^+ level to fall below control which might be expected if it were mediated by epinephrine, and that the elevation can occur in subjects whose ability to produce epinephrine and norepinephrine is greatly impaired. Respiratory alkalosis induced a rise in the serum K^+ concentration of an adrenalectomized subject and also a subject with idiopathic postural hypotension (Luft and von Euler, 1953). The cause and effect relationship of hyperventilation and increase in plasma K^+ is the subject of a further investigation discussed later in this section.

Na^+-K^+-ATPase

Wurth, Sayeed and Bane (1971) reported a significant increase in Na^+-K^+-ATPase activity in the liver of animals with prolonged haemorrhagic shock. The electrolyte shifts may well be related to changes in Na^+-K^+-ATPase activity. An increase in intracellular Na^+ consequent on an initially depressed activity of the sodium pump mechanism due to the haemorrhagic shock appears to be a plausible explanation of this increased activity. The mechanism of activation of the Na^+-K^+-ATPase by catecholamines is poorly understood but seems to involve cyclic-AMP-induced activation of a protein kinase (Scheid, Honeyman & Fay, 1979). The increased liver Na^+-K^+-ATPase activity during haemorrhagic shock is reversible

by reinfusion of the shed blood (Wurth, Sayeed and Bane, 1971), but whether the change in ATPase activity is a primary or a secondary response to electrolyte changes in shock is not certain.

The greater rise of plasma K^+ in the HIVC than in the aorta might suggest either an uptake by the heart or the lungs, or a dilution by blood with less K^+ concentration from the upper limbs, head and neck. The ensuing acidosis which replaced the initial transient alkalosis, from about the 5th minute might be a result of an accompanying anaerobic metabolism with production of lactic acid and a reduction in plasma HCO_3^- (Tung, Bettice, Wang and Brown, 1975).

Some adverse conditions contributing to the increasing acidosis have been reported. Alterations of the alveolar surfactant in haemorrhagic shock has been reported to add to the increasing oxygen deficit (Reffy, Takacs, Demel and Jozsa, 1980). Intracellular acidification which primarily occurs in haemorrhagic shock before extracellular acidosis has been shown in recent studies to increase the outward K^+ current (Sato et al., 1985). It might also lead to the impairment of glucose metabolism (Williamson et al., 1976), resulting in a decrease in the intracellular ATP level. This intracellular acidification may activate the ATP-regulated K^+ channel (Noma, 1983; Trube and Hescheler, 1984) and also a shortage of energy supply for the Na^+-K^+-ATPase activity which could cause the accumulation of intracellular Na^+ and Ca^{++}, and extracellular K^+. The increasing acidosis with accompanying hyperkalaemia recorded in the present study

in the later phase of haemorrhagic shock might be similar to the sequence of metabolic events caused by the impairment of glucose metabolism as described above.

Other investigators (Burget & Visscher, 1927; Watts, 1956; Page, 1946) have shown that as the pH of the blood decreases during haemorrhagic shock, vascular smooth muscles lose their responsiveness to catecholamines. Towards the 90th minute after blood withdrawal, there followed a seemingly increasing effect of acidosis and increasing CO_2 content on K^+ efflux as the K^+ uptake effect of the sympatho-adrenal response declined. This explanation is based on the available data from this section of the present study and does not exclude other mechanisms yet unknown, which may be responsible for the hyperkalaemia following the haemorrhage. Indeed, the role of endogenous opioids (as mimicked by intravenous morphine in a subsequent study), which are suspected to be secreted under the stress of haemorrhagic shock has been investigated and will be discussed later in this thesis.

1.18.5 HYPOTENSION MAINTAINED AT 80 mm Hg MABP

A prerequisite for elucidating the mechanism of haemorrhagic shock is a reproducible experimental model leading to a predictable outcome. The method employed here using fixed hypotension levels at different severities (40 and 80 mm Hg) proved a good predictor of the outcome. Other workers (Schoenberg *et al.*, 1985) have used other methods like employing a fixed hypotension level and determining total oxygen deficit and shed blood volume

uptake as hypotension continued. Uptake volume is the volume of blood required for subsequent reinfusion to maintain the desired MABP. They found that the non-surviving animals showed the need for a higher uptake volume to maintain MABP at the desired experimental shock level, indicating an incipient collapse of the microcirculation (Schoenberg et al., 1985). Decades of studies have solved some of the problems concerning the pathogenesis of haemorrhagic shock, but the mechanism of irreversibility is still not fully understood. Jesch et al (1973) subjected dogs to a continuous haemorrhagic hypotension of 40 mm Hg for 3 hours and observed that the dogs differed in the amount of withdrawn blood that had to be reinfused at the end of the hypotension phase to maintain the MABP at 40 mm Hg. By doing this, they were able to show that this so-called "uptake volume" correlated directly with the mortality rate. Crowell and Smith (1964) found an excellent correlation between oxygen deficit and irreversibility of haemorrhagic shock. In contrast, Rothe (1968) in dogs, and Smart and Rowlands (1972) in rats found no correlation between oxygen deficit and survival. The existence of conflicting and not easly interpreted data has been pointed out by Gump (1971).

Whereas Schoenberg et al. (1985) did not find any correlation between the duration of the hypotensive period, decrease in pH, oxygen deficit and survival of the animals except "uptake volume", the present study suggests a correlation between the degree of hypotension, the duration of haemorrhage, fall in pH, rise in plasma K^+ levels and

the survival of cats during haemorrhagic hypotension.

In the present study in which two fixed hypotensive levels were used, it took a longer duration (over 3 hours) before the higher irreversible level of K^+ occurred in cats with the MABP at 80 mm Hg. In cats at 40 mm Hg, it was just over 90 minutes before this occurred, and reinfusion of the shed blood at this stage failed to revive the animals. In the 80 mm Hg group of cats, the pH and its determinants turned acidic by the 180th minute, whereas this occurred by the 60th minute in the 40 mm Hg group of cats. The shifting of the slope of the pH parameters to the right in the 80 mm Hg group was a significant difference from the 40 mm Hg group. Another interesting difference between the two sets of animals was that the volume of topping up blood required to maintain the MABP at 40 mm Hg was up to 5% within the 120 minute period, while in the case of the 80 mm Hg group of cats smaller quantities of blood summing up to 2% extra was withdrawn to maintain MABP at 80 mm Hg, and it was only after the 180th minute that topping-up was required.

A major interesting finding in this study was that in the 80 mm Hg group of cats, after haemorrhage, the plasma K^+ levels fell to near control values without reinfusion of the shed blood. Indeed the aortic plasma K^+ level fell below the control value before returning to it. There was no such fall to the control values from the first level of plasma K^+ rise in the 40 mm Hg group. The plasma K^+

levels stayed high and increased further to irreversible stages in the 40 mm Hg group. In both the 40 mm Hg and 80 mm Hg groups of cats the initial response in plasma K^+ and the associated changes in acid-base balance were qualitatively similar but different in magnitude, that is, an early respiratory alkalosis which was followed by a gradually developing metabolic acidosis.

There is a correlation between the degree of haemorrhage and the time-course of events leading to irreversibility. Plasma K^+ elevation and fall in arterial PO_2 and pH seemed to be important factors in producing irreversibility, because even after reinfusion of the shed blood later in shock, the blood became more acidic with further rises in plasma K^+ although there was a transient rise in MABP. The marked Q-waves and the peaked T-waves persisting up to 25 min after loss of recording of arterial blood pressure and breathing suggest myocardial infarction in the presence of hyperkalaemia or acidaemia. (see review on ECG). Such development into myocardial infarction may be prevented if blood or fluid infusion is carried out during the first maximum rise in plasma K^+ before it forms a second plateau.

1.18.6 EFFECTS OF MECHANICAL HYPERVENTILATION ON MABP AND PLASMA K^+

The consistent observation in previous studies that a hyperventilatory phase followed haemorrhage and preceded a rise in plasma K^+ stimulated this further study. Reports on the association between hyperventilation and changes in plasma K^+ in both man and experimental animals are conflicting.

Consequently, in the present study, the time-course of hyperventilation and its relation to plasma K^+ changes was investigated with simultaneous monitoring of end-tidal CO_2 and plasma K^+. An attempt was made to investigate the relationship between these two responses using adrenoceptor- and opiate-receptor blockers.

The results of the present study show that mechanical hyperventilation with room air for 5 min. produced a significant rise ($\rho < 0.01$) in plasma K^+. The rise in plasma K^+ following hyperventilation appears to be mediated by endogenously released opioids and catecholamines. Involvement of the opioid-receptors is suggested by the significant reduction ($\rho < 0.01$) in the rise of hyperventilation-induced plasma K^+ by naloxone, an opioid-receptor blocker (Holaday, 1982). While prazosin or phentolamine, both α-adrenoceptor blocking agents did not significantly reduce the hyperventilation-induced rise in plasma K^+, propranolol, a β-adrenoceptor blocking agent significantly reduced ($\rho < 0.01$) the hyperventilation-induced hyperkalaemia.

Evidence that plasma levels of opioids and catecholamines increase during mechanical hyperventilation has been shown by the recent works of Mason and Medbak (1983), and Mason, Medbak and Rees (1987), all in greyhounds.

A relationship between the plasma level of K^+ and hyperventilation has been recently demonstrated by Linton and Band (1985); Band, Linton, Kent & Kurer (1985). These workers reported that intravenous injection of KCl raised

arterial K^+ which produced a burst of chemoreceptor activity leading to hyperventilation.

Involvement of β-adrenoceptors in the mediation of K^+ release from isolated liver slices has been reported by Castro-Travares (1975), Cocks et al., (1984) and Coats (1986). The marked species differences in the effects of adrenoceptor agonists on plasma K^+ in vivo has been highlighted by Coats (1986). It is therefore not surprising in the present study that β-adrenoceptors are found to mediate the mechanical hyperventilation-induced hyperkalaemia, whereas the same receptors mediate plasma K^+ uptake following adrenaline injection (Treasure, 1977; Coats, 1985) or moderate exercise (Linton et al., 1985).

Morphine has been reported to induce K^+ efflux in de-energized liver cell mitochondria (Chistyakov, 1980). This also lends some support to the present finding that naloxone reduced the hyperkalaemia resulting from hyperventilation which is suggested to be partly mediated by opioid receptors.

It is probable that the opioid- and adrenergic-receptors co-exist either in the same or different tissues of the body responsible for releasing K^+ during acute changes in the pH of their extracellular fluid. The work of Mason et al. (1987) and the present finding that naloxone or propranolol reduced the hyperkalaemia produced by the hyperventilation-induced alkalosis give some support to the above probability. In addition, the co-release of enkaphalin and catecholamines from adrenal chromaffin cells have recently been reported

by Livett et al. (1981), while Smith and McCabe (1986) have reported the mediation of K^+ conductance in the rabbit colon by β_1-adrenoceptors.

The consistent recording of higher levels of plasma K^+ in the HIVC than in the aorta lends further support to the view that the region drained by the hepatic vein contributes more K^+ than the head and neck, the lungs or the lower regions of the body to the rise in plasma K^+ during mechanical hyperventilation. The lower value of K^+ in the aorta may be due also to dilution by blood from the superior vena cava, vena azygos or uptake by the lungs or the heart.

With fast K^+ sensing electrode catheters as used in the present study, the rise in plasma K^+ produced by haemorrhage is always recorded 20 to 25 secs after hyperventilation has occurred producing an initial transient alkalosis (See Fig. 1.20). The underlying hypoxia which usually accompanies severe haemorrhage together with the resulting hyperkalaemia appear to be the sustaining stimuli for the continuing hyperventilation after haemorrhage.

Later, after the haemorrhage, the cause of the failure of the raised plasma K^+ to further enhance hyperventilation remains to be determined, for Band et al. (1985) have reported that hyperkalaemia produced by infusion of KCl caused a hyperventilatory response via the stimulation of the carotid body chemoreceptors. The results of the present study suggest that if such hyperkalaemia-induced hyperventilation exists, it depends on the PaO_2 and $PaCO_2$ of the blood at the point in time, because as haemorrhagic hypotension continues with decreasing PaO_2 and increasing

$PaCO_2$, hyperventilation fails to continue in the presence of increasing hyperkalaemia.

1.18.7 ECG IN THE CAT DURING HAEMORRHAGIC SHOCK AND THE ROLE OF K^+ IN THE CAUSE OF DEATH

In the present study, the way in which plasma K^+ disturbance is demonstrated by changes in the lead II ECG is considered along with other changes in blood pH/gases, end-tidal CO_2 and MABP. It is uncommon in both pathological and experimental situations to find a pure or single electrolyte disturbance, and disturbances in the other electrolytes including hydrogen ion can change the ECG in such a way as to simulate especially the pattern of hypokalaemia (Burgh, 1972).

The recording of the time-course of changes in the ECG and plasma K^+ has been facilitated by the use of the valinomycin-based K^+-selective electrode catheters suitable for continuous intravascular monitoring. The correlation of the lead II ECGs to changes in plasma K^+, MABP and blood pH which leads up to death in late and irreversible haemorrhagic shock is an important improvement on previous techniques and even enabled us to follow the plasma K^+ concentrations up to immediately after the cessation of respiration.

The results of the present study show that the T-waves decrease in amplitude at the same time as the decrease in the amplitude of the QRS-complex (Fig. 1.21) as opposed to the expected usual peaking of the T-wave in hyperkalaemia. As haemorrhagic hypotension continues with increasing levels of K^+, the peaking of the T-wave occurs independently of

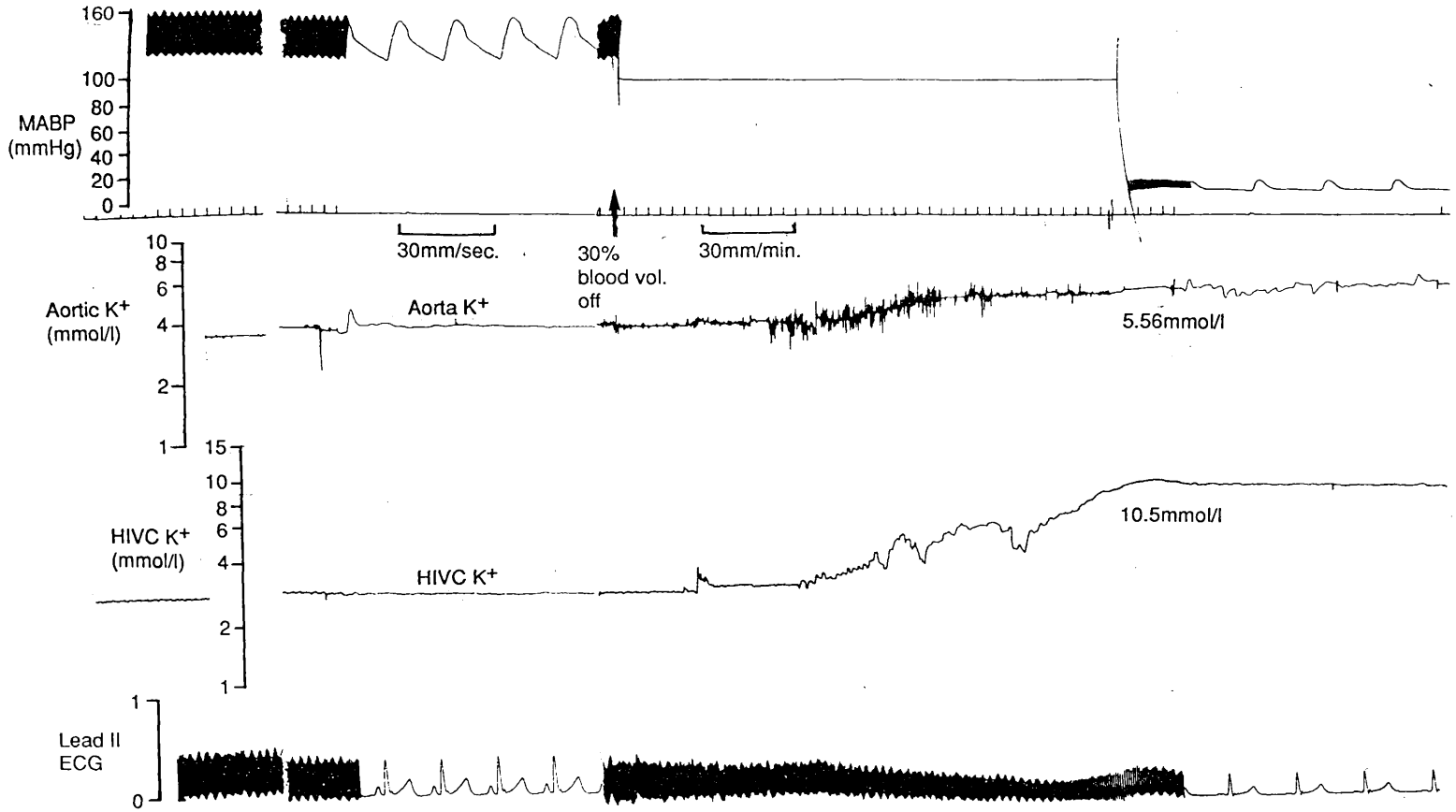


Fig.1-21. Effects of acute withdrawal of 30% total blood volume on the MABP, plasma K+ and the ECG, illustrating the decrease in the amplitude of the T-wave with the attendant bradycardia during the haemorrhage-induced hyperkalaemia.

the changes in amplitude of the QRS-complex accompanied by shortening and widening of the P-wave and prolongation of the P-R interval. Eventually the P-wave disappears along with the widening of the QRS-complex and irregular R-R intervals when the plasma K^+ level approaches 9 to 10 mmol/l.

In most cases an abrupt rise in K^+ occurs when breathing ceases while the heart is still beating, and the ECG continues to indicate slow ventricular ectopic beats with peaked T-waves and marked Q-waves some 15 to 25 min. after MABP has fallen to zero with cessation of breathing (Fig. 1.22).

The decrease in the amplitude of the T-wave instead of an increase in hyperkalaemia following severe haemorrhage seems to be an activity secondary to a primary effect of haemorrhage on the QRS-complex. Oberg and Thoren (1971) have reported that severe haemorrhage in cats causes the contraction of the nearly empty ventricular walls against themselves and thereby excites their vagal afferents which result in an inhibitory vaso-vagal reaction. This inhibitory vaso-vagal effect is shown in the lowering of the QRS-complex in the present study (see Figs. 1.18(g) and 1.21). The unusual low amplitude of the T-wave in the presence of hyperkalaemia under such a reduced QRS-complex has been reported to be secondary to the vaso-vagal effect on the QRS-complex by Burgh (1972). The primary effect of hyperkalaemia: decrease in the QRS-complex and elevation of the T-wave are usually observed later in haemorrhagic shock when acidosis is present as indicated by blood gas analysis. Modification of the effects of changes in K^+ metabolism by

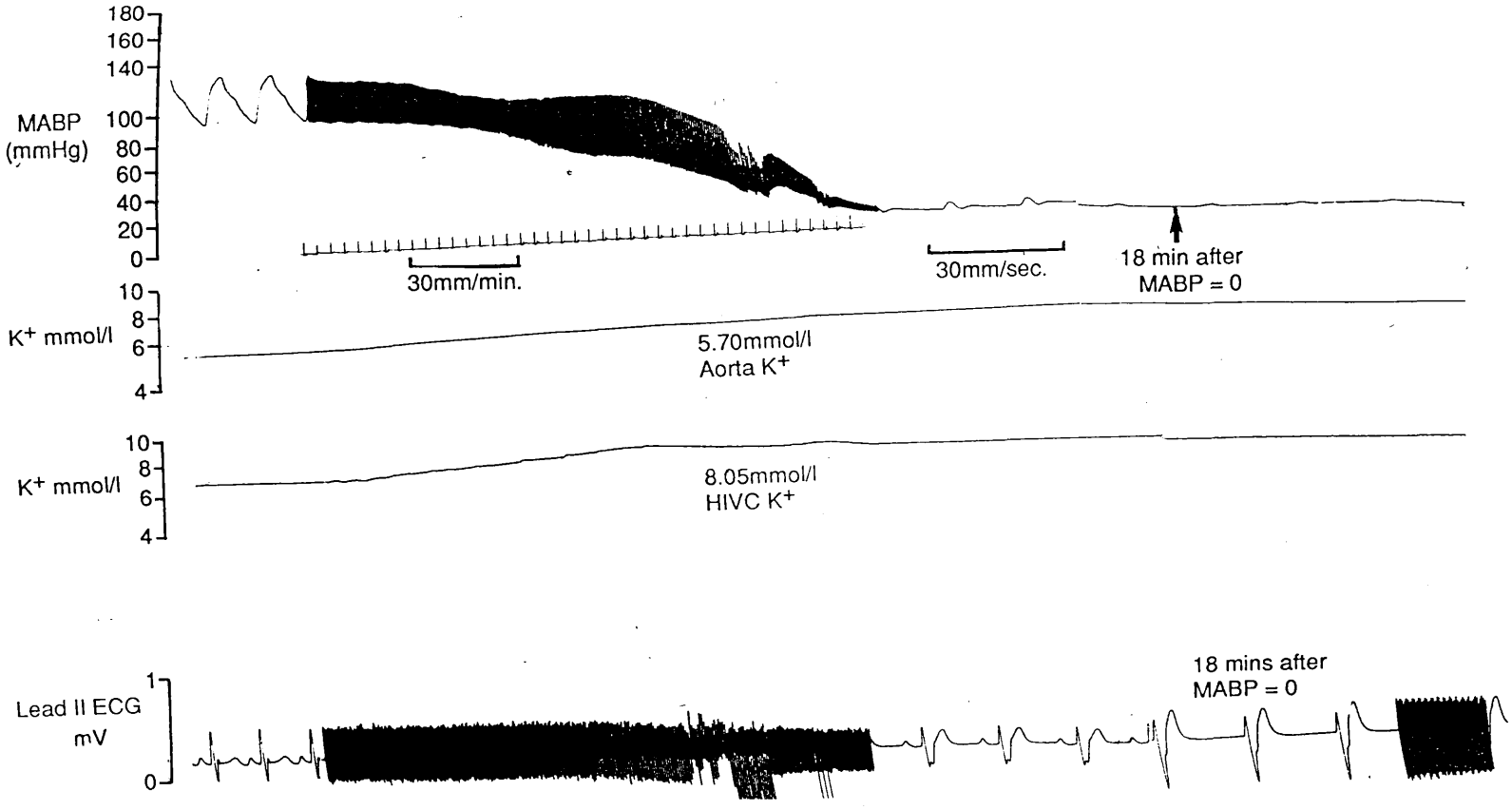


Fig.1-22. Late phase of haemorrhagic hypotension illustrating irreversible cardiac function in moderate hyperkalaemia (8.05mmol/l in the HIVC and 5.70 mmol/l in the aorta) with bradycardia and ventricular ectopic beats for over 15 mins after the MABP has fallen to zero.

changes in acid-base balance has been reported by Young, Sealy and Harris (1954). The present results therefore show that the electrocardiographic changes during haemorrhagic shock are caused by a combination of hypoxia (low PaO_2), and lack of washout (hyperkalaemia and acidosis) as indicated by increased $PaCO_2$ and low pH.

The action potentials of ischaemic cells have been shown to be smaller than those of normoxic cells at similar reduced levels of resting membrane potential by Morena et al. (1980).

Hirche et al. (1982) have reported that during severe ischaemia resulting from haemorrhage or coronary occlusion, Na^+ and Ca^{++} are taken up by the myocytes while K^+ and H^+ are released. Such ionic redistribution results in changes of the Na^+ and K^+ equilibrium potential which affects the ECG. In addition to this ionic redistribution, systemic and local myocardial catecholamine release has been shown to be another major factor in the genesis of postischaemic ventricular arrhythmias and ventricular fibrillation (Hirche et al., 1980; Abrahamsson, Almgren & Sensson, 1981.

Evidence has been presented that impairment of the myocardium is a factor in the unfavourable response to shed blood infusion after haemorrhage (Wiggers, 1945). In the present study, reinfusion of shed blood induces ventricular dysrhythmias which appear to depend upon different yet characteristic electrophysiological derangements which are different from those produced by sustained haemorrhagic shock as demonstrated by the lead II ECG. The dysrhythmia induced by haemorrhagic shock is found to be gradual in

onset and seems to be primarily a result of re-entrant excitation secondary to an increased conduction time throughout the ischaemic heart as shown by the prolongation of the P-R and the Q-T intervals. Such re-entrant excitation has been shown to be accompanied by asynchronous depolarization and shortening of the refractory period by Penkosko et al. (1978). On the other hand, dysrhythmia induced by re-infusion seems to depend on a "demand-supply-mismatch" whereby different regions of the myocardium with varying degrees of ischaemia might react differently to reinfusion and probably result in electrical inhomogeneity in the myocardium. Reinfusion arrhythmias apart from being sudden in onset usually deteriorate to ventricular fibrillation within 5-30 secs if hypotension has continued for over 90 min in the present study. The extent or uniformity of the return of blood flow to ischaemic tissue is not evaluated by the present study and so haemorrhagic or reinfusion ventricular arrhythmias cannot be correlated with the extent of ischaemic injury.

Thus the present study suggests that multiple mechanisms are responsible for reinfusion ventricular arrhythmias. First there is rapid washout of the metabolites, potassium and other accumulated substances that contribute to changes in the ECG observed during severe haemorrhage. This washout should facilitate the rapid recovery of electrophysiologic properties of affected myocardial cell membranes and presumably plays a role in the occurrence of the instantaneous

re-infusion arrhythmias. In the rapid transition from depression of electrical activity as shown in the reduced QRS-complex, to almost normal activity, there is a transient period of partial recovery highlighted by increased amplitudes of the P-wave, the QRS-complex and the T-wave, conducive to a re-entrant mechanism. Secondly, a rapid but non-uniform recovery of severely depressed tissue as occurs after prolonged haemorrhagic shock may result in greater inhomogeneity of the myocardial electrophysiologic properties contributing further to this arrhythmogenic environment of the heart. And, in fact, if blood flow is restored slowly, the incidence of re-infusion arrhythmias is less than with rapid re-infusion. Thirdly, it is also possible that acute re-infusion may subject the myocardium to additional hyperaemic and subsequently haemorrhagic injury as reported by Bresnahan, Roberts and Shell et al. (1974). Fourthly, enhanced α-adrenergic responsiveness during myocardial ischaemia could be responsible for the reinfusion ventricular arrhythmias induced by local myocardial catecholamines released during haemorrhagic hypotension and reinfusion. In cats, similar contribution by α-adrenoceptors to dysrhythmia during myocardial ischaemia and reperfusion has been reported by Sheridan et al. (1980). The limitations in methodology of the present study do not allow further speculation than to suggest the possible role of delayed or partial recovery of either myocardial or specialized Purkinje fibres as the etiology of the reinfusion arrhythmias.

Another finding of interest in the present study is that, occasionally the plasma K^+ concentration in some cats never exceeded 8 mmol/l (Fig. 1.22) and indeed is falling before the final gasp occurred. The reason why the K^+ rises higher than 8 mmol/l in some experiments but not in others is obscure. It seems reasonable to interpret the sequence involving plasma K^+ levels exceeding 8 - 10 mmol/l at death with deranged ECG complexes as a secondary reaction of the agonal electrocardiogram to the terminal release of K^+. Whether this release of K^+ is local or generalized is not known. It is true that these terminal increases in plasma K^+ levels might themselves be responsible for certain of the final stages in the ECG sequence, and that these changes may therefore simulate those of spontaneous K^+ poisoning. The occurrence of sharp increases in plasma K^+ concentrations with associated derangements in the ECG only after respiration has ceased in some animals in the present study makes it tempting to suggest that hyperkalaemia is the effect and not the cause of death in such cases. That apart, in many instances unusually high plasma K^+ levels were recorded while the heart was still beating after severe haemorrhage with the only changes in the ECG being lowered amplitude of QRS-complex, depression of the S-T segment and decrease also in the amplitude of the T-wave (See Fig. 1.23). All of these changes are reversible with reinfusion of the shed blood or dextran 110 if the plasma K^+ level is not allowed to rise further to new higher concentration levels.

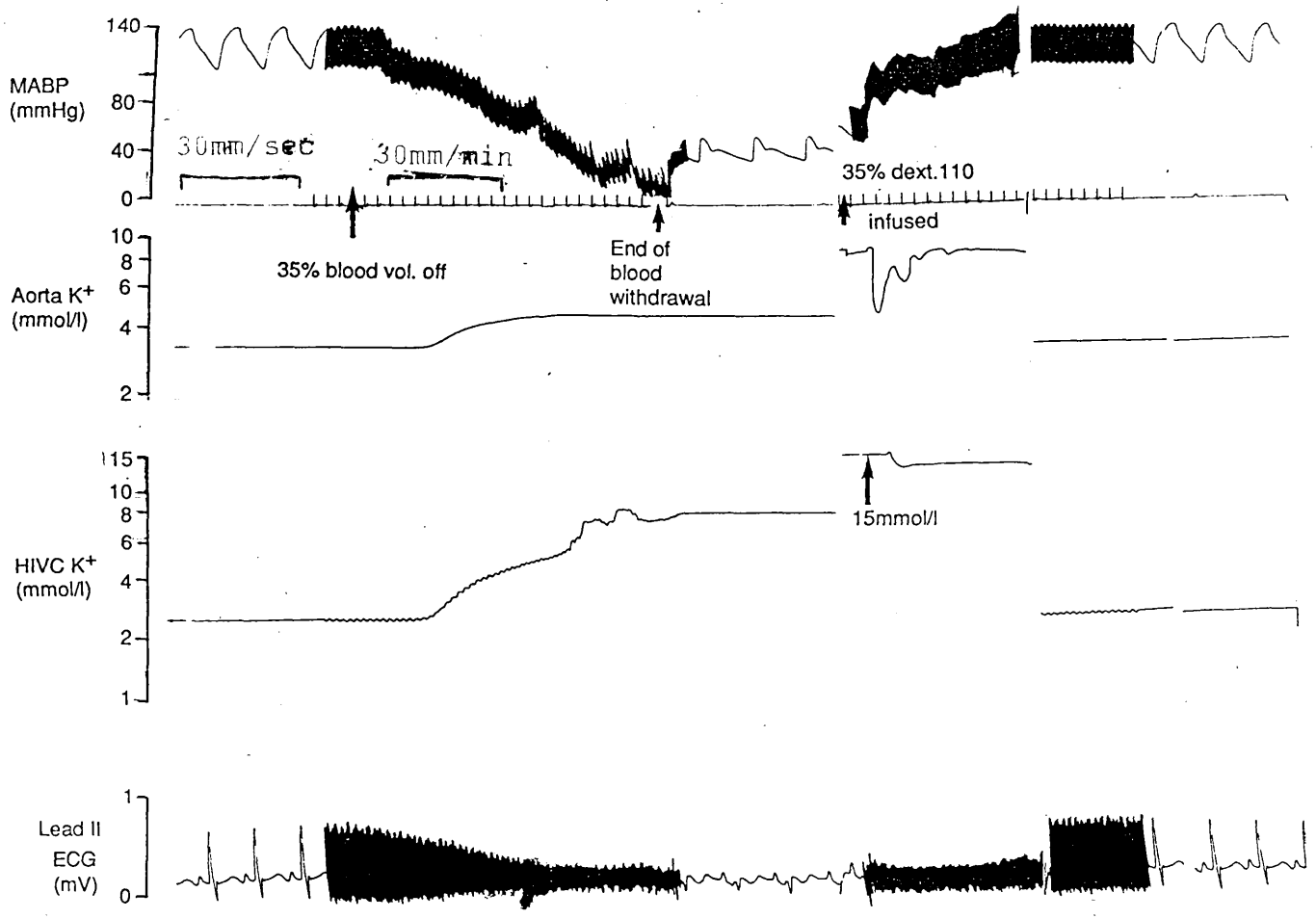


Fig.1-23. Severe hyperkalaemia foliowing acute withdrawal of 35% total blood volume and the restoration of normal MABP, plasma K+, and the ECG after volume replacement with dextran 110 early in the haemorrhagic hypotension.

1.19 SUMMARY

A study of the effects of haemorrhagic shock on plasma K^+ using ion-selective electrode catheters has been described.

These potassium-electrodes (valinomycin-based) were found to be sensitive to K^+ beyond 90% of the standard Nernstian response for monovalent cations.

Increasing the amount of total blood volume withdrawn from 5 to 35% caused corresponding increases in the elevation of plasma K^+. Significant fall in MABP and rise in plasma K^+ occurred from loss of 15% of total blood volume and over. Maximum levels of plasma K^+ ranged from 8 to 12 mmol/l after 35% haemorrhage, and over 14 mmol/l at death.

Blood pH/gas analysis during haemorrhage showed that the initial rise in plasma K^+ was always preceded by a hyperventilation-induced alkalosis. The hyperventilation resulted from hypoxia following haemorrhage. The simultaneous determination of pH parameters lent some credence to this finding. The later time-course of events associated with hyperkalaemia were found to be associated with the increasing metabolic acidosis as shock progressed.

The hyperventilation-induced hyperkalaemia was found to involve β-adrenergic and opiate receptors because this effect was significantly reduced by propranolol, and naloxone, respectively.

The site of release of K^+ was suggested to be the region drained by the hepatic vein. This was consistently indicated by higher levels of K^+ in the HIVC which drains from the hepatic vein as compared to the levels in the aorta

or the lower inferior vena cava plasma K^+ below the hepatic vein.

Two plateaux of plasma K^+ levels were observed during haemorrhagic shock. The end of the first and/or the beginning of the second was found to be the warning sign of end of reversibility of haemorrhagic shock.

The lead II ECG showed that rising levels of plasma K^+ above 7 mmol/l started to affect the amplitudes and the duration of the waves and segments, respectively, of the ECG. Continued rise in plasma K^+ levels associated with metabolic acidosis complicated the agonal ECG, leading to atrial and ventricular dysrhythmias followed by death. The sudden sharp increases in plasma K^+ levels recorded in some cases only after respiratory failure with accompanying arrhythmias suggested that these effects were only results and not causes of death, though the raised levels of K^+ preceding the sharp rise during shock might have contributed to the deterioration of myocardial function.

SECTION TWO

2. COMPARATIVE STUDIES OF THE EFFECTS OF ADRENALINE, MORPHINE, ASPHYXIA AND HAEMORRHAGE ON MABP AND PLASMA K^+

2.1 INTRODUCTION

This section of the present study includes an investigation of the changes in MABP with the rise in plasma K^+ produced by asphyxia, haemorrhage, adrenaline and morphine injections. Asphyxia-like conditions with very low PaO_2 and high $PaCO_2$ levels have been observed in Section One of this study and the review of the literature shows that increased plasma levels of catecholamines and endogenous opioids occur following haemorrhage. The present study is therefore carried out to investigate and compare the changes in MABP and plasma K^+ produced by adrenaline, morphine and asphyxia with the haemorrhage-induced changes.

The effects of haemorrhage, adrenaline, noradrenaline and morphine on MABP and plasma K^+ have been reviewed, investigated and discussed separately in Sections One and Three of this thesis. Therefore a brief review of only asphyxia is given here.

2.2 ASPHYXIA

In asphyxia both oxygen lack and CO_2 excess occur simultaneously causing hyperpnoea. This was demonstrated by Haldane and Priestley in 1935 in their experiments in which they allowed their subjects to breathe air containing varying amounts of carbon dioxide up to 6 per cent.

The answer to the problem whether the two stimuli of oxygen lack and CO_2 excess are simply additive or, whether

the response to CO_2 is amplified or sensitized by hypoxia has been provided by the work of Nielson and Smith (1951) and by Lloyd, Jukes and Cunningham (1958). Both these teams of investigators examined the effect of increasing the alveolar PCO_2 on breathing while keeping the alveolar PO_2 steady at various values. The response of respiration to CO_2 was strikingly greater when the alveolar PO_2 was maintained throughout at 40 mm Hg than when it was kept at the normal value of 100 mm Hg. Anoxia and hypercapnia interact; it appears that anoxia sensitizes the response of the respiratory mechanism to excess CO_2 (and H^+), and so the two stimuli are not simply additive.

Other responses to asphyxia include vasoconstriction with a rise of MABP, a feature of the early stages of asphyxia produced, for example by occlusion of the trachea (Ghosh and Koley, 1980). These workers reported that asphyxia produced an immediate increase in MABP in cats, and such a vasopressor response was blocked by rogitine, an α-blocking agent, and not by propranolol, a β-receptor blocker. The pressor response was followed by a slowing of the heart via the carotid sinus and aortic arch reflexes, because when these reflexes were abolished by section of the vagi the rise of MABP during asphyxia was much greater.

A great increase in plasma lactate level during asphyxia has been reported by Dellenbach (1982). These findings are so consistently reproducible that Dellenbach suggested that a rise in lactate levels could be an indicator for foetal and neonatal asphyxia.

The cardiovascular response to asphyxial challenge in chronically hypokalaemic dogs rendered so by frusemide infusion has been investigated by Wong, Port and Steffins (1983). These workers found that in both normokalaemic dogs and those rendered hypokalaemic, serum K^+ levels at the end of clamping of the trachea were significantly above control levels of K^+. A sudden rise in plasma K^+ during asphyxia, and a fall in anoxic hypoxia have both been reported by Ferguson and Smith (1958). I am unaware of any one study measuring the changes in MABP, plasma K^+, ECG, and expiratory CO_2 continuously and simultaneously during asphyxia.

The present study was conducted to follow the time-course of changes in MABP, plasma K^+ concentration and lead II ECG during asphyxia, and to compare with data from similar studies obtained by adrenaline or morphine injection and those produced by haemorrhagic hypotension. An attempt was then made to investigate the mechanism of K^+ release by the above challenges using adrenoceptor- and opioid-receptor blocking agents.

2.3 MATERIALS AND METHODS

The basic set-up and surgical procedures were the same as those for the experiments in Section One, where haemorrhage (25 per cent here), and i.v. adrenaline injections (2 µg/kg) were used, and the same as those used for the experiments in Section Three where morphine, 8 mg/kg i.v. was given.

As shown below, the alpha-adrenoceptor blockers, phentolamine and prazosin, the beta-adrenoceptor blocker propranolol, or the opioid receptor blocker, naloxone were given before the respective agonist, adrenaline or morphine was administered. After observing the effects produced by haemorrhage or asphyxia, either an adrenoceptor or an opiate receptor antagonist was then given and each procedure was repeated separately in the presence of the required blocking agent.

The orderly sequence of procedures involving drugs, asphyxia or haemorrhage were as follows:

2.3.1 ASPHYXIA: Under deep anaesthesia produced by intraperitoneal injection of pentobarbitone sodium, 45 mg/kg, spontaneous inspiration of room-air was cut off for 2 min by plugging the inlet of the tracheal tube with a rubber plug. The outlet into the CO_2 analyser via the side-arm tubing was left open during this period of asphyxia. After the 2 min period the animal was allowed to breathe room air spontaneously or in some instances mechanically hyperventilated with room air to see if there is any difference in the direction or magnitude of change in plasma K^+ by the mechanical assistance.

2.3.2 HAEMORRHAGE: 25 per cent of the cats' total blood volume was quickly removed and observations were made for 5 min and the shed blood was then reinfused. In the presence of either an adrenoceptor blocking agent or an opioid receptor blocker, the above procedure was repeated.

2.3.3 ADRENALINE: 2 µg/kg adrenaline was injected intravenously and observation made for 5 min. This was repeated after either an adrenoceptor or an opioid receptor blocking agent was injected.

2.3.4 MORPHINE: 8 mg/kg was injected intravenously and observation made for 5 min. Repeat observations were then made in the presence of an adrenoceptor or an opioid receptor blocking agent.

Results are expressed as means ± standard errors of the mean, and p values less than 0.05 are taken as significant.

2.4 RESULTS:

2.4.1 EFFECTS OF ASPHYXIA

The results of the experiments on asphyxia are shown in Figures 2.1 and 2.2.

When the tracheal cannula was occluded, the respiratory movements observed in the cat were at first increased in depth and rate. The breathing became very forceful and the expiratory efforts more and more powerful. This type of breathing was followed by a stage of slow deep inspiratory efforts. Within 1 min of occlusion of the tracheal cannula, there was a rise in the MABP from a control level of 103 ± 6.5 mm Hg to 158 ± 5.44 mm Hg in the hyperpnoeic phase accompanied by a HIVC plasma K^+ rise from 3.68 ± 0.48 mmol/l to 8.82 ± 0.34 mmol/l (ΔK^+ = +5.14 ± 0.41 mmol/l), and an aorta K^+ rise from 3.19 ± 0.10 mmol/l to 4.3 ± 0.60 mmol/l (ΔK^+ = +1.11 ± 0.35 mmol/l). A few seconds later, the MABP started to fall more steeply to 43 ± 8.70 mm Hg within 1 min from the beginning

Fig.2-1a.

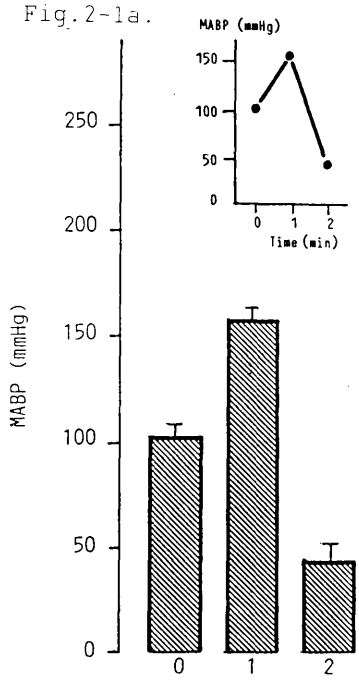
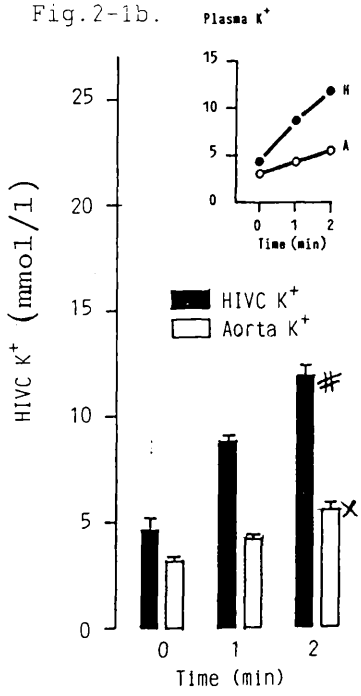


Fig.2-1b.



# P < 0.001, experiment compared with controls.

x P < 0.01, HIVC K^+ compared with aortic K^+ values.

Fig.2-1a. Histograms showing the changes in the MABP produced by asphyxia for 2 minutes. Inset is a curve describing the same changes

Fig.2-1b. Histograms showing the changes in the HIVC and the aortic plasma K+ produced by asphyxia for 2 minutes. Inset are curves describing the same changes.

P values < 0.05 are significant

of the fall, while the HIVC K^+ continued to rise to 11.90 ± 0.55 mmol/l, and the aortic K^+, to 5.62 ± 0.43 mmol/l (see Figs. 2.1(a,b,c)). Lead II ECG showed atrial fibrillation with disappearance of the P-wave and slowing of the heart rate (see Fig. 2.1(c)). If the asphyxia was not terminated during the falling phase of the MABP within 90 secs from the maximum rise in MABP, the MABP fell to zero resulting in the death of the cat.

Sudden termination of asphyxia and allowing the animal to breathe room air spontaneously or putting it on mechanical ventilation usually caused a further small transient rise in both HIVC and aortic K^+ before they both fell to control levels. The ECG also returned to normal. Administration of either prazosin (0.2 mg/kg. i.v.), propranolol (0.2 mg/kg. i.v.) or naloxone (0.4 mg/kg. i.v.) before the period of asphyxia did not produce any significant change in the pattern of responses of the MABP, HIVC K^+ or aortic K^+ to asphyxia. However, when asphyxia was induced as above in a totally different set of experiments in which the cats had received a bilateral cervical vagotomy the rise in MABP and the accompanying plasma K^+ levels were more marked (see Fig. 2.1(d)).

2.4.2 EFFECTS OF HAEMORRHAGE

The results of withdrawing 25% total blood volume are shown in Figures 2.3 and 2.5(a, b). When the MABP was suddenly lowered from a control value of 108 ± 4.6 mm Hg to 46 ± 2.24 mm Hg by withdrawing 25 per cent of total blood volume, hyperventilation preceded the rises in HIVC K^+ from 3.19 ± 0.12 mmol/l to 6.72 ± 0.68 mmol/l (ΔK^+ = +3.53 ± 0.40 mmol/l), and in aortic K^+ from 2.31 ± 0.11 mmol/l to

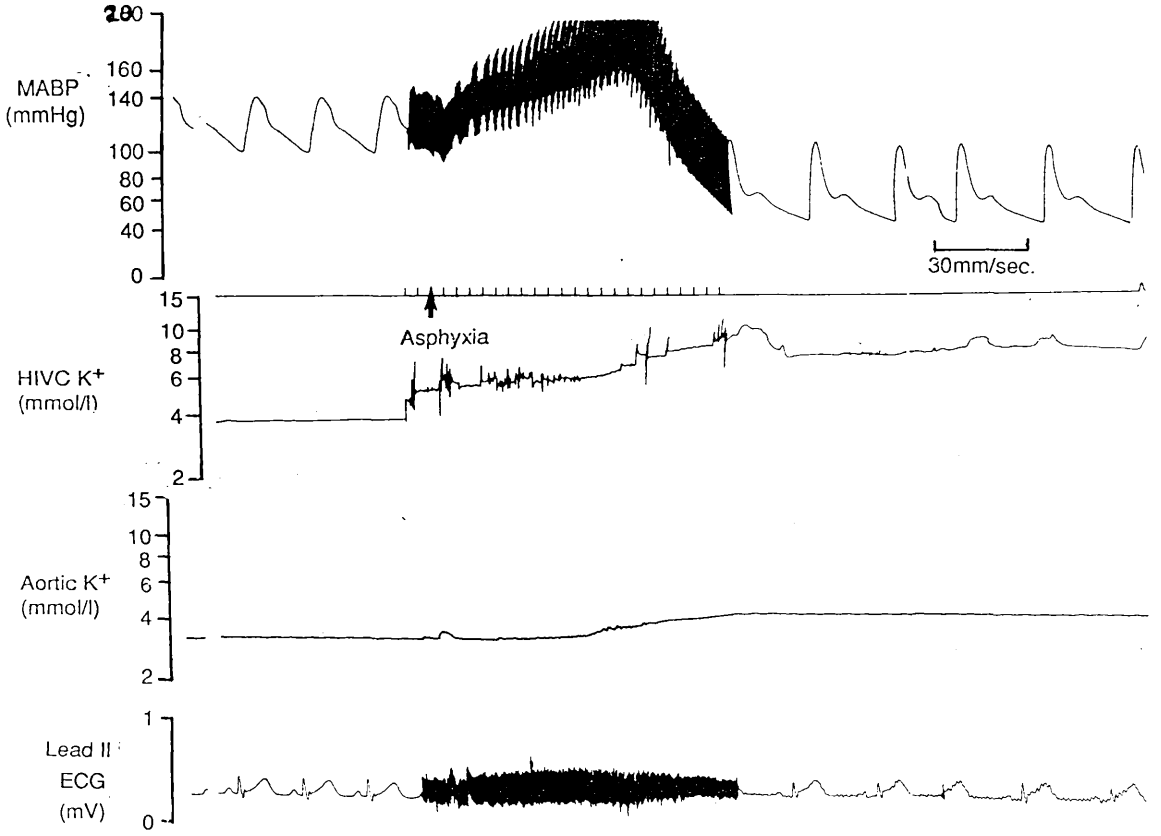


Fig.2-1c. Continuous monitoring of the effects of asphyxia on the MABP, the HIVC and aortic plasma K+, and the ECG. Note the occurrence of atrial fibrillation in the falling phase of the MABP and the attendant hyperkalaemia.

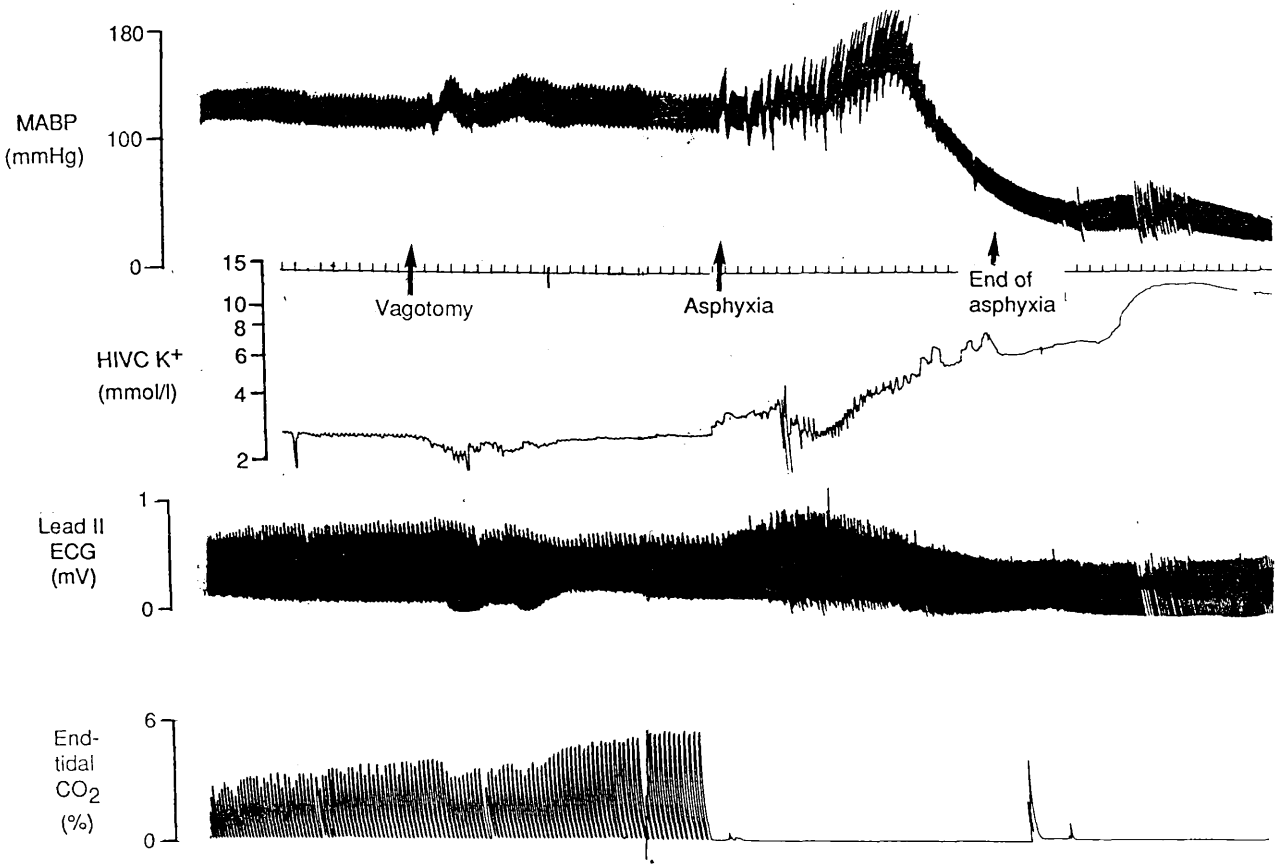


Fig.2-1d. The effects of asphyxia following bilateral cervical vagotomy on the MABP and the HIVC plasma K+. Note the greater increase in plasma K+ than the response in Fig.1-2c where the vagi are intact.

Fig. 2-2a:

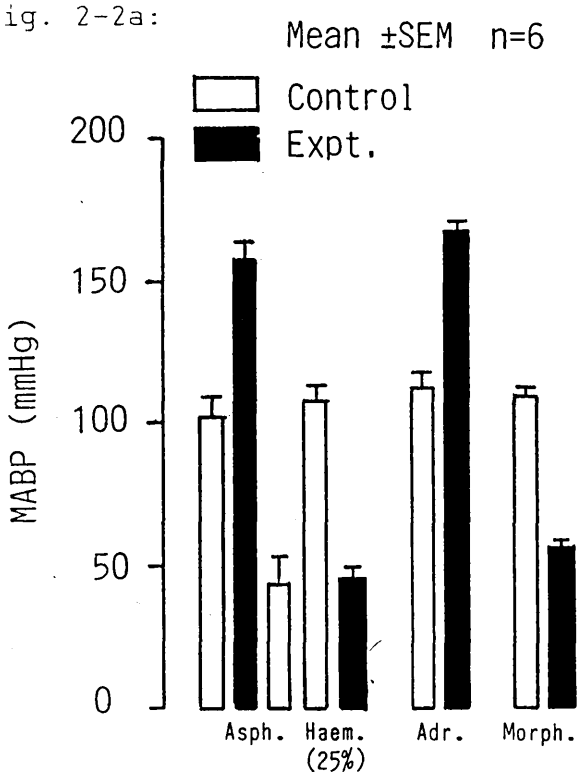
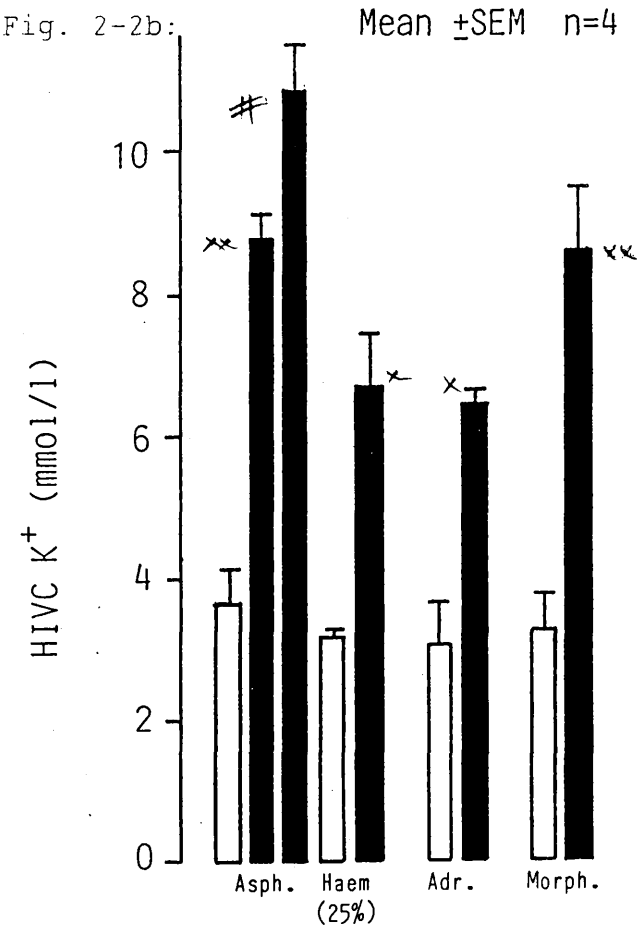


Fig. 2-2b:



# P < 0.001
x P < 0.02
xx P < 0.01
compared with control values

Figs. 2-2 (a & b) Patterns of changes (a) in MABP and (b) Plasma K^+, produced by Asphyxia (Asph), 25% haemorrhage (Haem), 2ug/kg i.v. adrenaline (Adr), and 8mg/kg morphine (Morph). Note the differences in the magnitude and direction of changes in MABP, which were all accompanied by a rise in plasma K^+.

P values < 0.05 are significant.

Fig. 2-3a:

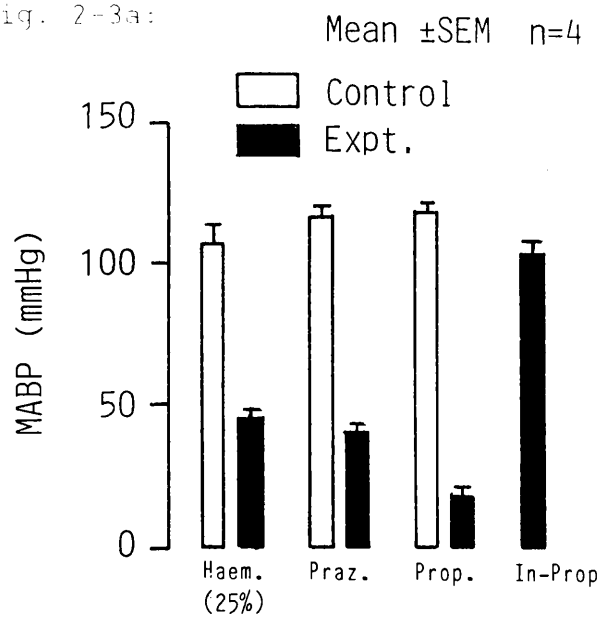
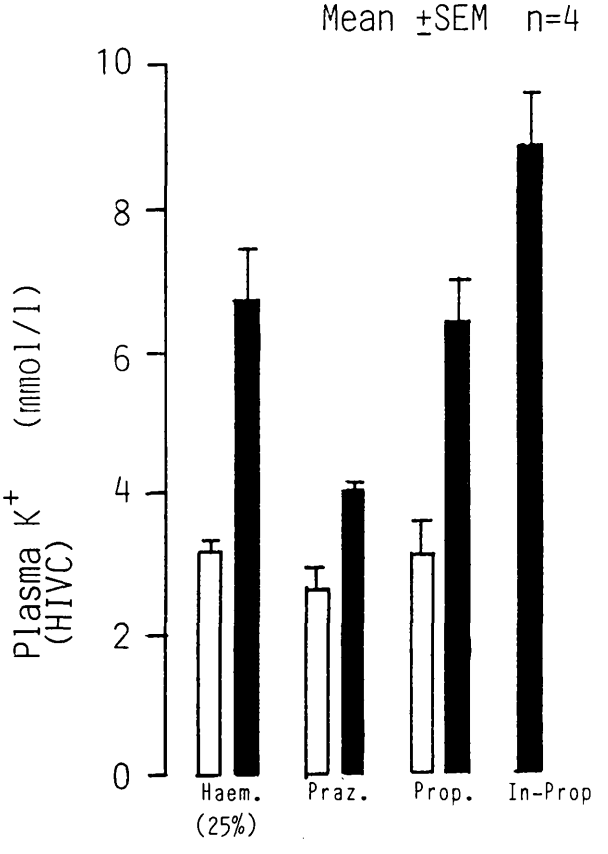


Fig. 2-3b:



(b)
Effects of 25% Haem. before and after Praz. and Prop. on HIVC K^+.

"In-Prop." shows the sustained K^+ following infusion of shed blood in the presence of Prop. (see also fig.2-4). Prazosin significantly reduced haemorrhage-induced hyperkalaemia ($P<0.01$), propranolol did not.

Figs. 2-3

(a) Effects of 25% Haemorrhage before (Haem 25%) and after Prazosin (0.2mg/kg i.v.) (Praz),and Propranolol (0.2mg/kg i.v.) (Prop). "In-Prop".= Blood Infusion after propranolol to show the specific effect of reinfusion on plasma K^+ in the presence of Propranolol(see below). Reinfusion after haemorrhage or prazosin returned MABP and K^+ to control levels, and are not shown.

4.31 ± 0.21 mmol/l (ΔK^+ = +2.00 ± 0.16 mmol/l), within the 5 min. period of observation (see Figs. 2.3(a,c)).

Reinfusion of the shed blood returned all measured values back to near control levels.

Pretreatment of the cats with 0.2 mg/kg i.v. prazosin (allowed to act for 10 min) before 25% haemorrhage produced a significant reduction ($\rho < 0.02$) in the rise of HIVC K^+. In this instance it rose from 2.65 ± 0.34 mmol/l to 4.05 ± 0.14 mmol/l (ΔK^+ = +1.40 ± 0.24 mmol/l), and also a significant reduction in the rise of aortic K^+ from 2.25 ± 0.18 mmol/l to 3.45 ± 0.09 mmol/l (ΔK^+ = +1.20 ± 0.13 mmol/l). (See Fig. 2.3(d)).

In the presence of prazosin, the MABP fell to a greater extent than in the absence of prazosin from 117 ± 3.4 mm Hg to 41 ± 2.40 mm Hg. It is noteworthy that in the face of the increased fall in MABP produced by prazosin the rises in plasma K^+ levels were reduced. However when 30-35% total blood volume was withdrawn in the presence of prazosin, there was no reduction in the plasma K^+ rise by prazosin with this degree of haemorrhage. There was a significant rise ($\rho < 0.01$) in HIVC plasma K^+ and aortic K^+ to the same levels as 30-35% haemorrhage-induced plasma K^+ in Section One of this thesis. Reinfusion of the shed blood restored the values to near control levels after a longer interval than when haemorrhage was performed without any prazosin present.

Phentolamine, another α-adrenoceptor antagonist produced similar effects to prazosin on the 25% haemorrhage-induced

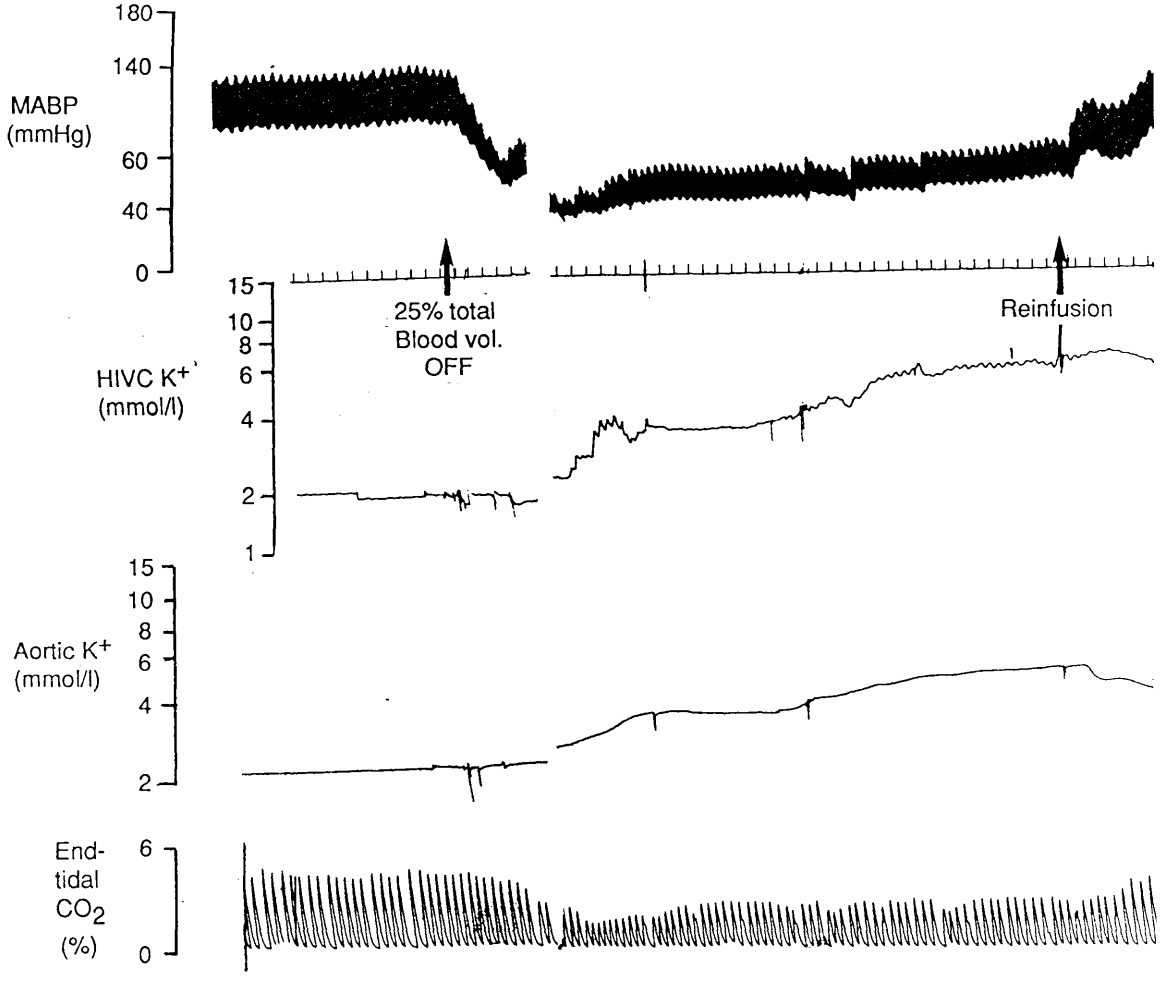


Fig.2-3C. continuous monitoring of the effects of 25% haemorrhage on the MABP, HIVC and aortic plasma K+ and the end-tidal CO2. Note the rise in the HIVC K+ to approximately 6 mmol/l.

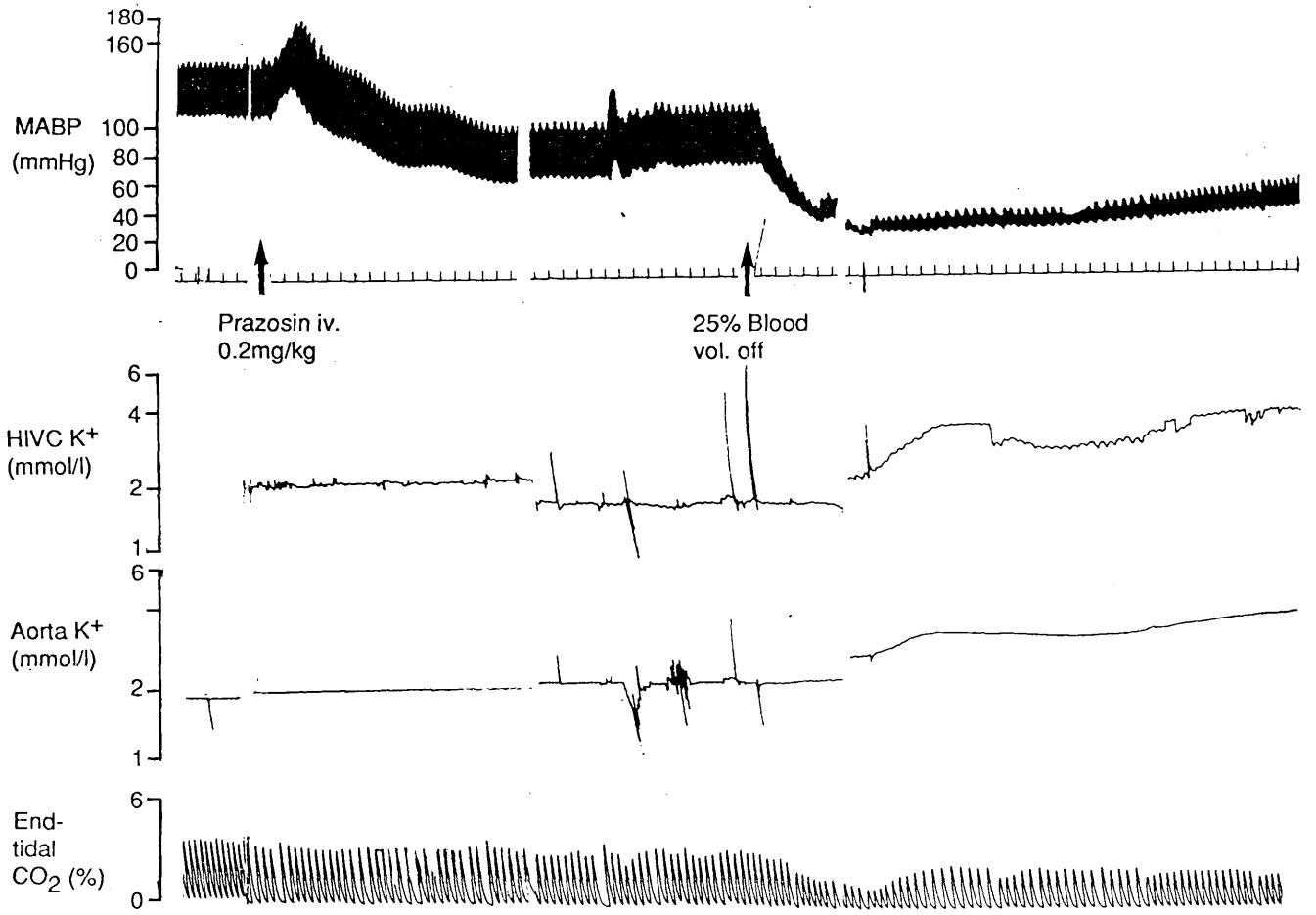


Fig.2-3d. Effects of 25% haemorrhage on the MABP, HIVC and aortic plasma K+ and the end-tidal CO2 in the presence of prazosine, an alpha 1-adrenoceptor blocker. Note the significant (p 0.02) decrease in the rise of the HIVC plasma K+ (less than 4 mmol/l).

plasma K^+ elevation except that phentolamine has a shorter latent period of action. Phentolamine acts within 2 min while prazosin takes about 10 min to produce its effects. Phentolamine also produced no effect on the 30-35% haemorrhage-induced hyperkalaemia (See Fig. 2.3(e)).

In the presence of 0.2 mg/kg i.v. propranolol (β-adrenoceptor antagonist) however, 25% haemorrhage caused a greater fall in the MABP from 119 $\pm$ 2.48 mm Hg to 19 $\pm$ 2.80 mm Hg with accompanying rises in HIVC K^+ from 3.11 $\pm$ 0.46 mmol/l to 6.38 $\pm$ 0.64 mmol/l (ΔK^+ = +3.27 $\pm$ 0.55 mmol/l), and in aortic K^+ from 2.43 $\pm$ 0.24 mmol/l to 3.78 $\pm$ 0.22 mmol/l (ΔK^+ = +1.35 $\pm$ 0.23 mmol/l). Animals went into apnoea from hyperventilation as hypotension continued in the presence of propranolol. On reinfusion of the shed blood after 5 min of observation, MABP returned to 104 $\pm$ 2.88 mm Hg (control 119 $\pm$ 2.40 mm Hg) in 25 $\pm$ 4 min, but the reinfusion produced further increases in the already raised HIVC K^+ from 6.38 $\pm$ 0.64 mmol/l to 8.88 $\pm$ 0.66 mmol/l, and in the aortic K^+ from 3.78 $\pm$ 0.22 mmol/l to 5.12 $\pm$ 0.44 mmol/l (see Fig. 2.3(f)). These higher levels of plasma K^+ were sustained for 2.5 $\pm$ 0.50 min. before falling again to control levels.

2.4.3 EFFECTS OF ADRENALINE INJECTION

In the normal anaesthetized cat, a 2 μg/kg i.v. injection of adrenaline produced a rise in MABP from 112 $\pm$ 5.8 mm Hg to 168 $\pm$ 2.6 mm Hg while HIVC K^+ rose from 3.10 $\pm$ 0.56 mmol/l to 6.44 $\pm$ 0.24 mmol/l, and the aortic K^+ rose from 2.66 $\pm$ 0.21 mmol/l to 5.82 $\pm$ 0.54 mmol/l. Lead II ECG showed sinus

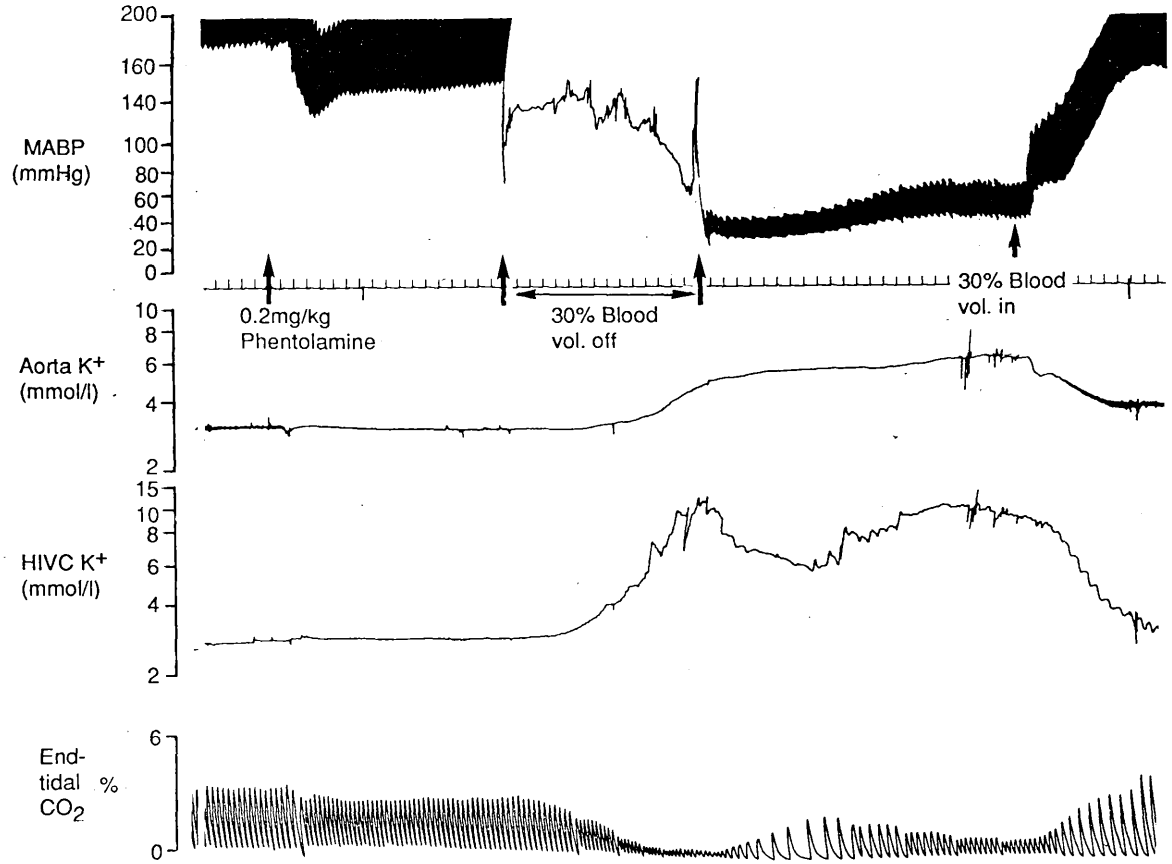


Fig.2-3c. Effects of withdrawal of 30% total blood volume on the MABP, HIVC and the aortic plasma K+ and the end tidal CO2 in the presence of 0.2 mg/kg phentolamine i.v. Note the obvious reduced effect of phentolamine, an alpha-adrenoceptor blocker in preventing the rise in plasma K+ when the degree of haemorrhage is increased (compare with the effect of prazosine on the response to 25% haemorrhage in Fig.2-3c).

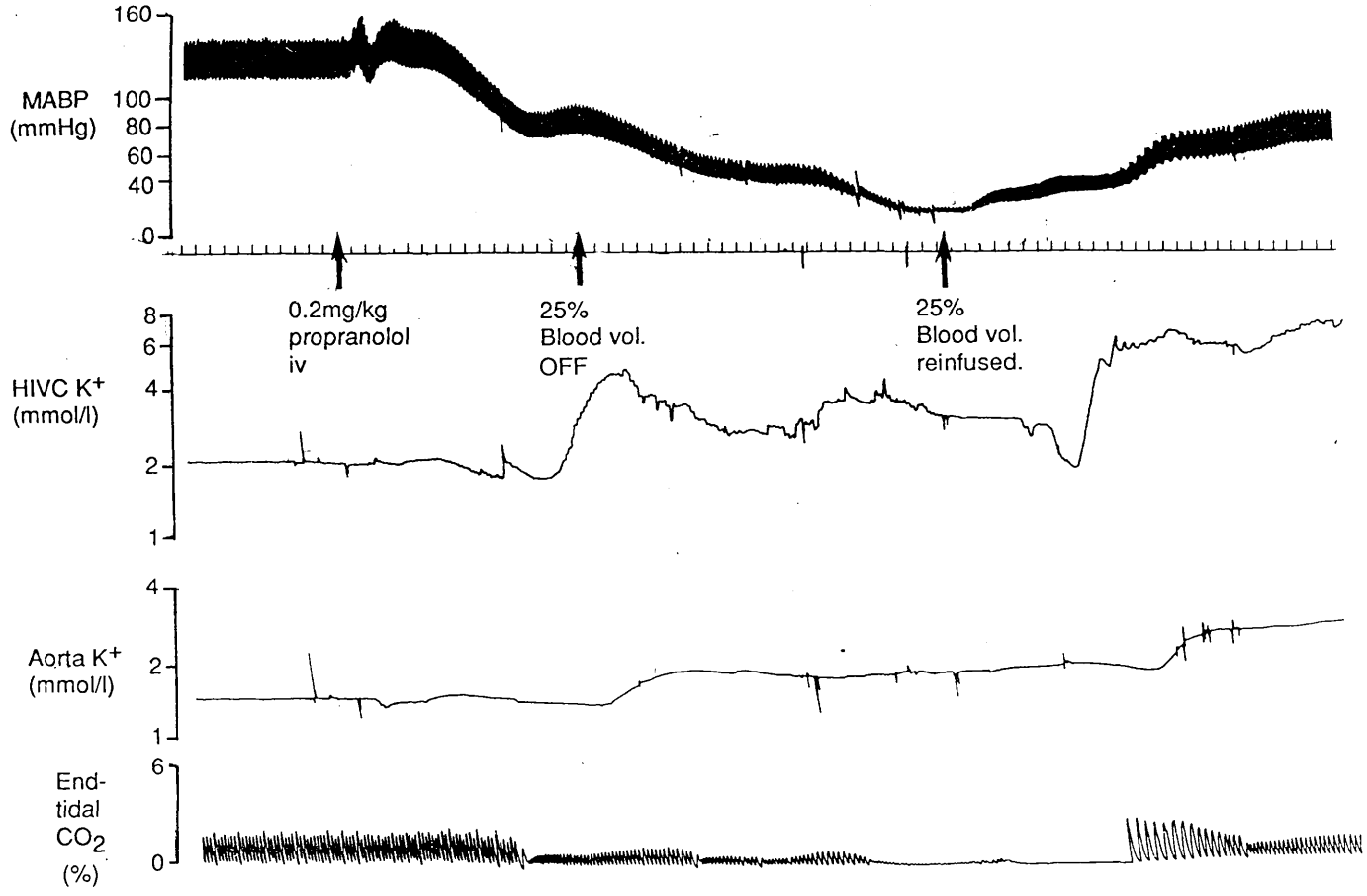


Fig.2-3f. The effects of 25% haemorrhage and the reinfusion of the shed blood on the MABP, HIVC and aortic plasma K+ and the end-tidal CO2 in the presence of propranolol, a beta-adrenoceptor blocker. Note the further increase in the HIVC plasma K+ after the reinfusion of the shed blood.

rhythm with tachycardia, and end-tidal CO_2 traces showed tachypnoea. There was no phase of apnoea as there was with haemorrhage (30-35%), and with 0.8 mg/kg i.v. morphine injection. The plasma K^+ levels later fell below the control values, before returning to control and at the same time MABP and the ECG gradually returned to normal. Both alpha-adrenergic antagonists, phentolamine and prazosin (0.2 mg/kg i.v.) reduced the adrenaline-induced hyperkalaemia significantly ($\rho < 0.01$). Propranolol, a β-adrenoceptor antagonist on the other hand increased the plasma K^+ elevation produced by adrenaline significantly ($\rho < 0.02$) and prolonged the elevated phase of plasma K^+ in both HIVC and aorta. There was no phase of hypokalaemia following the return of the K^+ values to control levels.

2.4.4 EFFECTS OF MORPHINE INJECTION

8 mg/kg i.v. injection of morphine produced effects similar to those described in Section Three of this thesis. This dose was chosen following a preliminary dose-response curve used to determine a dose which would be sublethal but potent enough to produce a significant increase in plasma K^+ and a reduction in MABP. Within 1.5 min of injection, morphine reduced MABP from 109 $\pm$ 2.6 mm Hg to 56 $\pm$ 1.8 mm Hg with an accompanying rise in HIVC K^+ from 3.25 $\pm$ 0.46 mmol/l to 8.6 $\pm$ 0.90 mmol/l. Lead II ECG showed tachycardia followed by bradycardia while the respiratory frequency went into end-inspiratory apnoea before recovering to deep slow breaths. Recovery in the ECG and breathing occurred in 15 to 20 min while HIVC K^+ returned to control within 10 min. MABP

never recovered fully (99 ± 1.5 mm Hg; control 109 ± 2.6 mm Hg).

Pretreatment of the animal with 0.2 mg/kg i.v. each and separately of phentolamine, prazosin or propranolol had no significant effect on the morphine-induced hyperkalaemia as shown earlier in this section. Only 0.1 mg/kg i.v. injection of naloxone reversed the depressor and hyperkalaemic responses to morphine. However naloxone could not prevent the initial elevation of plasma K^+ following blood withdrawal but it did reverse hyperkalaemia after it had occurred. In this case naloxone completely prevented the morphine-induced hyperkalaemia (see Fig. 3.7).

2.5 DISCUSSION

The results in this section of the present study indicate that adrenaline, morphine, asphyxia and haemorrhage, all produce hyperkalaemia but this is accompanied by different patterns of change in MABP. Morphine and haemorrhage decrease the MABP and increased the plasma K^+ concentrations. Their initial effects on ECG and respiration are similar. Asphyxia produces initial effects resembling those produced by adrenaline i.e. increased K^+ and increased MABP and later effects resembling those produced by haemorrhage and morphine, i.e. increased K^+ and decreased MABP.

The greater increases in MABP and plasma K^+ produced by asphyxia (in this section), and by morphine and adrenaline in Section Three after vagotomy suggest that the parasympathetic system has some inhibitory role on the plasma K^+ release induced by endogenous catecholamines and opioids. It is tempting to suggest that the initial effects of asphyxia may be mediated at the early phase by endogenous

catecholamines released from sympathoadrenal stimulation by the "stress" of asphyxia. The removal of the vagal effects is normally expected to disinhibit sympathetic outflow, and Ghosh et al. (1980) have demonstrated the involvement of α-adrenoceptors in increasing the MABP in asphyxia. The steep fall in MABP in the later phase of asphyxia that accompanies the continuing rise in plasma K^+ is suggestive of vagal stimulation via vagal efferents to the heart which is superimposed on the effects of endogenous opioids which are acting either directly on the heart or on the vasomotor centre. The continuous bathing of this centre with blood lacking O_2 and with CO_2 excess as asphyxia continues may exhaust the vasomotor centre and therefore may further weaken the heart in the later phase of asphyxia.

The lack of significant effects of the adrenergic receptor - or opioid receptor - blockers at the concentrations of which these drugs were administered in the present study might not be enough to suggest that the above described early and late effects on MABP and plasma K^+ produced by asphyxia do not involve the adrenergic or the opiate-receptor systems because Ghosh et al. (1980) reported that rogitine, an α-adrenoceptor blocker prevented the early rise in MABP produced by asphyxia which suggested that α-adrenoceptors do mediate the elevation in MABP during asphyxia. Ghosh et al. used higher concentrations of the adrenergic blocking agent. Also, in the present study, the ability of prazosin, or phentolamine to block or significantly reduce the rise in plasma K^+ induced by

withdrawal of 25% blood volume, and their failure to do so after 30-35% haemorrhage suggests the competitive nature of these adrenergic receptor blockers, prazosin and phentolamine. The endogenous receptor agonists e.g. catecholamines produced by the severe and acute nature of the stress of asphyxia (Ghosh *et al.*, 1980), might have overcome the effectiveness of the antagonists employed.

The effect of increasing acidosis in reducing the potency of prazosin and naloxone has been discussed elsewhere in Section Three in this thesis. It could also be speculated that the tissue receptors might lose their sensitivity to the receptor blocking drugs as acidosis increases.

2.6 CONCLUSION

It appears that the direction of change in MABP is not an indication of a corresponding direction of change in plasma K^+ in emergency situations like haemorrhagic shock, asphyxia or the "fright, fight or flight" state. The body responses to asphyxia, excess endogenous opioids and catecholamines seem to combine to produce an overall reaction to acute severe haemorrhage. The correction of these stimuli must be kept in mind together with fluid replacement in resuscitatory manoeuvres instead of only restoring MABP and correcting acid-base shifts which are necessarily normal physiological responses to the prevailing stress of haemorrhage at the time. A common factor of liver tissue anoxia in all these conditions which produce

an increase in K^+ release is suggested.

2.7 SUMMARY

The changes in MABP associated with the rise in plasma K^+ caused by different challenges, for example asphyxia, haemorrhage, injection of adrenaline and morphine, have been investigated. Adrenoceptor and opioid receptor blocking agents have been employed to throw some light on the mechanism of release of K^+ by the various stimuli as compared to haemorrhage. The continuous monitoring of the plasma K^+ levels with valinomycin-based K^+-selective electrode catheter enabled an accurate recording of the time-course of events throughout the periods of observation for each stimulus.

In response to haemorrhage and morphine injection, there is a fall in MABP accompanied by a rise in plasma K^+ level, while in response to adrenaline injection there is a transient rise in MABP accompanied also by a transient rise in plasma K^+ level. Asphyxia, early in its effect produces a rise in MABP with an accompanying rise in plasma K^+ level as exhibited by adrenaline injection, while later it produces a response that is similar to haemorrhage and morphine injection.

Haemorrhagic hypotension seems to cause a release of endogenous opioids and catecholamines whose receptors seem at some stage to be involved in the release of K^+ as indicated by the significant prevention of the excessive rise in plasma K^+ by naloxone, prazosin or phentolamine following haemorrhage, and the injection of morphine and adrenaline.

Section of the vagi in the neck appears to disinhibit sympathetic outflow. This causes a greater rise in the plasma K^+ levels in response to asphyxia, haemorrhage, injection of adrenaline and morphine. The vagi have therefore been suggested to modulate some of the mechanisms involved in the release of K^+.

A combination of physiological adjustments by the cardio-respiratory system and changes in fluid electrolytes including a rise in plasma K^+ appear along with other changes to be the early body response to haemorrhage. These reactions and changes are in part produced by endogenous opioids and catecholamines. Any regime of treatment or resuscitation directed towards improving these seeming abnormal states of low MABP and elevated plasma K^+ should consider correcting the stimuli causing them. The "abnormal states" are themselves normal physiological responses to the challenging condition, for example haemorrhage as in the present study.

3. INTRODUCTION

3.1 NALOXONE AND HAEMORRHAGIC SHOCK

The experiments to be described in this section were carried out because of the consistent observation that the direction of change in MABP associated with the rise in plasma K^+ levels differed and depended on the stimulus or challenge applied. For example, catecholamines caused a rise in MABP, haemorrhage produced a fall while asphyxia produced an initial rise in MABP followed by a precipitous fall, but all were associated with a rise in plasma K^+. In addition, a review of the literature (see Holaday, 1983) showed that naloxone, an opioid receptor antagonist is capable of increasing arterial blood pressure to near normal levels after haemorrhage.

There are several reports that naloxone raises the MABP during hypovolaemic shock (Faden & Holaday, 1978; Holaday & Faden, 1979; Holaday, 1983), but there is lack of information on the effects of naloxone on the increase of plasma K^+ after haemorrhage. Faden and Holaday (1979) reported that the opiate antagonist, naloxone reversed hypovolaemic shock in conscious rats. MABP, pulse pressure and survival time were significantly increased compared with controls. This original discovery of Faden and Holaday about naloxone has been supported by subsequent evidence in other animals subjected to haemorrhagic shock, for example, in cats (Curtis & Lefer, 1980; Feuerstein, Ailman & Bergman, 1980), in dogs (Gurll *et al.*, 1981, 1982; Vargish *et al.*, 1980), and in monkeys (Vargish, 1983).

The majority of these studies were performed on anaesthetized animals. Other reports have contradicted the studies mentioned above. For example, it has been shown that naloxone has little effect on the cardiovascular response in hypotensive baboons (Golanov, Parin & Suchkov, 1983; Isoyama, Tanaka, Sato & Shatney, 1982). Brown and Burr (1986), have reported that naloxone failed to reverse haemorrhagic hypotension in rats. They found that injection of equal volumes of either saline or naloxone in saline produced similar pressor responses in rats subjected to haemorrhagic hypotension. Brown and Burr (1986) suggested that the difference in the results of the various groups of workers were due either to the difference in the animal species used, or the difference in the anaesthetics used.

Dashwood and Feldberg (1976) showed that a pressor effect of naloxone on anaesthetized intact cats could not be demonstrated unless the animals had been pretreated with morphine or opioid peptides. Moreover certain factors like rapid bleeding, low environmental temperature (van der Meer *et al.*, 1986) and acidosis (Kaufman, 1982) seemed to reduce or abolish the effect of naloxone. Bennett and Gardiner (1982) found that naloxone had no effect on MABP after rapid bleeding but produced an increase in MABP after slow bleeding. If shed blood is not infused, naloxone could only prolong survival time. A similar increase in MABP was found in cats, rabbits and baboons (McIntosh *et al.*, 1984). Schadt and York (1981) found that pentobarbital anaesthesia reduced the effects of naloxone on MABP in rabbits but did not completely suppress it. In their 100

percent lethal haemorrhagic shock model which involved the withdrawal of 50% total blood volume, Van der Meer et al. (1986) found no increase in MABP by naloxone. It is therefore not absolutely certain that naloxone acts only by antagonizing the effects of released endorphins. Naloxone has been reported to influence pharmacological responses to a variety of non-opiate drugs (Sawynok, Pinsky & La Bella, 1979). Some of these responses mentioned below may be mediated by endogenous opiates and the effect of naloxone could be a consequence of the displacement of these substances from opiate receptors, while other effects may not involve an effect on opiate receptors. For instance in isolated rat aortic strips, naloxone antagonized the contractile effects of K^+ and noradrenaline as well as the effect of other substances in the morphine family such as l-pentazocine (Lee & Berkowitz, 1976), while in the guinea-pig vas deferens, naloxone potentiated the contractile response of noradrenaline, acetylcholine and KCl (Ramaswamy, Nazimudeen & Kameswaran, 1979).

Naloxone is a competitive inhibitor of opiate responses and the inhibitory concentration depends upon the level of agonist in the receptor environment (Sawynok et al., 1979). Sawynok et al. (1979) have suggested that in order to implicate endogenous opiates in a process, evidence in addition to blockade by naloxone is required. Such evidence could include

(a) the demonstration of cross tolerance with morphine;

(b) similar responses with other opiate antagonists;

(c) the lack of an effect with non-antagonist isomers;

(d) evidence that agents which inhibit the breakdown of endogenous opioid peptides potentiate the response and (e) evidence of a direct release of endogenous opioids. Two of such pieces of evidence were involved in the present study. The release of endogenous opioids in haemorrhagic shock as mimicked by morphine injection, and the cross-tolerance with morphine in the elevation of plasma K^+, were used to try to prove that endogenous opioids are partially responsible for the elevation of plasma K^+ during shock.

Since the modification by naloxone of physiologic responses suggests involvement of opioid action (Akil et al., 1976), a role for the endogenous opioids in blood pressure and plasma K^+ in response to haemorrhage was investigated in the present study. It was also decided to investigate whether naloxone would reverse the raised plasma K^+ after haemorrhage since it would raise the MABP without reinfusion of the shed blood.

3.2 ENDOGENOUS OPIOIDS IN CIRCULATORY SHOCK

In 1980, Dashwood and Feldberg reported that if in a particular experimental condition naloxone antagonizes an action which can be mimicked by morphine or opioid peptides, then this suggests that the action is probably caused by the release of opioid peptides.

Severe stress including shock from haemorrhage produces endogenous opioid peptides (Guilleman et al., 1977; Holaday, 1983). Such peptides have recently been isolated and identified (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975; Li & Chung, 1976; Bradbury, Smyth & Snell, 1976).

It has been reported that catecholamines and opioid peptides are stored and released together from the chromaffin cells of adrenal medulla (Schultzberg et al., 1979; Viveros et al., 1979). This could suggest that the functions of these substances may be synergistic or antagonistic. Since it has been reported in Sections One and Two of this thesis that haemorrhagic shock increases plasma K^+ levels in the HIVC, it is possible that this K^+ release could be a direct effect of a substance or substances, for example, catecholamines, opioids or hypoxia on the liver cells themselves. Alternatively, it could be the action of these substances on the liver vasculature whose response to actions of catecholamines could modify the liver cells which may directly cause the release of K^+.

3.2.1 K^+ CONDUCTANCE INCREASED BY OPIOIDS

Electrophysiological experiments have shown that opioids increase membrane K^+ conductance in a variety of tissues (Duggan & North , 1983). In both liver and cultured mouse dorsal root ganglion, the results of experiments with selective agonists and measurements of naloxone dissociation equilibrium constants indicate that the receptor involved is the μ-type (Werz & MacDonald, 1983a,b). Williams and North in 1984 implicated the μ-type of opioid receptor in their studies with naloxone on neurones of rat locus coerulens. The alteration of K^+ conductance by opioids has been found to be via different receptors by different workers. For example, activation of delta-receptors to

increase K^+ conductance in submucous neurones of guinea pig caecum (Mihara & North, 1986), and of μ-opioid receptors and α_2-adrenoceptors on myenteric neurones of guinea pig small intestines (Surprenant & North, 1985) have been reported. Opioids inhibit the firing of nerve cells in various regions of the mammalian nervous system. Williams, Egan and North (1982) have reported that the inhibition of firing results from a membrane hyperpolarization in neurones of the rat.

Pepper and Henderson (1980) obtained similar results from the guinea pig. North et al. (1979) and Pepper et al. (1980) have produced evidence from voltage clamp recordings that the membrane hyperpolarization results from an increased K^+ conductance of cell membranes. Such hyperpolarization induced by opiates was essentially unchanged in calcium-free solution. Thus the action of opioids is not mediated by the release of intermediate transmitters which then increase the K^+ conductance of the impaled neurone. The opening of K^+ channels by opiates may underlie not only the inhibition of cell firing but could contribute to the reduced transmitter release observed in several preparations (Kosterlitz et al., 1975). This effect also appears to account for many of the pharmacological actions of exogenously administered opiate drugs and may also represent a physiological action of endogenous opioid peptides (Duggan & North, 1983).

3.2.2 THE EFFECT OF MORPHINE ON LIVER

Incubation of isolated rat liver mitochondria in the presence of morphine or its derivatives - ethylmorphine, methylmorphine and nalorphine - resulted in a disturbance

of oxidative phosphorylation due to induction of permeability of the mitochondrial membranes to K^+ (Chistyakov & Genana 1980). The opiates induced the efflux of K^+ from de-energized mitochondria and caused the accumulation of free fatty acids in the mitochondria. These workers suggested that opiates caused the formation of a K^+ carrier in mitochondria by releasing Ca^{++} which activated the enzyme phosphorylase A_2, and that such effects of opiates increased with the increase of their hydrophobic properties.

The present study therefore involved the use of an opioid alkaloid, morphine, and the opioid receptor antagonist, naloxone to separate the adrenoceptor-mediated from the opioid-receptor-mediated changes in plasma K^+ and MABP during and after haemorrhage. Whether any effect on plasma K^+ by naloxone after haemorrhagic hypotension is due to the MABP raising effect of naloxone per se or not will also be highlighted.

3.3 MATERIALS AND METHOD

The set-up and the surgical procedures were basically the same. Cats deeply anaesthetized with pentobarbitone sodium, 45 mg/kg intraperitoneally were used, and the insertion of the potassium selective electrode catheters into the high inferior vena cava (HIVC) and the aorta was as described in Section One.

Withdrawal of 30% total blood volume which has been found in the preliminary studies of Section One to significantly reduce the MABP and raise the plasma K^+ was employed throughout this section unless otherwise stated. In order

to find out whether naloxone, the opioid receptor antagonist which has been reported to raise arterial blood pressure during hypovolaemic shock would reverse the raised plasma K^+ following haemorrhage without reinfusion of the shed blood, it (naloxone) was slowly infused intravenously at 0.1 mg/kg - min for 60 min at the same time as haemorrhage was started. Freshly prepared doses of naloxone in 10 ml of normal saline were used throughout this section of the study, and so a control study was done on a set of 4 cats giving slow i.v. infusion of 10 ml of saline at the same time as blood withdrawal was started as for naloxone. In further attempts to study the possible role played by the adrenergic receptors in mediating the rise in MABP, the release and the uptake of plasma K^+ produced by naloxone infusion following haemorrhage, the effects of naloxone in the presence of some alpha- and beta-adrenoceptor blocking agents were investigated. Such adrenoceptor blocking drugs used included the intravenous injection of 0.2 mg/kg each and separately of prazosin, an α_1-adrenoceptor blocker, phentolamine, an α_1 and α_2 adrenoceptor blocker, and propranolol, a β-adrenoceptor blocker. Also the effect of adrenaline, an α- and β-adrenoceptor agonist in the presence of naloxone on MABP and plasma K^+ was investigated in the same light.

In order to throw more light on the idea that modification by naloxone of physiologic responses suggests involvement of opioid action, the effects of morphine, an opioid alkaloid on MABP and plasma K^+ was investigated in the presence or absence of naloxone. A dose-response curve was constructed

for morphine and the dose of 8 mg/kg i.v. morphine was chosen for this investigation throughout this section, because it was found to be sublethal but potent enough to produce significant changes in MABP and plasma K^+. In 4 experiments involving the withdrawal of 30% total blood volume, 8 mg/kg of morphine was injected i.v. after blood withdrawal to observe if there is any contribution to the rise in plasma K^+ by endogenous opioids during haemorrhagic hypotension. In 4 other cats injection of 0.4 mg/kg i.v. naloxone as a single dose was given either before or 30 min after blood withdrawal to find out whether haemorrhagic hypotension was necessarily a prerequisite for the action of naloxone or not. The role played by any endogenously released catecholamines whether synergistically or antagonistically with any endogenously released opioids following haemorrhagic hypotension was also investigated by the administration of a single dose of 2 µg/kg adrenaline and 8 mg/kg morphine, i.v. together, 4 different times in 2 cats which had not undergone blood withdrawal.

The report that opiates cause a transient rise in MABP before the prolonged depressor effect and that this effect is mediated by the parasympathetic system was investigated to find out if any correlation exists between the involvement of the parasympathetics and the action of any endogenously released opioids to produce hyperkalaemia following haemorrhagic hypotension. In order to investigate any role played by the parasympathetics in the depressor and the hyperkalaemic effects of morphine, the vagi in the cervical region were sectioned and the effects of morphine alone, or morphine in

the presence of the adrenoceptor and opioid receptor blocking agents, as well as the effects of blood withdrawal after vagotomy were studied. Animals pretreated with prazosin were given the drug 10 min before the start of blood withdrawal or any drug administration, other blocking agents were administered 2 min before haemorrhage or agonist administration.

The results are expressed in means ± standard error of the mean. P values less than 0.05 are accepted as significant.

3.4 RESULTS

3.4.1 EFFECTS OF WITHDRAWAL OF 30% TOTAL BLOOD VOLUME AND THE SLOW INFUSION OF NALOXONE, ON MABP AND PLASMA K^+

When the MABP was lowered from 139 ± 14 mm Hg to 20 ± 4.0 mm Hg by withdrawal of 30% total blood volume, the HIVC K^+ rose from 3.61 ± 0.41 to 10.50 ± 1.68 mmol/l, while the aortic plasma K^+ increased from 2.82 ± 0.18 to 5.56 ± 1.40 mmol/l at the end of blood withdrawal (time zero) (see Fig. 3.1). The plasma K^+ levels stayed about these values until the 5th min. Between the 10th and the 20th min the HIVC and aortic K^+ rose again from 11.20 ± 0.75 mmol/l and 5.90 ± 0.57 mmol/l to 14.80 ± 0.98 mmol/l and 9.65 ± 0.54 mmol/l, respectively. If the shed blood was not infused some cats died before the 25th min with the HIVC K^+ at 15 ± 0.68 mmol/l and the aortic K^+ at 9.95 ± 0.98 mmol/l.

However, when 0.1 mg/kg.min of naloxone was given by slow intravenous infusion for 60 min starting at the same time as the haemorrhage was started, MABP fell from the

Fig. 3-1:

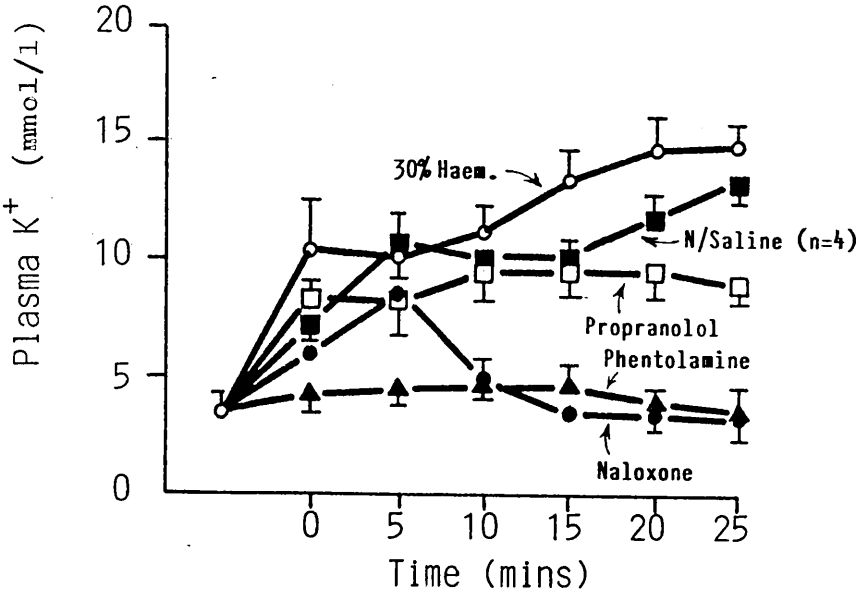
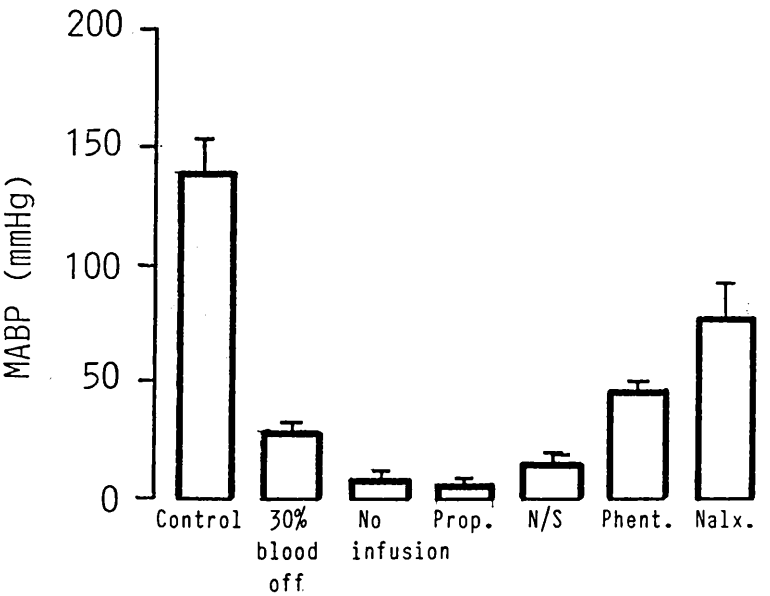


Fig. 3-2:



MABP at the 25th min. after 30% Haemorrhage.

Figs. 3-1 & 2.

Effects of Slow i.v. Naloxone, Before and after Pretreatment with Adrenoceptor Blockers During Haemorrhage, on (1) HIVC plasma K^+ and (2) MABP. Mean ± SEM. n=6.

Contr.	= Control.
30% Blood Off.	= 30% Haemorrage.
No Infusion	= No Drugs/Fluid Replacement.
Prop.	= Propranolol i.v. before Naloxone.
N/S	= Saline Infusion.
Phent.	= Phentolamine before Naloxone.
Naloxone	= Naloxone Infusion Alone.

control value to only 42 ± 4.85 mm Hg, while plasma K^+ in the HIVC and the aorta only rose from the controls to 6.0 ± 1.67 mmol/l and 5.21 ± 1.29 mmol/l, respectively, by time zero. The aortic plasma K^+ remained at this level while the HIVC K^+ further increased to 8.98 ± 1.20 mmol/l by the 5th min when both levels started gradually to fall significantly ($p < 0.01$) to control levels by the 25th min. At the same time, the MABP rose from 42 ± 4.8 mm Hg to 77 ± 14.7 mm Hg i.e. 62.3% of control (see Figs. 3.1& 2). When 0.4 mg/kg i.v. naloxone was given as a single dose and blood was withdrawn, a quick rise in HIVC plasma K^+ from 3.15 ± 0.25 mmol/l to 12.86 ± 0.34 mmol/l occurred which returned to control level without reinfusion of the shed blood. The MABP fell from 112 ± 2.8 mm Hg to 32 ± 4.0 mm Hg, and remained about this level while the HIVC plasma K^+ level returned to the control (Fig. 3.2(a)). In two cats in which blood was withdrawn and hypotension continued for over 30 min, the administration of a single dose of 0.1 to 0.2 mg/kg i.v. of naloxone produced a sudden further transient rise in plasma K^+. During this transient rise in plasma K^+ there was also a transient increase in MABP showing that the K^+ rise was not due to an increase in hypotension. In another two subsequent experiments where the withdrawal of blood was started with i.v. naloxone infusion and the MABP was maintained at 40 mm Hg by further removal of blood, the raised plasma K^+ due to the haemorrhage still fell to the control values indicating that the K^+ lowering effect of naloxone was not

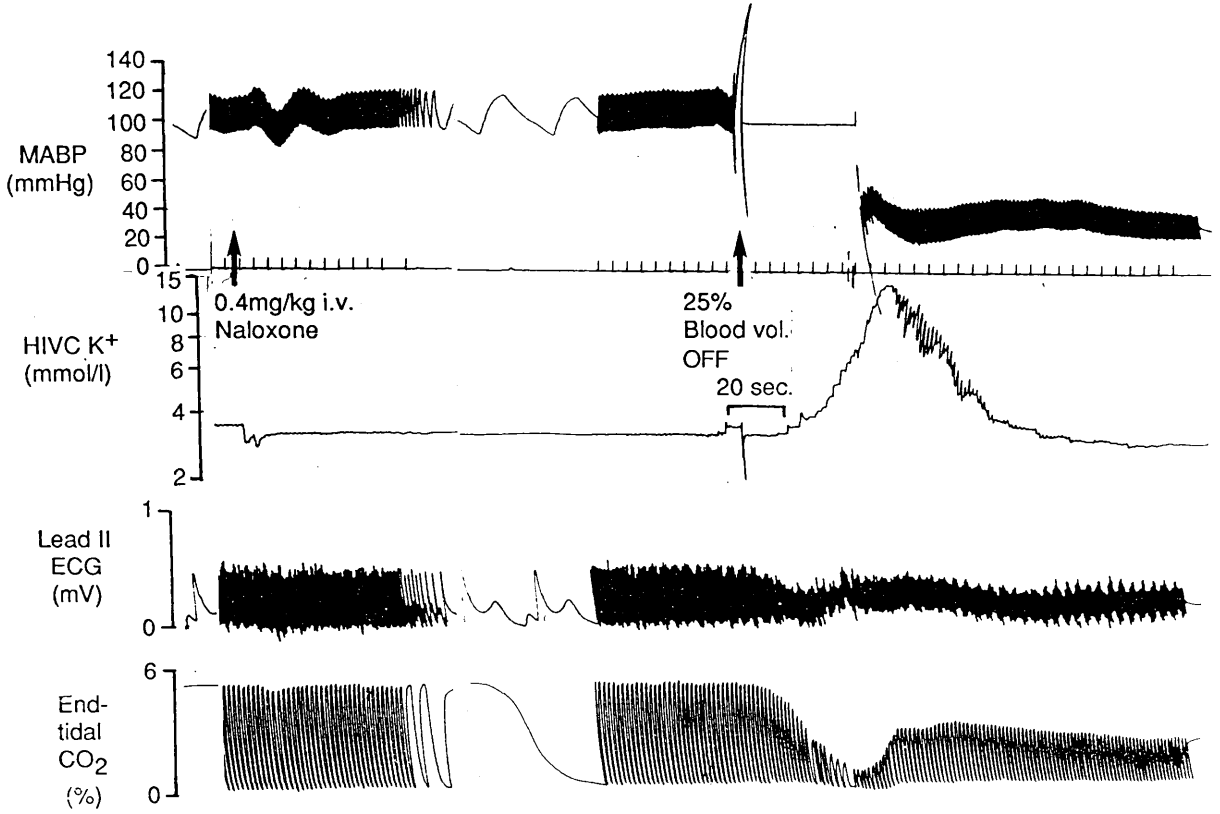


Fig.3-2a. Effects of a single dose of naloxone, 0.4mg/kg i.v. before the withdrawal of 30% total blood volume on the MABP, HIVC plasma K+, ECG and the end-tidal CO2. Note the abrupt rise in the HIVC plasma K+ within 20 secs of blood withdrawal and the unusual fall in the plasma K+ level to the control value without the reinfusion of the shed blood, and at a MABP level below 40 mmHg.

associated with its effect in raising the blood pressure (see Fig. 3.3). If 30% of blood volume was removed and hypotension allowed to continue beyond 120 min, the blood pH/gas analysis showed a fall in the arterial pH (7.21 ± 0.02). If single doses of naloxone of increasing concentrations from 0.2 to 0.4 mg/kg i.v. were then administered, they no longer raised the MABP nor did they lower the raised plasma K^+ back to normal values.

These varying results with naloxone demonstrate clearly that the effects of naloxone will vary depending on the time of administration and on the severity and duration of haemorrhagic hypotension.

Another observation consistently made was the extra increase in plasma K^+ both in the HIVC and the aorta when naloxone, 0.1 or 0.2 mg/kg was given as a single dose in the presence of adrenaline, or when adrenaline, 2 µg/kg was given i.v. in the presence of naloxone. A typical example of such a recording is shown in Figures 3, 4a and b. Naloxone by itself, before haemorrhage or any drug pretreatment had no effects on MABP or plasma K^+.

3.4.2 THE EFFECTS OF SLOW INFUSION OF NORMAL SALINE DURING AND AFTER HAEMORRHAGE

Naloxone in the previous experiments was dissolved in 10 ml of 0.9% NaCl, and therefore in 4 control cats 10 ml of 0.9 NaCl was slowly infused from the beginning of haemorrhage in the same way as the naloxone solution. At time zero, the MABP had fallen to 29.8 ± 3.5 mm Hg and the HIVC and aortic plasma K^+ had risen to 7.30 ± 0.39 mmol/l and 3.80 ± 0.77 mmol/l, respectively. However, by the 25th min.

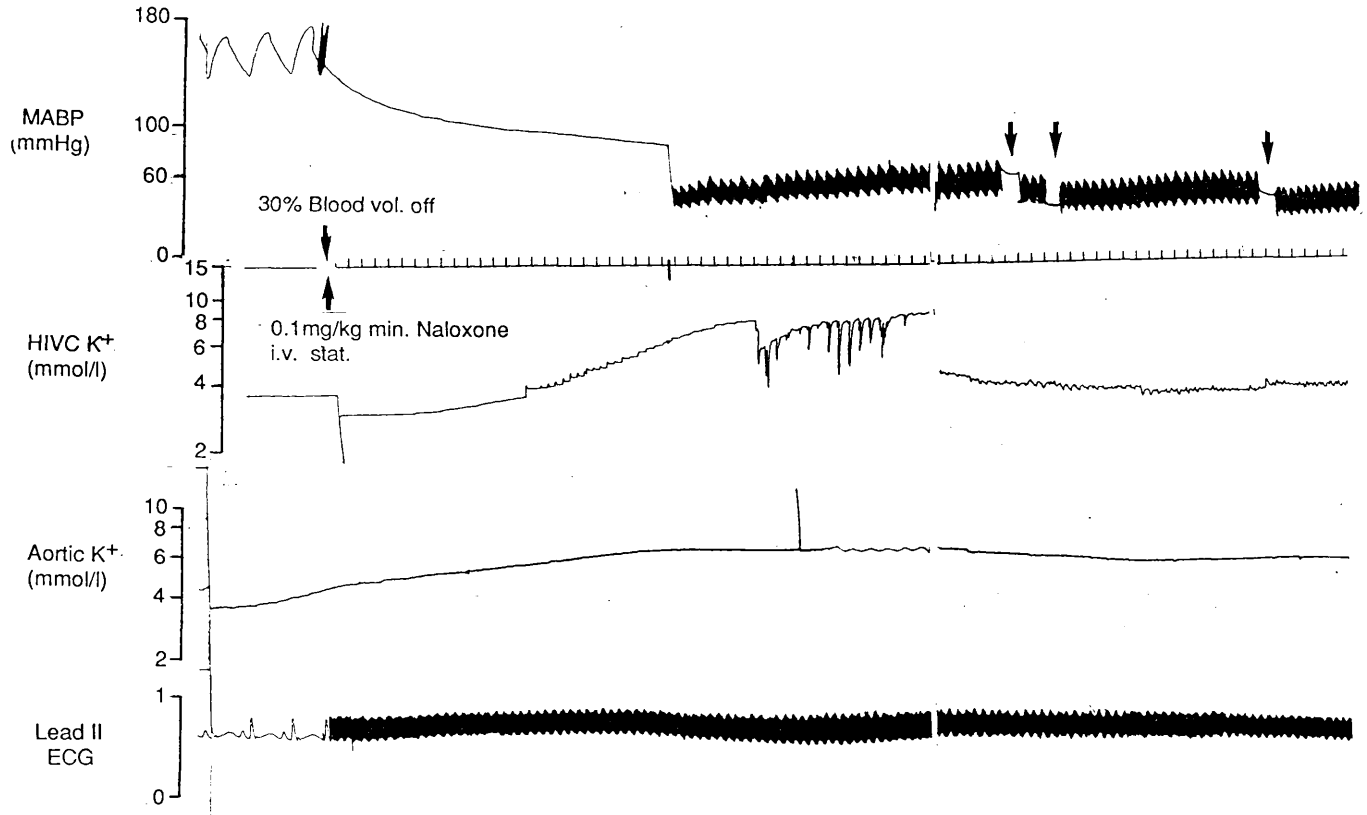
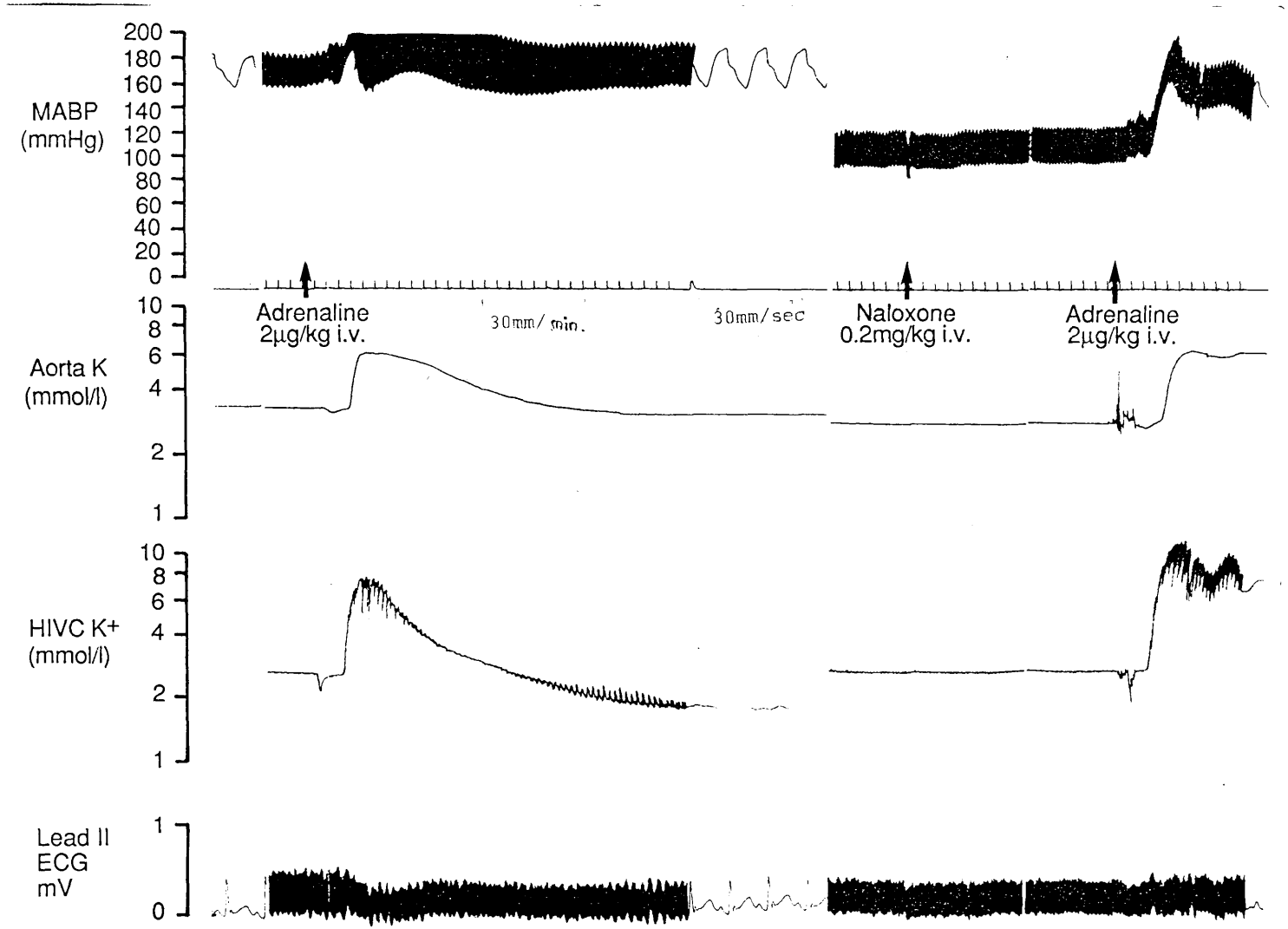


Fig.3-3. The effects of maintaining the MABP low follow
ing haemorrhage by further withdrawal of small volumes
of blood as appropriate, on the plasma K+ lowering
effect of slowly infused naloxone. Arrows indicate points
of further withdrawal of 5ml of blood (see also Fig.3-1a)



Figs. 3-4(a)&(b). Effects of adrenaline (a) alone, on MABP and plasma K^+ and (b) after naloxone. Note the marked rise in plasma K^+ due to the same dose of adrenaline after naloxone.

instead of a rise in MABP there was a further fall (see Figs. 3.1 and 3.2) to 15 ± 5.0 mm Hg, and the plasma K^+ levels in the HIVC increased to 13.50 ± 0.52 mmol/l, and in the aorta to 8.00 ± 0.88 mmol/l, during the same period. There was no significant difference in the changes in MABP and plasma K^+ between blood withdrawal without infusion and blood withdrawal accompanied by a slow infusion of 10 ml of normal saline.

3.4.3 EFFECTS OF PHENTOLAMINE ADMINISTRATION PRIOR TO SLOW NALOXONE INFUSION DURING AND AFTER HAEMORRHAGE

In order to differentiate changes in plasma K^+ involving any adrenoceptors from endogenously released opioid-mediated changes affected by the naloxone infusion, the slow infusion of naloxone was repeated in the presence of phentolamine, an α-adrenoceptor blocking agent.

In cats which were pretreated with 0.2 mg/kg i.v. phentolamine before the haemorrhage and slow i.v. infusion of naloxone were started simultaneously, haemorrhage produced a fall in MABP to 25 ± 8.6 mm Hg and the HIVC K^+ rose to only 4.25 ± 0.32 mmol/l while there was no significant change in the aortic K^+ at time zero. The HIVC K^+ remained around 4.75 ± 0.32 mmol/l until the 25th min. when it returned to the control level, with no further significant change in the aortic K^+. At the 25th min. MABP rose to only 46.6 ± 4.8 mm Hg, i.e. 33.6% of the control value. Thus, though slow infusion of naloxone alone during and after 30% haemorrhage could not prevent the initial rise in plasma K^+ and the formation of a plateau, this rise and plateau formation in plasma K^+ was significantly prevented ($p < 0.01$) by the presence of phentolamine (see Fig. 3.1).

3.4.4 EFFECTS OF PROPRANOLOL ADMINISTRATION PRIOR TO SLOW NALOXONE INFUSION DURING AND AFTER HAEMORRHAGE

In further attempts to study the possible role played by the adrenergic receptors in mediating the rise in MABP, and the release and uptake of K^+ produced by naloxone following haemorrhage the effect of propranolol, a β-adrenergic blocking agent on the response of plasma K^+ to naloxone was investigated. In the presence of 0.2 mg/kg of propranolol, withdrawal of 30% total blood volume with slow i.v. infusion of naloxone caused the MABP to fall to 4.68 ± 2.0 mm Hg from the control with an accompanying rise in HIVC plasma K^+ from 3.61 ± 0.41 mmol/l to 8.40 ± 0.55 mmol/l and in the aortic K^+ from 2.82 ± 0.18 mmol/l to 3.60 ± 0.15 mmol/l at time zero (see Figs. 3.1 and 3.2). In the HIVC, the plasma K^+ remained at this level until the 5th min. while the aortic K^+ level continued to rise slowly to reach 4.75 ± 0.65 mmol/l in the 20th min and it remained there with no significant change until the 25th min. However, the HIVC K^+ plateaued a second time at 9.60 ± 0.89 mmol/l between the 10th and the 20th min and it remained there with no significant change until the 25th min. Thus, propranolol, unlike phentolamine, did not prevent a rise and the formation of a K^+ plateau although the rise in plasma K^+ and the plateau formed were significantly lower ($p < 0.02$) than those produced by 30% haemorrhage without drugs.

3.4.5 EFFECTS OF MORPHINE ON MABP AND PLASMA K^+

Morphine in doses of 8 mg/kg administered intravenously caused a fall in MABP from 139 ± 14 mm Hg to 54 ± 7.8 mm Hg. The MABP then slowly rose to 59 ± 6.9 mm Hg, but did not return to the control level. A small transient initial rise in MABP usually preceded the drop in pressure.

The fall in MABP was accompanied within 50 sec of the injection by a rise in the HIVC plasma K^+ from 3.61 ± 0.41 mmol/l to 6.69 ± 0.74 mmol/l before dropping to the control value (see Figs. 3.5a, b). Intra-arterial injection of the same dose produced an increase in plasma K^+ to 8.20 ± 0.66 mmol/l within 20 sec. Spontaneous fluctuations and sometimes a sustained rise in MABP occurred and there was no return to the control values. Other cardiorespiratory effects observed after morphine injection were tachycardia followed by bradycardia (Lead II ECG), and an end-inspiratory apnoea which was followed by slow respiration (CO_2 analyser) (see Figs. 3.5a, b). Intravenous injections of 8 mg/kg of morphine in successive doses at intervals of 30 min resulted in diminishing effects on MABP and HIVC K^+. Such effects are illustrated in Fig. 3.6a.

A single dose of subcutaneous injection of 2 mg/kg morphine did not significantly change the MABP in the cat and caused a very slow rise in HIVC K^+ from 4.88 ± 0.4 mmol/l to only 5.70 ± 0.2 mmol/l in 120 min. Successive increasing doses of 2, 4, 6 and 8 mg/kg morphine i.v. caused increasing falls in MABP but varying HIVC K^+ changes as shown in Figure 3.6b. 2 to 4 mg/kg i.v. morphine caused a significant fall in MABP with a moderate rise in HIVC K^+ ($p < 0.05$).

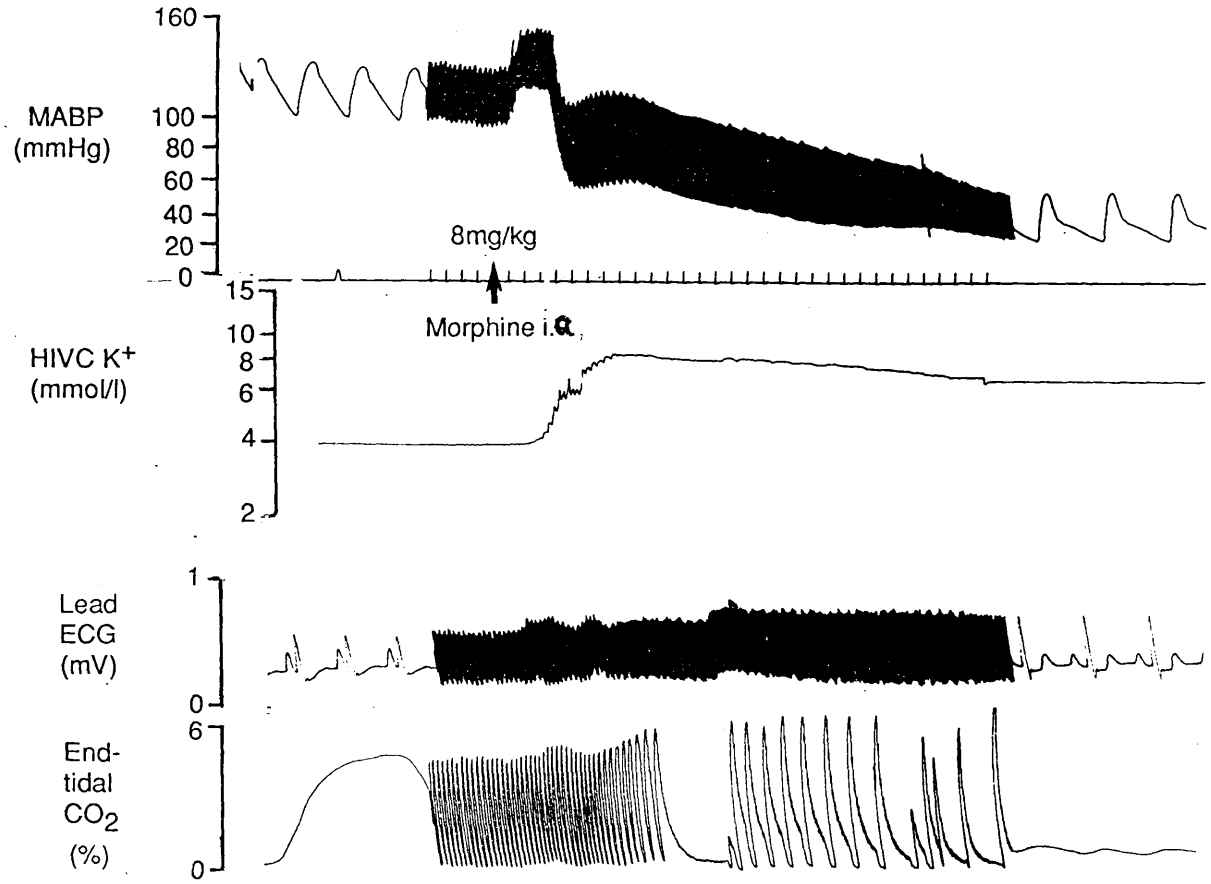


Fig.3-5a. Time-course of the response to intra-arterial injection (i.a) of morphine. Note the abrupt rise in the HIVC plasma K+ occurring within 20 secs of the i.a. injection accompanied by a transient rise in the MABP before a fall, tachycardia followed by bradycardia and an increase in the breathing rate followed by an end-inspiratory apnoea before resuming into a slow rate (compare with Fig.3-5b).

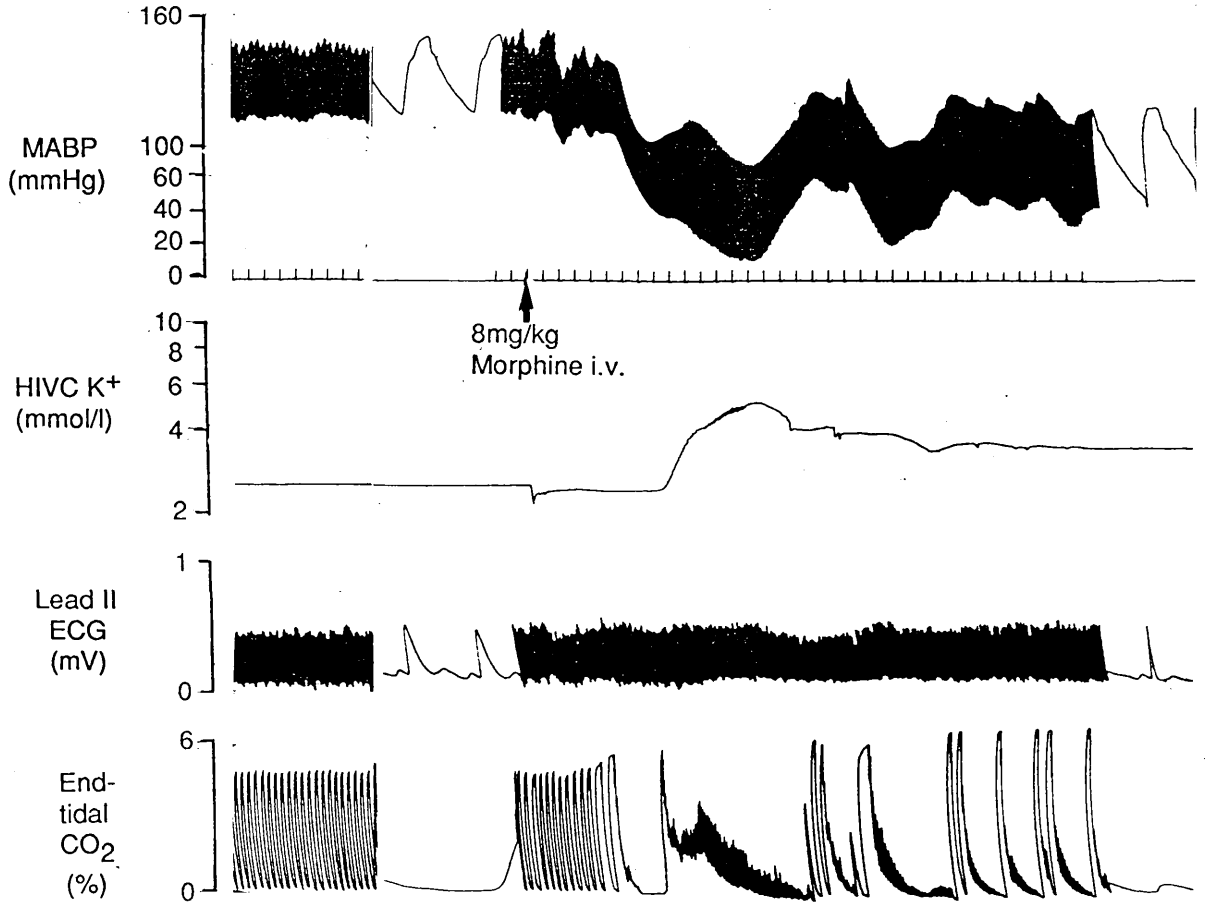


Fig.3-5b. Time-course of the response to intravenous injection of morphine. Note the rise in the HIVC plasma K^+ occurring within 50 secs of the injection accompanied by fluctuations in the MABP (compare with Fig.3-5a, and the longer period of apnoea than in the intra-arterial injection.

Fig.3-6a

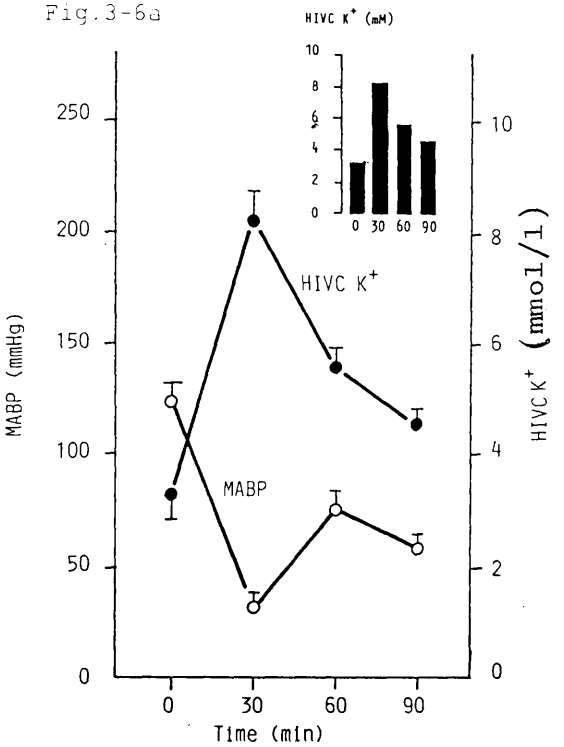


Fig.3-6b.

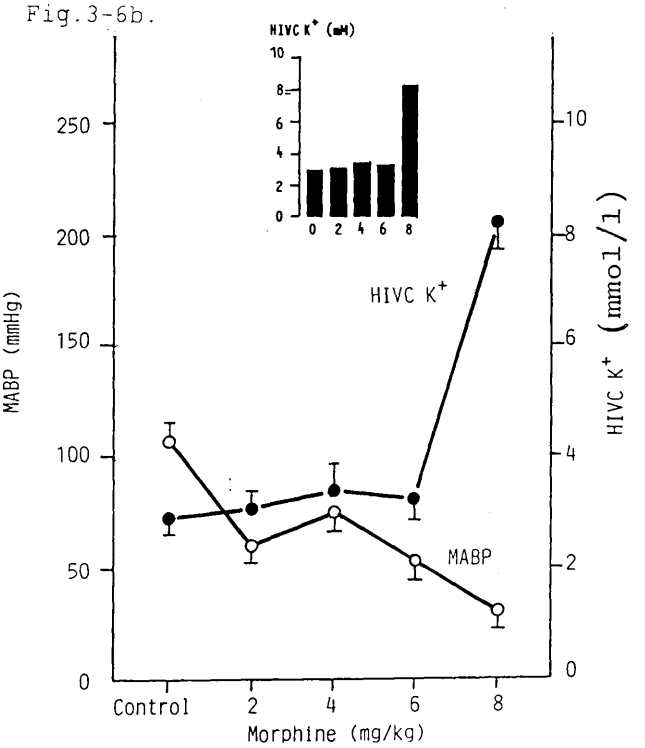


Fig.3-6a Dose-response curves for changes in the MABP and the HIVC plasma K+ produced by repeated intravenous injection of morphine 18mg/kg) at intervals of 30 minutes Inset is a histogram illustrating the same changes in the HIVC plasma K+.

Fig.3-6b. Dose-response curves for the changes in the MABP and the HIVC plasma K+ produced by i.v. morphine of various concentrations increasing from 2 to 8 mg/kg. Inset is a histogram illustrating the same changes in the HIVC plasma K+ only.

6 mg/kg i.v. caused a significant fall in MABP ($\rho < 0.02$) with no significant difference in HIVC K^+ from that produced by 4 mg/kg i.v., though a repeated injection of the same dose (6 mg/kg i.v.) caused less of an effect as explained for 8 mg/kg i.v. injections above. Doses above 8 mg/kg i.v. caused sudden death of two cats after a sharp drop in MABP with arrest of breathing which were irreversible with naloxone. Therefore i.v. injections of 8 mg/kg of morphine which is sublethal dose but potent enough to produce significant changes in plasma K^+ was used throughout this part of the present study.

3.4.6 EFFECTS OF MORPHINE IN THE PRESENCE OF NALOXONE, PROPRANOLOL, PRAZOSIN OR ADRENALINE ON MABP AND PLASMA K^+

In order to ascertain whether the effects of morphine on MABP and plasma K^+ occurred through opioid receptors alone or synergistically with catecholamines via adrenoceptors, adrenaline, a catecholamine which is known to cause a transient rise followed by a fall in plasma K^+ was employed. Also, the α_1-adrenoceptor blocking agent prazosin, and the β_1- and β_2-adrenoceptor blocking agent, propranolol, were used to investigate whether the adrenoceptors play any role in mediating the responses to morphine, while naloxone, the opioid-receptor antagonist was used to pharmacologically separate the opioid-receptor mediated effects from the adrenoceptor effect.

Examples of traces obtained for each of the drugs used, agonist or antagonist, are illustrated and explained in the following Figures 3.6 - 3.9. The results were very consistent and reproducible.

3.4.7 MORPHINE EFFECTS IN THE PRESENCE OF NALOXONE

Administration of 0.4 mg/kg i.v. naloxone before morphine, 8 mg/kg i.v. significantly reduced ($p < 0.01$) the effects of morphine on MABP, heart rate and end-tidal CO_2 (see Fig. 3.7).

3.4.8 MORPHINE EFFECTS IN THE PRESENCE OF PROPRANOLOL

When 0.2 mg/kg i.v. propranolol was given before morphine, there was an initial delay of about 2 min from the morphine injection before the HIVC K^+ rose from 3.35 $\pm$ 0.46 mmol/l to 8.2 $\pm$ 0.38 mmol/l. Conspicuous oscillations on the HIVC K^+ traces corresponding in time with breathing appeared at the maximum point of the rising phase of HIVC K^+. These oscillations went through a 180° change in direction at the lowest level of fall in HIVC K^+ (see Fig. 3.8). The CO_2-analyser traces showed tachypnoea which was followed by observed deep fast breathing. The lead II ECG showed a bradycardia at the same time as the MABP fell from 129 $\pm$ 4.8 mm Hg to 26 $\pm$ 2.8 mm Hg.

3.4.9 MORPHINE EFFECTS IN THE PRESENCE OF PRAZOSIN

In the presence of 0.2 mg/kg i.v. prazosin, morphine produced a greater fall in MABP from 112 $\pm$ 4.0 mm Hg to 24 $\pm$ 2.8 mm Hg and there was no significant reduction in the rise in HIVC K^+ produced by morphine. There was a measurable fall in MABP (ΔMABP = +28 $\pm$ 4.2 mm Hg) produced initially by prazosin alone without a corresponding rise in HIVC K^+. Prazosin produced similar decreases in MABP in earlier studies in which it significantly reduced the plasma K^+

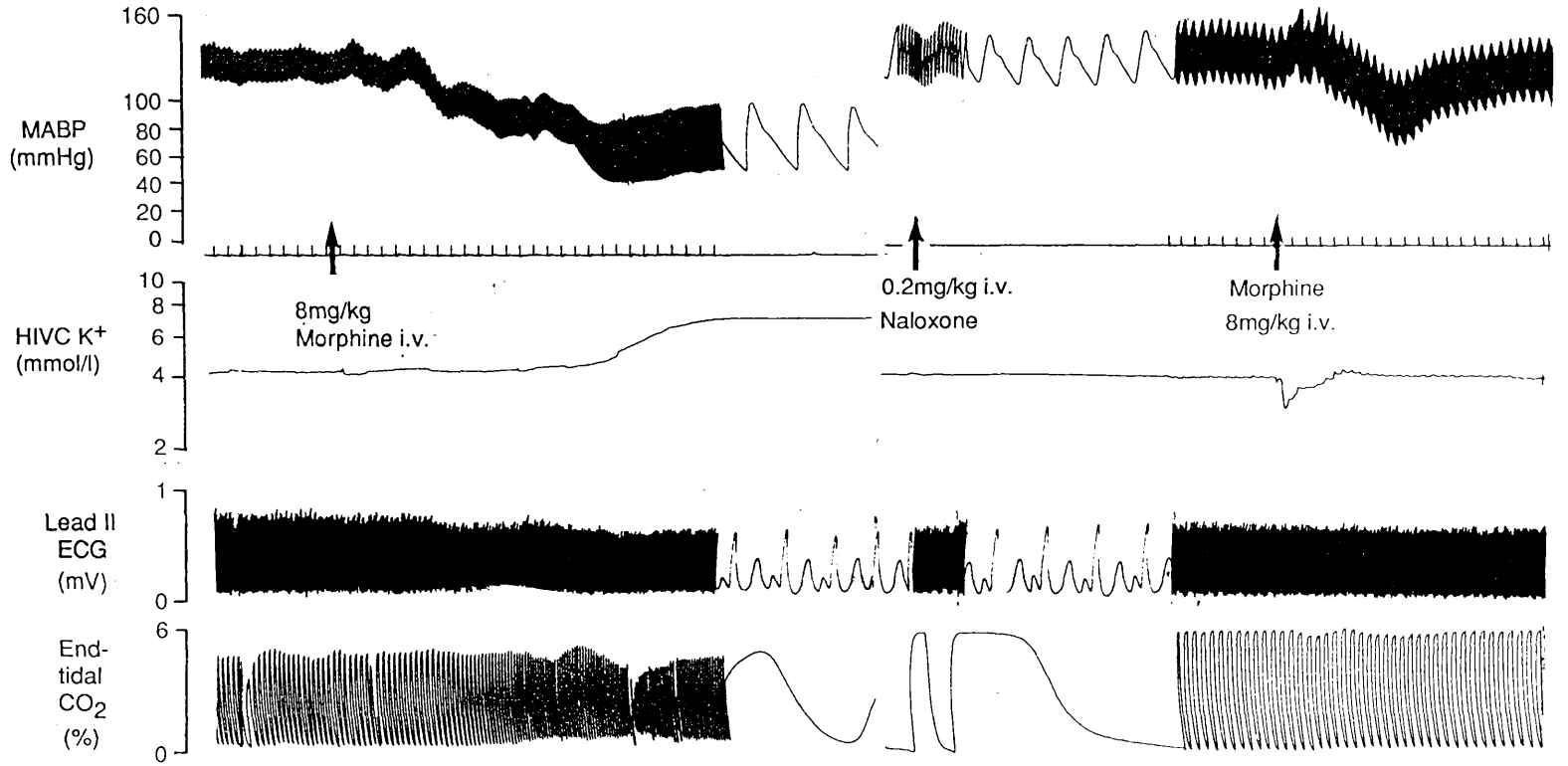


Fig.3-7. Effects of morphine before and after naloxone pretreatment on the MABP, HIVC plasma K+, the ECG and the end tidal CO2. Note the lack of effect of naloxone on its own on the MABP, plasma K+, ECG and the end tidal CO2. However, the effects of morphine are abolished in the presence of naloxone.

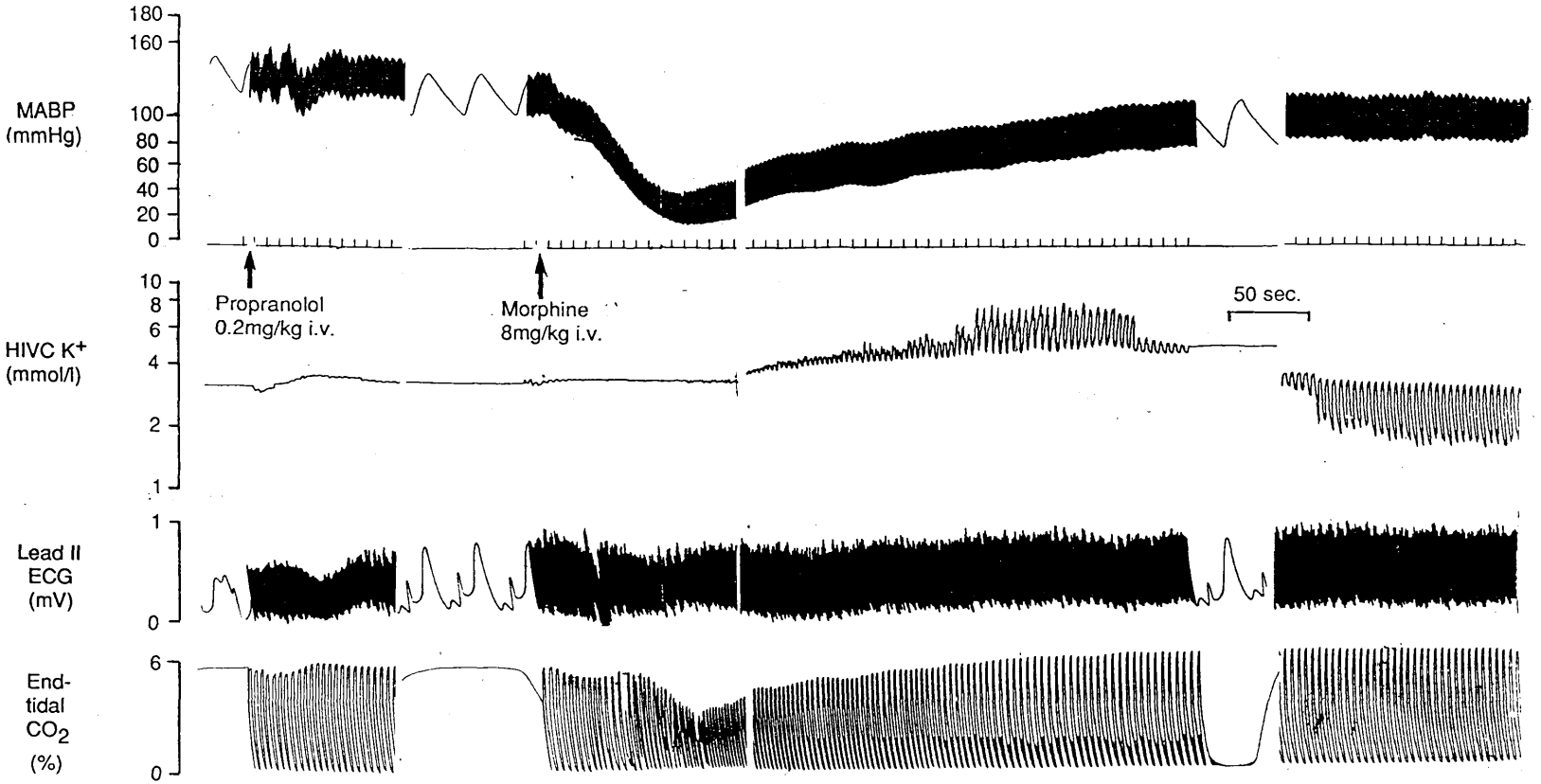


Fig.3-8. Morphine response in the presence of propranolol, illustrating also the oscillations in the HIVC plasma K+ trace corresponding with the respiratory oscillations in the end-tidal CO2 trace. Note the 180° change in direction of the oscillations as plasma K+ is taken up towards the control level.

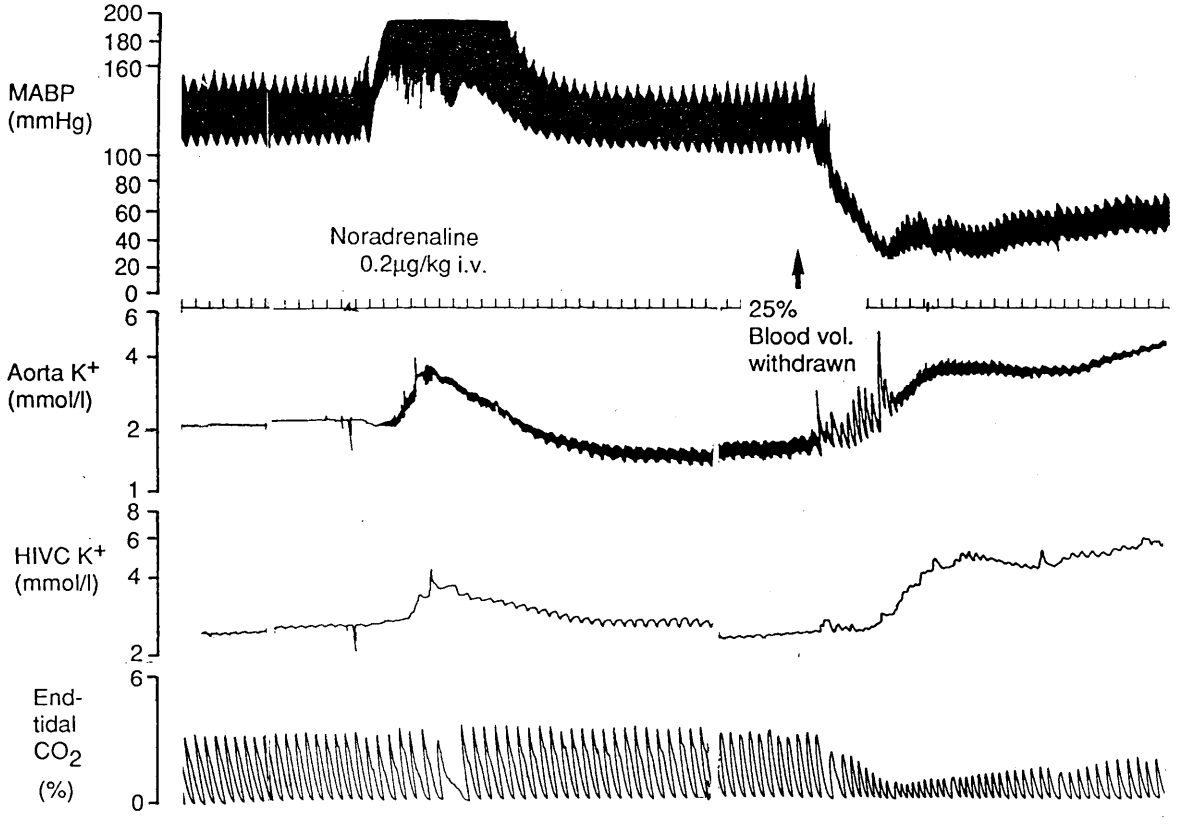


Fig. 3 2a

Illustration of oscillatory excursions in both the aortic and the EIVC plasma K+ traces produced by noradrenaline injection, and blood withdrawal. In this only case throughout the study where oscillations were also observed in the aortic plasma K+ trace, the tip of the aortic plasma K+-selective electrode catheter was found to be situated in the left ventricle at postmortem examination.

rise produced by adrenaline, showing that the morphine-induced rise in K^+ was mediated through receptors which are different from those mediating the responses to adrenaline.

The CO_2 recordings, when morphine was given in the presence of prazosin, showed that hyperventilation accompanied the fall in MABP and the rise in HIVC K^+ (See Fig. 3.9).

3.4.10 MORPHINE EFFECTS IN THE PRESENCE OF ADRENALINE

When 2 μg/kg i.v. adrenaline and 8 mg/kg i.v. morphine were given together in a single dose, the effect on the MABP, end-tidal CO_2 and heart rate were more like those of adrenaline than morphine. The combined effect on the rise in HIVC K^+ was less than the sum of the effects of the two given separately, yet greater than the effect of the individual drugs given alone. For example, 2 μg/kg adrenaline i.v. produced a rise in HIVC K^+ from 3.00 ± 0.14 mmol/l to 7.73 ± 0.56 mmol/l, while 8 μg/kg morphine i.v. produced a rise from 3.61 ± 0.41 mmol/l to 6.69 ± 0.74 mmol/l. The combination of the two drugs as a single dose produced a greater rise in K^+ from 3.72 ± 0.52 mmol/l to 8.66 ± 0.68 mmol/l (Fig. 3.10).

3.4.11 EFFECTS OF VAGOTOMY ON MORPHINE RESPONSE

In order to investigate any role played by the parasympathetics in the depressor and the hyperkalaemic effects of morphine the vagi in the cervical region were sectioned, and the effects of morphine, alone, and in the presence of the adrenoceptor and opioid-receptor blocking agents were studied as in the previous series of experiments reported

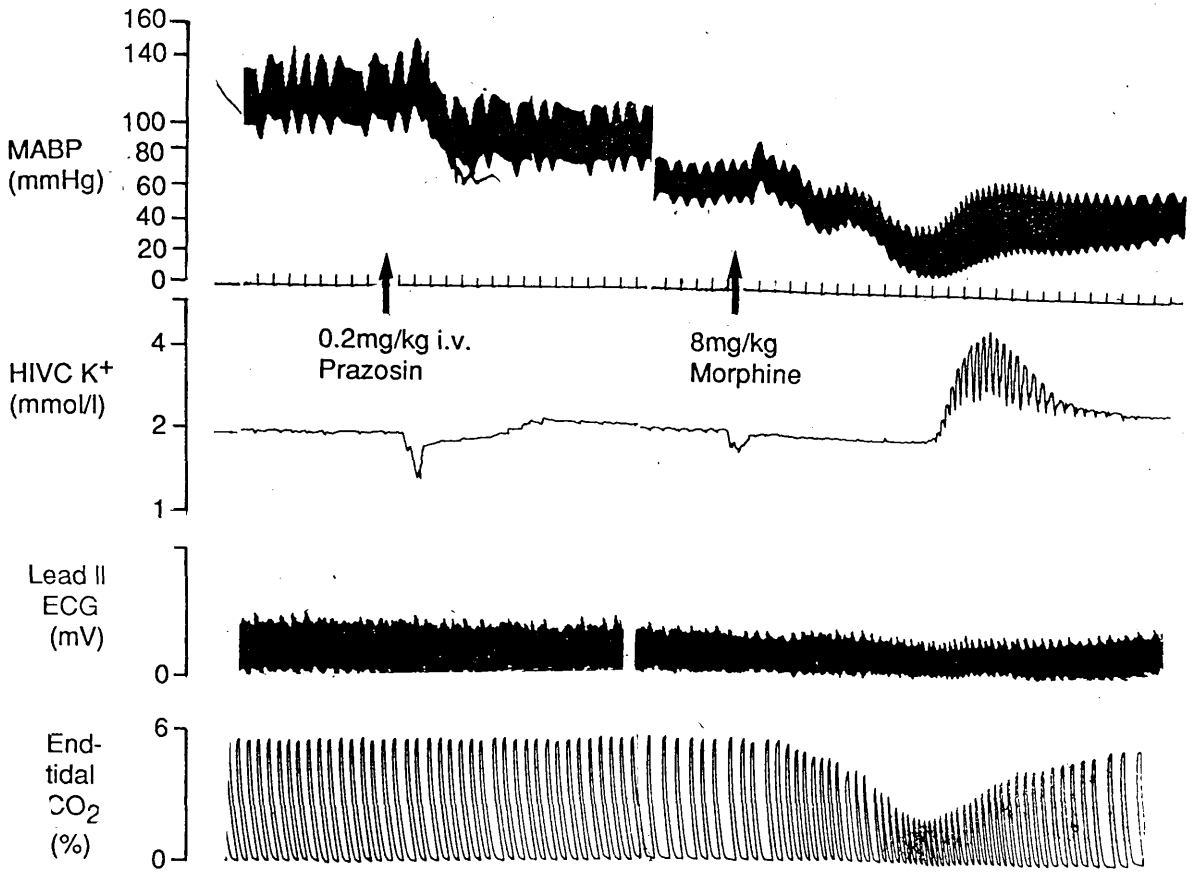


Fig.3-9. Effects of morphine after pretreatment with prazosin on the MABP, HIVC plasma K+ and the end-tidal CO2. Note the marked fall in the MABP produced by prazosin on its own without a significant increase in the plasma K+. The transient hyperventilatory effect of morphine is also illustrated. Prazosin has no significant effect on the hyperkalaemia induced by morphine.

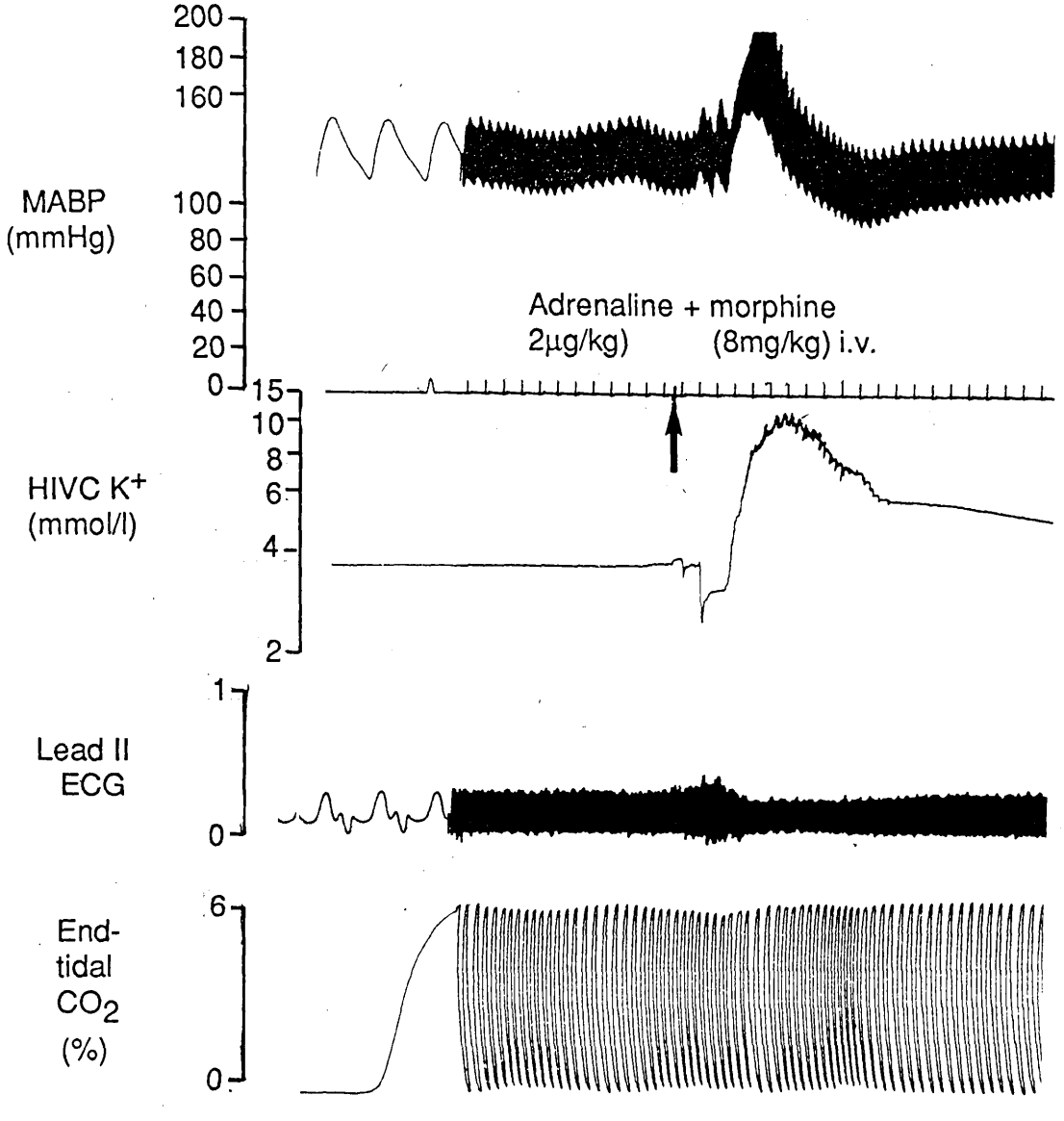


Fig.3-10. Effects of the intravenous injection of the combined dose of morphine and adrenaline on MABP and HIVC plasma K+. Note the greater pressor and the hyperkalaemic effects of the combined dose with no significant effect on the breathing frequency and the end-tidal CO2. (Compare with the individual drug dose responses in Figs.3-4a and 3-6a).

above. The vagi have been reported to modulate the transient pressor as well as the prolonged depressor responses to opiates.

When 8 mg/kg morphine was given i.v. there was a small transient rise in MABP followed by a gradual fall in BP. When the vagi were sectioned during this falling phase a sudden rise in MABP occurred, and oscillations on the HIVC K^+ traces caused by rises and falls in K^+ accompanied the rise in MABP. The MABP also fluctuated at the same rhythm as that of the HIVC plasma K^+ (see Fig. 3.11).

Accompanying these events, the breathing rate declined, then stopped. After about 25 seconds respiration resumed with slow deep breaths and with apnoeic intervals of 20 to 40 sec. At the same time, i.e. after sectioning the vagi, lead II ECG showed periods of tachycardia accompanied by a reduced amplitude of the QRS-complex, alternating with periods of bradycardia with an increased amplitude of the QRS-complex. Throughout this period from the sectioning of the vagi to the periods of bradycardia, the HIVC plasma K^+ rose from a control value of 2.38 ± 0.66 mmol/l to 9.9 ± 0.68 mmol/l and afterwards fell again to control levels. The fluctuating peaks in MABP, HIVC K^+, lead II ECG and end-tidal CO_2 and the way they correspond with one another are illustrated in Figure 3.11. These were the results when vagotomy was performed in the presence of morphine. However, when morphine was given later after the initial effects of vagotomy had subsided, there seemed to be some adaptation in the responses to morphine, that is the effects

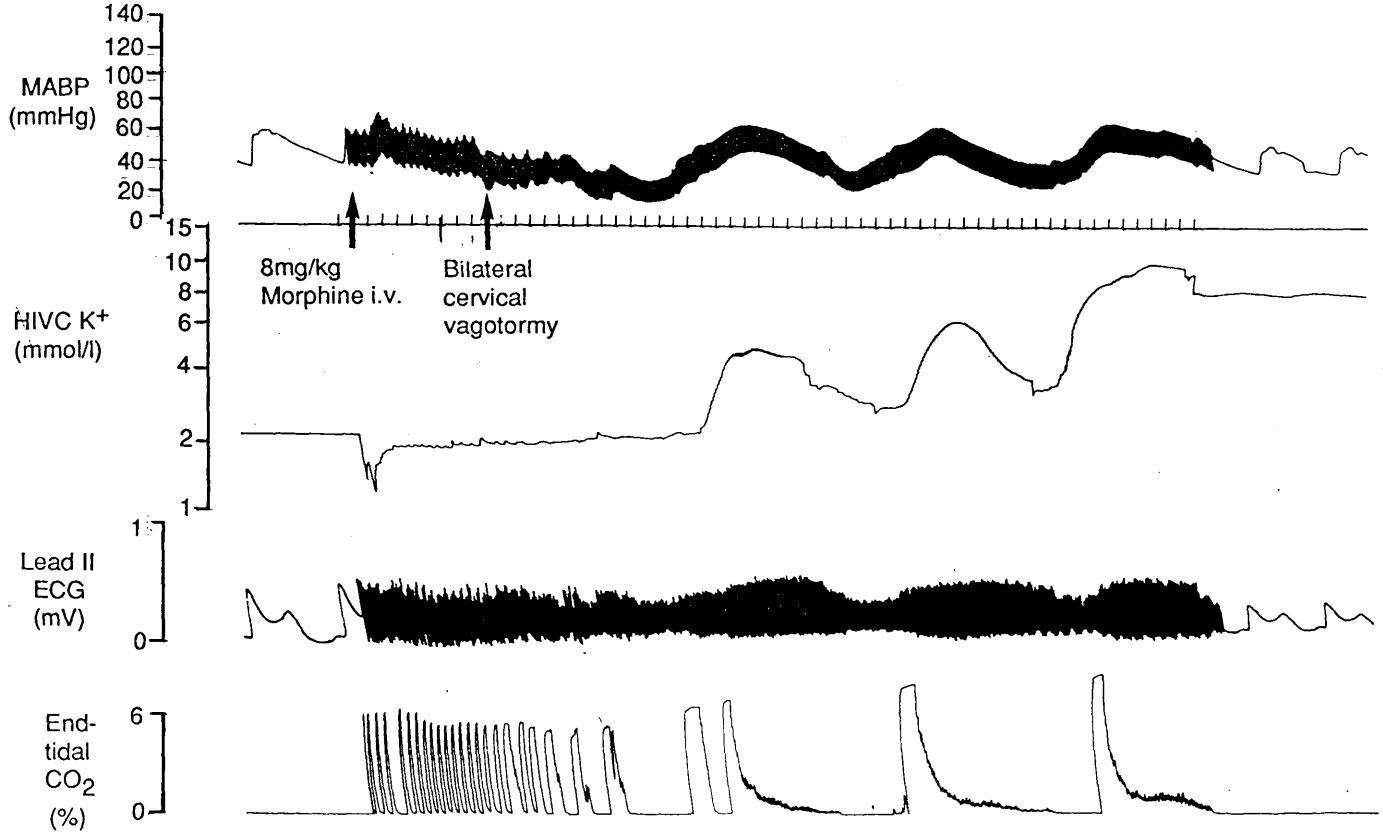


Fig.3-11. Effects of bilateral cervical vagotomy on morphine response. Note how the rising phases of the fluctuations in the MABP correspond with the phases of hyperkalaemia, apnoea and high amplitude of the QRS-complex in the lead II ECG.

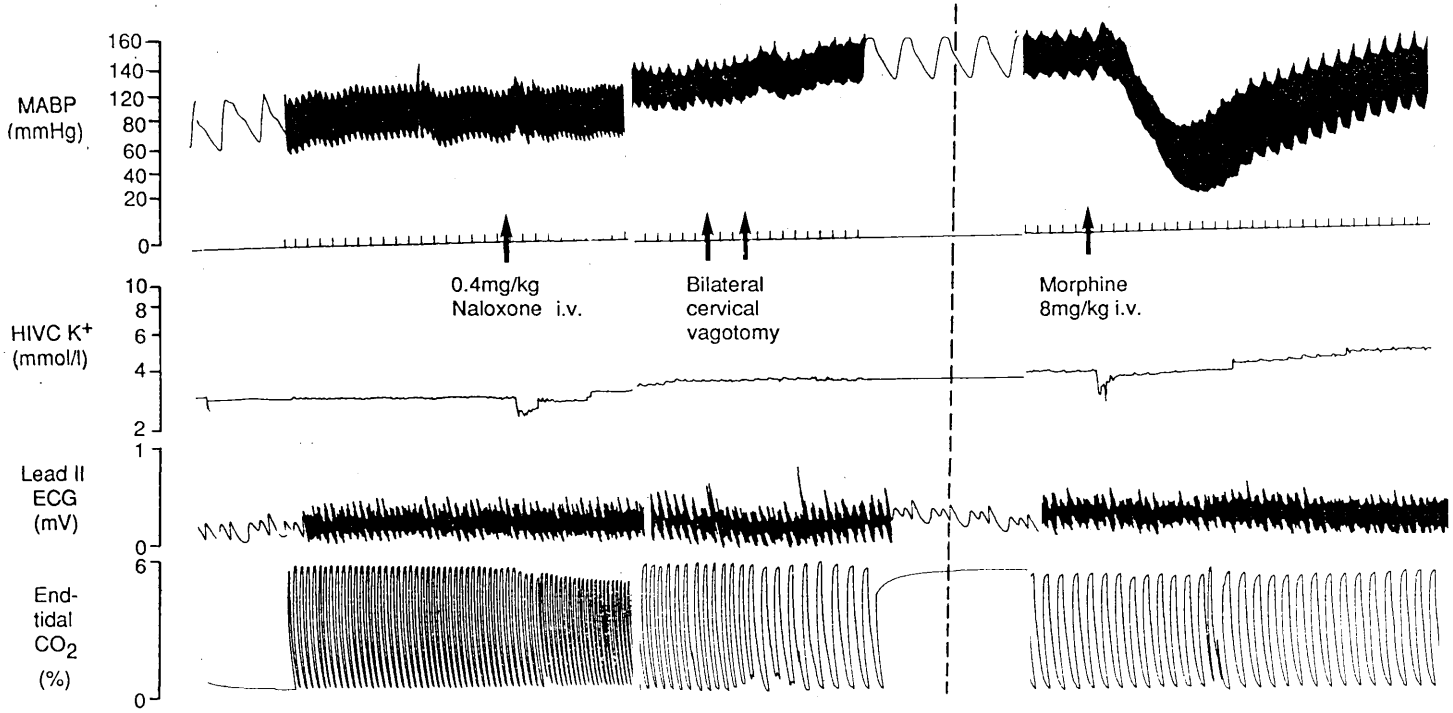
on MABP and plasma K^+ were qualitatively similar to that described above but smaller in magnitude. The control respiration rate after vagotomy was reduced from 26 $\pm$ 4 breaths per minute to 10 $\pm$ 2 breaths per min.

In order to test whether these effects of morphine in the absence of the vagi were a result of the disinhibition of the sympathetic outflow by vagotomy or a primary effect of morphine enhancing any endogenously released opioids after vagotomy, naloxone, the opioid receptor antagonist, and adrenaline, a sympathetic agonist were given in the presence of morphine following vagotomy in another set of 4 cats. It has been observed in the present study that when naloxone is given i.v. to a deeply anaesthetized cat under control conditions, it produces no effect on the MABP, heart rate, plasma K^+ but causes a tachypnoea. When vagotomy was performed in the presence of 0.4 mg/kg naloxone i.v. a significant rise ($p < 0.02$) in MABP from 111 $\pm$ 9.8 mm Hg to 127 $\pm$ 6.4 mm Hg occurred and a marked fall in breathing rate from 26 $\pm$ 4 to 10 $\pm$ 2 breaths/min. There was no significant effect on the end-tidal CO_2 (see Fig. 3.12). The breaths were observed to be very deep. When the administration of naloxone was repeated, this time after the vagotomy, there was no significant effect on HIVC K^+, MABP or heart rate.

In the presence of naloxone after vagotomy an injection of 8 mg/kg morphine i.v. caused the MABP to fall rapidly from 127 $\pm$ 6.4 mm Hg to 43 $\pm$ 4.4 mm Hg and returned gradually to 112 $\pm$ 8.6 mm Hg. Under these circumstances the effects

Fig. 3 - 12

Fig. 3 - 13



Figs.3-12 & 13:
3-12. Effects of vagotomy on naloxone response. Note the lack of effect of naloxone on its own on the MABP, HIVC plasma K+ and heart rate, except causing an increase in the breathing frequency. Vagotomy in the presence of naloxone produced only a rise in the MABP but a fall in the breathing fequency.

3-13. Morphine in the presence of naloxone after vagotomy significantly ($p<0.01$) reduced the MABP with no effects on the heart rate or the breathing frequency, nor on the plasma K+. (See also Fig.3-14).

of morphine on plasma K^+, heart rate, breathing rate and end-tidal CO_2 were not significant (see Fig. 3.13).

Also, in Figure 3.14 can be seen the effect of 2 μg/kg i.v. adrenaline on HIVC plasma K^+ after vagotomy, and after the response to morphine had been blocked by naloxone. An increase in plasma K^+ (ΔK^+ = +6.17 mmol/l) was recorded in this case. This increase is similar to the rise recorded as reported above when naloxone was given before adrenaline (see Figs. 3.4a,b). The large HIVC K^+ oscillations were observed again and they corresponded with each breath of respiration. Naloxone appears to enhance the effects of adrenaline on MABP and plasma K^+, with or without vagotomy. However, following vagotomy, the presence of naloxone had no effect on the depressor response to morphine but significantly reduced the hyperkalaemic response to morphine.

Some other observations included episodes of sudden spontaneous increases in MABP (ΔBP = +21 $\pm$ 4.2 mm Hg) accompanied by marked increases in plasma K^+ (ΔK^+ = +5.2 $\pm$ 0.66 mmol/l) and tachycardia. These episodes were always preceded by a period of apnoea of 30-60 secs duration (see Fig. 3.15).

3.4.12 EFFECTS OF HAEMORRHAGE AFTER VAGOTOMY

If the vagi played any role directly or indirectly, in the regulation of changes in MABP and plasma K^+ during haemorrhage, then in the absence of the vagi such changes produced by haemorrhage would either be increased or decreased. Indeed, removal of 25% of the total blood volume after vagotomy in the present study resulted in responses similar

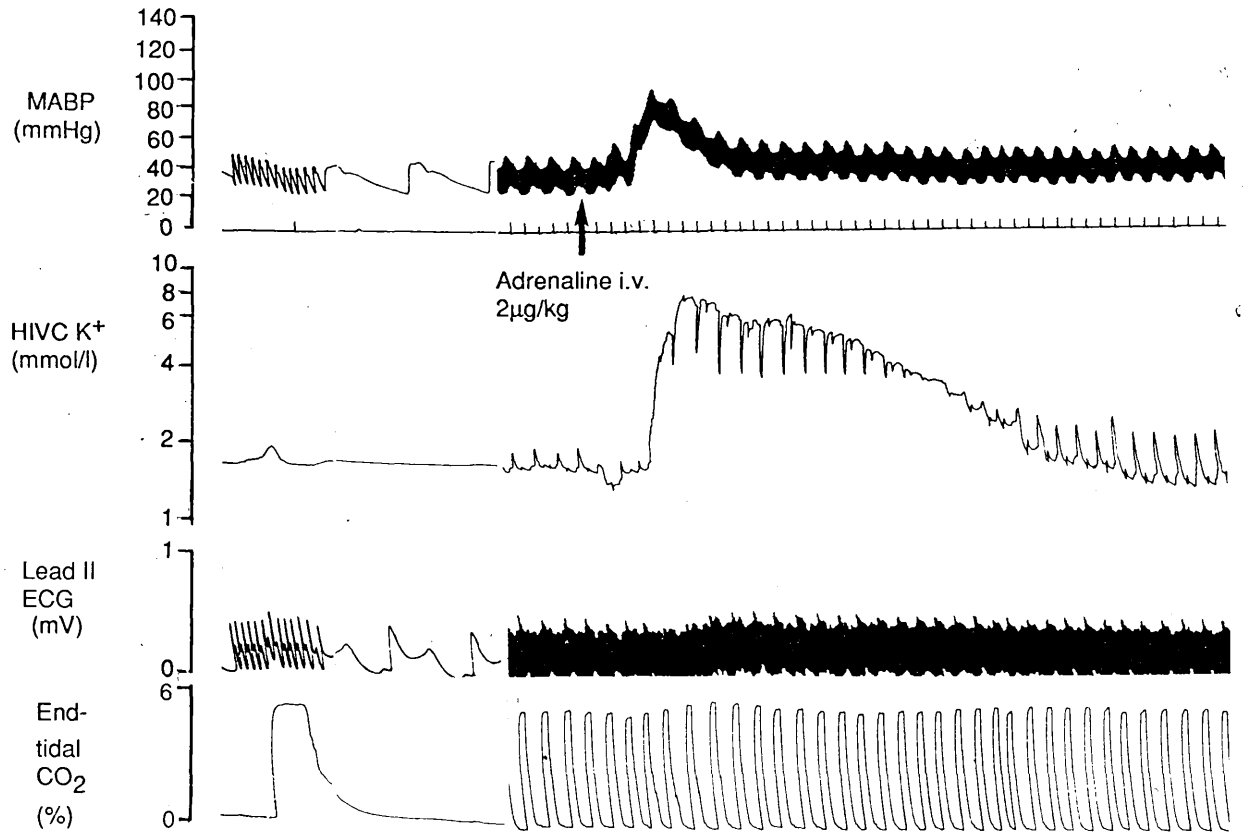


Fig. 3-14. Effects of adrenaline in the presence of naloxone after vagotomy on the MABP, HIVC plasma K+, and the end-tidal CO2. Note the marked rise in the HIVC plasma K+ and the MABP with no change in the breathing rate (compare with Figs. 3-4b and 3-13).

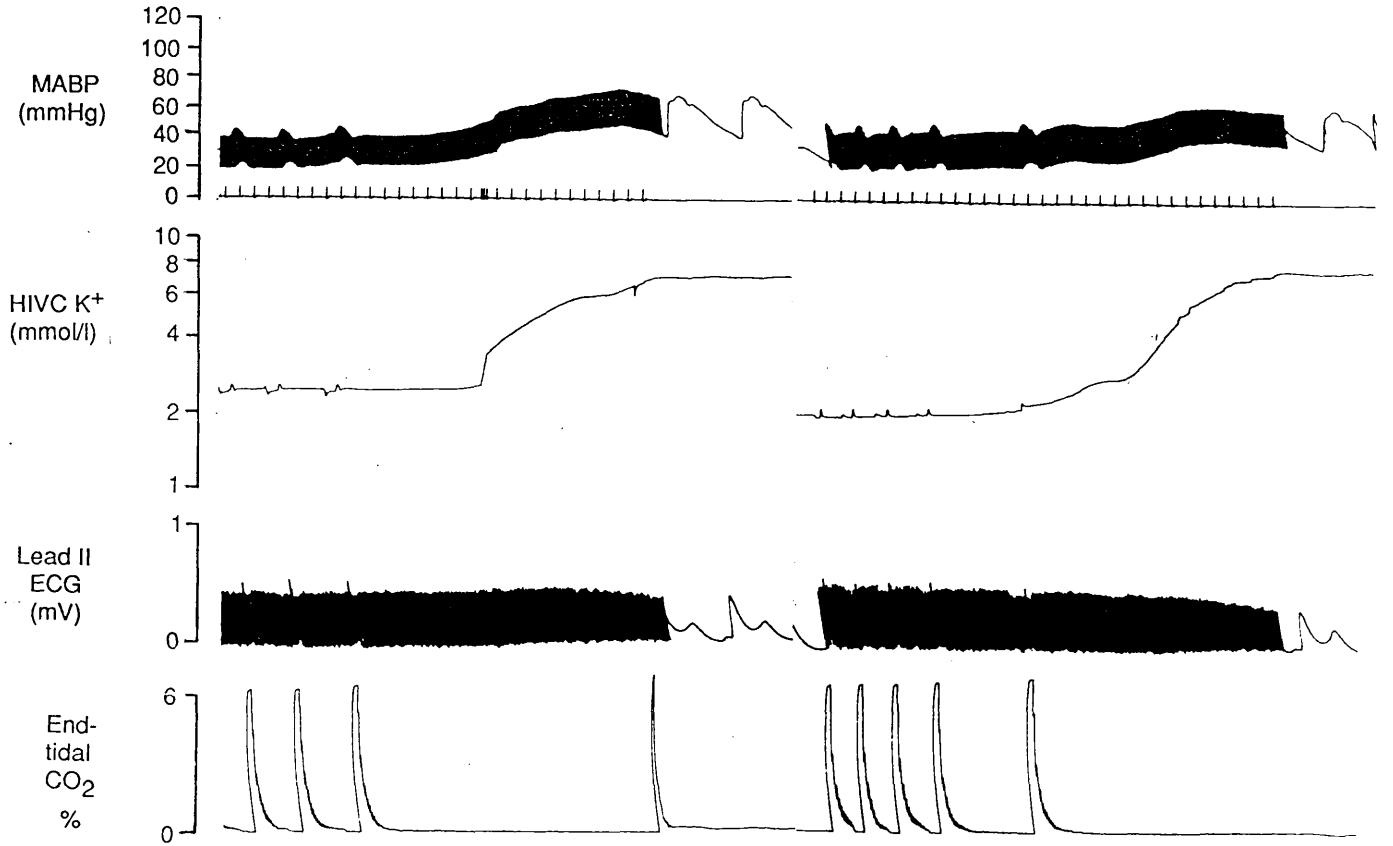


Fig.3-15. Episodes of spontaneous rises in the MABP and HIVC plasma K+ preceded by periods of apnoea following bilateral cervical vagotomy.

in magnitude to 30-35% haemorrhage with the vagi intact, viz., a fall in MABP from control to zero then a rise to 4.0 mm Hg, a marked rise in HIVC K^+ which stayed at the raised level for the <5 min period of observation, and apnoea with accompanying bradycardia.

It is clear from these results that despite pretreatment of the cats with adrenoceptor and opioid receptor antagonists and after bilateral cervical vagotomy, the rise in plasma K^+ after withdrawal of blood cannot be totally abolished by any single or combination of these antagonists. Attempts made to effect a complete blockade of the K^+ release by increasing the dose of the blocking agents resulted either in death or non-recovery of the MABP to control values from severe hypotension.

3.5 DISCUSSION

The results from these studies showed that naloxone, apart from raising the MABP in haemorrhagic hypotension also returned the raised plasma K^+ levels resulting from haemorrhage back to control values. These dual effects of naloxone appear to occur through different mechanisms and depend on the severity, the duration and the time of injection of naloxone in the course of the hypotension. For example, naloxone still reversed the elevated plasma K^+ levels even when its effect of raising the MABP was prevented by withdrawing more blood. Also when vagotomy was performed in the presence of naloxone, naloxone could no longer prevent the fall in MABP produced by morphine but still abolished

the morphine-induced rise in plasma K^+. The mechanism involved in the dissociation of the MABP raising effect of naloxone from its plasma K^+ lowering effect in the above circumstances is obscure. However, it seems that the failure of naloxone to be effective in severe and prolonged shock may be due either to a loss of potency in an increasing acidotic medium or the amount of plasma opioids released as a result of prolonged hypotension (Holaday, 1983), may have increased and may have overcome the effect of naloxone, which is a competitive antagonist. Alternatively, the body tissues may no longer respond to naloxone in such conditions. The lowering of the *in vivo* potency of morphine and narcotic antagonists, and even abolishing naloxone activity by acidosis has been reported by Kaufman (1982). Holaday and Faden (1981) had to administer sodium bicarbonate in their endotoxic and haemorrhagic shock experiments to correct progressive acidosis, or their animals became less responsive to naloxone. Bull *et al.* (1982) have reported that some endogenously released opioids like the enkephalins are not antagonized by naloxone because their responses are mediated by δ-receptors and not by μ-receptors to which naloxone is more antagonistic, and that higher doses of naloxone would be needed to counteract such opioids. These workers further demonstrated that increased levels of plasma K^+ in turn stimulated the release of met-enkephalins from guinea-pig striated slices and these opioids released did not respond to naloxone, or to M_r 2034, another opioid receptor blocker.

If such K^+-induced opioid release occurred, then the increased level of plasma K^+ produced by haemorrhage suggested to be caused by the release of endogenous opioids and catecholamines might in fact be causing a positive-feedback effect on itself, leading to more opioid release and therefore in turn more plasma K^+. The dose of 0.1 to 0.5 mg/kg i.v. of naloxone either slowly infused or given as a single dose might therefore not be adequate to antagonize the effects of the endogenously released opioids if they are increasing in such a vicious cycle as haemorrhagic shock continues.

Therefore naloxone may block opioid receptors and raise the falling MABP, and reduce the rising plasma K^+ following haemorrhage. This effect of naloxone appears to be true because morphine, an opioid alkaloid which mimicks the effects of haemorrhage by decreasing the MABP, and increasing plasma K^+ was prevented by naloxone from producing these responses. Morphine when given during the hypotensive phase following blood withdrawal further accentuated the raised plasma K^+ level which also supports the idea that opioids released endogenously contribute to the release of K^+ during haemorrhagic hypotension. The present study also shows that naloxone has some potentiating effects on adrenergic receptors. This is illustrated by the consistent observation that naloxone increases the plasma K^+ level when given before adrenaline injection and this is followed by a greater hypokalaemia. Also, when naloxone was given in a single dose intravenously before blood withdrawal the rate

and magnitude of rise in plasma K^+ was enhanced (Fig. 3.1(a)). More support was lent to this idea by the consistent observation that naloxone on its own did not reverse the initial rise in the plasma K^+ level following haemorrhage, but it markedly attenuated this early rise in the presence of phentolamine, a predominantly α_1-receptor blocking agent. If propranolol, a β-adrenoceptor blocker was injected before naloxone, naloxone did not prevent either the early or the later phases of rise in plasma K^+. The lack of any significant effects on the MABP and plasma K^+ by slow infusion of 10 ml normal saline, the solvent for naloxone, during and after haemorrhage, confirms that the observed effects following i.v. naloxone in normal saline are produced by naloxone.

The vagi were found to be involved in the attenuation of some, and the enhancement of other morphine effects, for after bilateral cervical vagotomy, the effects of morphine in raising plasma K^+ increased, while the MABP lowering effect of morphine was changed to fluctuating rises and falls before returning to control. The greater effect of adrenaline injection, 2 μg/kg i.v. in raising the plasma K^+ after vagotomy also illustrated further that the parasympathetic system may have an indirect inhibitory role in the release of K^+ opposing the effects of the sympathoadrenal system in K^+ release. In support of the suggestion of such a role for the vagi, muscarinic agonists have recently been reported to block K^+ conductance by Cassell and McLachlan (1987). Acetylcholine released natrually e.g. by reflexes from chemoreceptors (Jänig, Kauspe &

Wiedersatz, 1983) can activate muscarinic receptors, evoking discharges in some postganglionic neurones. It is likely that the vagotomy in the present study may have removed the blockade of K^+ conductance by the vagi because morphine, adrenaline and less severe haemorrhage cause an increase in the release of K^+. These results do not agree with the reports of Hohnloser, Verrier and Lown (1986), that parasympathetic nervous system activation does not influence serum K^+ concentration after the injection of metacholine, a muscarinic receptor agonist. The same workers suggested that the lack of significant effect of metacholine on serum K^+ might be dose-related.

That there exists a combined effect of opioids and catecholamines in the release of K^+ was suggested by the greater increase in the HIVC K^+ rise after a combined dose of the two drugs than if either was injected alone. Co-release of enkephalin and catecholamines from cultured adrenal chromaffin cells (Livett et al., 1981), and the co-existence of μ-opioid receptors and α_2-adrenoceptors in guinea-pig myenteric plexus (Surprenant & North, 1985) have been reported. In further support of the contributory role of opioids in the release of K^+, Mihara et al. (1986) have reported an increase in K^+ conductance by opioids in the guinea pig caecum, while the induction of K^+ efflux from de-energized mitochondria in liver cells by opiates has been reported by Chistyakove et al. (1980). In addition to studies on organs of the splanchnic region, the opening

of K^+ channels by enkephalins in mammalian central neurones has been reported by Williams et al. (1982), while Curratu and Mitoto-Chieppa (1982) reported the inhibition of K^+ ionic currents in the frog node of Ranvier by an opioid receptor antagonist, naloxone.

Dashwood et al. (1980) demonstrated that cutting the vagi, among other surgical procedures in the cat e.g. evisceration and tying the sinus nerves induced the release of endogenous opiate peptides. The stimulus for such release of opioids was attributed to the interruption of afferent sensory pathways from viscera or the "stress" associated with the surgical procedures. Similarly, the surgical procedures involved in the present study including deep anaesthesia and the bilateral cervical vagotomy might be enough "stress" to stimulate the release of endogenous opioid which in turn may cause the release of K^+ as do other stimuli like the catecholamines. The pressor and the hypokalaemic responses to naloxone in the conditions of the present study are thought to be antagonistic effects of naloxone to the opioids released.

Other mechanisms have been reported by several workers to explain the pressor role of naloxone in shock. Some of these mechanisms discussed below have been demonstrated in the present study and investigated to explain the plasma K^+-reducing effect of naloxone following haemorrhage. In Section One of this thesis, hyperventilation has been shown to transiently induce hyperkalaemia within 5 min., and in

cats made hyperkalaemic by haemorrhage, hyperventilation causes a further small transient rise in K^+ followed by a fall in K^+ to near control levels. This means that hyperventilation causes a transient rise in plasma K^+ from a normokalaemic state, and in a hyperkalaemic state induces a normokalaemic effect.

The role of naloxone in disinhibiting the suppressor effects of opioids on the carotid chemoreceptors in hypoxia and hypercapnea has been reported by Porkorski and Lahiri (1981). These workers have shown that in hypoxic and hypercapnic cats, ventilation is stimulated by an injection of naloxone even in the absence of significant stimulation of carotid chemoreceptors. They suggested that ventilation was normally suppressed by the effect of endogenous opiates released in the carotid body type I glomus cells, and also in the central nervous system as a result of hypoxia, and was disinhibited by naloxone. It is therefore reasonable to suggest that in the present study naloxone appears to counteract the inhibitory effects on ventilation of endogenously released opioids resulting from the hypoxia of acute haemorrhage to enhance the hyperventilatory and pressor responses observed, and in doing so counteracts and reduces any opioid-induced hyperkalaemia.

Another possible mechanism for the increased pressor response and the normokalaemic effects of naloxone could be an increase in circulating levels of catecholamines in response to the increased blood flow to the adrenal gland following naloxone injection in shock (Lechner *et al.*, 1985).

However, Patton et al. (1983) reported that naloxone exhibited its usual beneficial effects in adrenalectomized dogs pretreated with cortisol. These workers argued that the haemodynamic response to naloxone was due to increased plasma cortisol since glucocorticoids have been shown to increase the inotropic effects of catecholamines when given two hours after hypovolaemia, and this inotropic effect might be due to the corticosteroids' ability to attenuate the desensitization of β-adrenergically mediated adenyl cyclase activity which results from hypovolaemia (Sherma, 1979; Davies & Lefkowitz, 1983). Again, in dogs incapable of synthesizing cortisol (Patton et al., 1983), naloxone was found to improve the cardiovascular performance, and so the effect was not due to an increase in plasma cortisol.

A combination of responses to naloxone in hypovolaemia, viz. an increase in cardiac, adrenal, hepatic and intestinal blood flow, as well as a significant increase in plasma cortisol level, has been suggested by Lechner et al. (1985) to be responsible for the improved cardiovascular function. In the present study since naloxone had no significant effect on the cardiovascular performance or plasma K^+ level of normovolaemic cats, it appears that animals must be in shock for naloxone to exert a pressor and normokalaemic effect. It is suggested that the increase in arterial pressure by naloxone and the increase in the cardiac and the splanchnic blood flow (Lechner et al., 1985), may result in an increased tissue perfusion rate and thereby induce plasma K^+ uptake, as well as the blockade of any further opioid-induced release

of K^+ by naloxone.

The sudden episodes of spontaneous and transiently sustained rises in MABP and plasma K^+ towards the terminal stages of the haemorrhagic hypotension following vagotomy may result from the activation of the CNS ischaemic response which causes extreme stimulation of the sympathetic nervous system to produce vasospasm of the arterioles. This effect of the CNS ischaemic response has been termed the "last ditch stand" of the sympathetic reflexes in their attempt to keep the MABP from falling too low (Guyton et al., 1961).

3.6 CONCLUSION

It is suggested that naloxone caused a normokalaemic effect to facilitate improved cardiac function in shock by antagonizing the hyperkalaemic tendency of endogenous opioids released in shock. The results of the present study do not show whether naloxone causes an increase in plasma catecholamines, but certainly naloxone potentiated the effects of plasma catecholamines. This was shown by the greater increase in MABP and plasma K^+ by adrenaline in the presence of naloxone. Propranolol, a β-adrenergic blocking agent prevents the pressor as well as the normokalaemic effects of naloxone following haemorrhage.

It is certain that the release of K^+ resulting from haemorrhagic shock is mainly from the region drained by the hepatic vein as consistently shown by the greater levels of plasma K^+ in the HIVC than in the aorta, but it is not clear whether this extra K^+ is released by the liver itself

or delivered to the liver via the portal vein from other splanchnic organs.

The residual rise of plasma K^+ following haemorrhage even after the individual or combined blockade of opiate-receptors and adrenoceptors suggests that either the blockade is pharmacologically not complete, or there are still some unknown mechanisms involved in causing hyperkalaemia during haemorrhagic shock.

The diversity of hypothesis merely reflects present uncertainty over the causes of the onset of plasma K^+ elevation during haemorrhage. If the multitude of factors which may initiate a plasma K^+ rise are incorporated into a causal hypothesis, that hypothesis must be sufficiently flexible to account for the different patterns of time-course of related events like the MABP, acid/base changes and the ECG.

3.7 SUMMARY

The effects of naloxone and to some extent the mechanism of raising the MABP and lowering the raised plasma K^+ following haemorrhagic hypotension have been investigated in the cat using K^+-selective electrode catheters.

Naloxone improved cardiac function and lowered hyper-kalaemia by potentiating the effects of circulating plasma catecholamines or perhaps rendering the body tissues sensitive to catecholamines once more following haemorrhage.

Opioid receptor antagonism appears to be one of the mechanisms involved in raising the MABP and lowering of the raised level of plasma K^+ following haemorrhage.

The vagi were found to be indirectly involved in the depressor effects of morphine and by inference endogenously released opioids following haemorrhage. Vagotomy was suggested to either increase the release of endogenous opioids and hence potentiate the hyperkalaemic response to injected morphine, or it disinhibited the sympathetic outflow to cause a transient increase in plasma K^+ levels and a rise in MABP.

It is suggested that the lack of effect of naloxone on the MABP and hyperkalaemia in the later phases of haemorrhagic shock may be due either to a lack of potency of naloxone due to increasing acidosis or a failure of the body tissues to respond to naloxone, or indeed an increase in the plasma concentration of the agonist, the endogenous opioids, as hypotension proceeded.

The extra plasma K^+ following haemorrhage was found to be coming from the region drained by the hepatic vein. Whether it was released by the liver itself or delivered to the liver via the portal vein by other visceral organs is not known with certainty.

CONCLUSIONS

The present study was originally undertaken to study the changes in plasma potassium and to investigate the role of potassium in the cause of irreversibility in haemorrhagic hypotension, monitoring continuously with valinomycin-based ion-selective electrode catheters. The potassium-selective electrodes seems to be useful in the measurement of the frequent and rapid changes in the plasma potassium level of flowing blood though with an important prerequisite of the understanding of the nature of potassium in the blood and how activity and concentration of the ion are related. The electrodes produced stable and reproducible Nernstian responses, and are therefore suitable for biological measurements *in vivo*.

The technique of measuring plasma potassium levels simultaneously and continuously in different intravascular sites - arterial and venous - proved very successful. The results from this technique proved that as a result of a variety of metabolic, physiological and pharmacologic stimuli potassium moves from one part of the body to another. The rapid changes in plasma potassium observed in the inferior vena cava and the arterial circulation would not necessarily be noticed in blood usually drawn from an upper limb in medical practice. The occurrence of oscillations in the HIVC plasma K^+ corresponding with respiratory movements indicates the difference in potassium content of hepatic venous and abdominal vena caval blood, and is evidence of the changing nature of the body's potassium balance. The

simultaneous recordings of potassium in more than one site confirm the potassium mobility and illustrate redistribution in the cat under a variety of circumstances.

It appears that the potassium level in the blood in healthy animals including man is kept constant because the normal physiological release of potassium from one area is matched by an equal uptake of potassium elsewhere in the body. The donor and recipient tissue vary from one time to another depending on the prevailing circumstances, but over any reasonable period of time each organ remains in a non-static (dynamic) balance. Although potassium was released by the region drained by the hepatic vein following haemorrhage, hyperventilation, asphyxia, adrenaline or morphine injection, it was unusual throughout this study to produce a similar increase in the potassium level in blood returning from the periphery compared to that in the HIVC except in irreversible states.

The initial increase in plasma K^+ produced by acute blood withdrawal was observed to be accompanied by a hyperventilation-induced respiratory alkalosis. The conclusion that hyperventilation *per se* produces an increase in plasma K^+ concentration was drawn from the observation of consistent rise in plasma K^+ following mechanical hyperventilation with room air for 5 min. in the cat. Such hyperventilation-induced hyperkalaemia was significantly reduced by opioid receptor and beta-adrenoceptor blockers which suggest that endogenous opioids and catecholamines may be released to cause the increase in plasma potassium

concentration during hyperventilation. The haemorrhage-induced hyperkalaemia was prevented by alpha-adrenoceptor blockers and reversed by an opioid receptor blocker, naloxone. However, the beta-adrenoceptor blocker, propranolol prevented the uptake of potassium following haemorrhage and the reinfusion of the shed blood. The further reproducible observations that morphine, an opioid alkaloid, and adrenaline, a catecholamine, produced significant transient rises in plasma K^+ which were prevented by naloxone and alpha-adrenoceptor blockers (phentolamine and prazosin), respectively, led to the general conclusion that adrenergic and opioid receptors are involved at some stage in the release of potassium.

The mechanism of naloxone in the reversal of hyperkalaemia following haemorrhage, or hyperkalaemia produced by morphine following cervical vagotomy is found to be different from the action of naloxone in raising the arterial blood pressure. This dissociated dual action of naloxone is obscure. However, naloxone which is ineffective on its own consistently increased the plasma K^+ level before reversal to the control level, and the arterial blood pressure in cats with prior injections of adrenaline. This led to the conclusion that naloxone either potentiates the effects of circulating catecholamines, or renders the hypoxic tissues following haemorrhage once more sensitive to catecholamines, in addition to its antagonistic effects on endogenously released opioids. Conversely, the lack of effect of naloxone in the reversal of hyperkalaemia and/or hypotension following

prolonged haemorrhagic hypotension with severe metabolic acidosis is suggested to be due to either the reduction in the potency of naloxone in severe acidosis, lack of response by the body tissues to naloxone action or indeed due to a greater antagonism by endogenously released opioids as hypotension progressed because naloxone is a competitive antagonist.

The vagi are suggested to play an indirect role in the modulation of changes in plasma K^+ levels following haemorrhage. Significant increases in the MABP and plasma K^+ were observed after sectioning the vagi in the neck in the presence of morphine, and less severe haemorrhage produced rises in plasma K^+ levels similar to those produced by more severe haemorrhage with the vagi intact. The spontaneous episodes of rise in MABP with accompanying increase in plasma K^+ concentration following vagotomy as observed in the later stages of haemorrhagic hypotension suggest a disinhibition of sympathetic outflow and appears to illustrate a central nervous system ischaemic response to the prolonged hypotension in the cat.

The differences in the direction and magnitude of change in MABP associated with a variety of stimuli like asphyxia, haemorrhage, adrenaline or morphine injection all resulting in hyperkalaemia as observed in the present study led to the conclusion that the direction of changes in MABP has no cause and effect relationship to the simultaneous changes in plasma K^+ concentration.

The concentration of plasma K^+ recorded immediately after death ranged from 7.05 to 15 mmol/l in the cat. Unusually high levels of plasma K^+ were recorded in some cases while the heart was still beating, and in some a sharp rise in plasma K^+ occurred only after the arrest of breathing. The reason why the K^+ level rises higher than 7 mmol/l in some experiments but not in others before death occurs is obscure. However the occurrence of sharp increases in plasma K^+ concentrations with associated derangements in the ECG only after respiratory arrest in some animals suggests that hyperkalaemia is the effect and not the cause of death in such cases. There is no doubt that the raised level of K^+ preceding the sharp rise during the haemorrhagic hypotension may have contributed to the deterioration of myocardial function.

Generally, two levels of increased plasma K^+ were observed during haemorrhagic hypotension. The end of the first and/or the beginning of the second which depends on the severity and the duration of hypotension was found to be the warning sign of end of reversibility. This level of hyperkalaemia which was always associated with acidosis is suggested to be due to a global ischaemia, resulting in the generalized hyperkalaemia which is refractory to volume replacement and other resuscitory measures.

It is concluded that the irreversibility resulting in death is not solely due to hyperkalaemia but a combination

of it, anoxaemia and severe acidosis with gross base deficit.

The implications of this study from a laboratory investigation perspective, and from a clinical perspective are indicated in the concluding paragraphs of the abstract at the beginning of this thesis.

Injection: Adrenaline (2 µg/kg,iv)	Plasma K^+ (mmol/l)			MABP (mm Hg)	
	Control	Max.	Min.	Control	Max.
Aorta K^+	2.92 ± 0.12	5.31 ± 0.15	2.90 ± 0.14	147 ± 6.0	170 ± 6.0
HIVC K^+	3.00 ± 0.14	7.73 ± 0.56	2.80 ± 0.08		
Injection: Noradrenaline (2 g/kg,iv)					
Aorta K^+	2.90 ± 0.13	5.91 ± 0.64	2.88 ± 0.11	136 ± 9.0	176 ± 2.0
HIVC K^+	3.02 ± 0.09	6.54 ± 0.18	2.86 ± 0.08		

Table 1.3: The effects of adrenaline and noradrenaline (2 µg/kg i.v. each) on aortic and high inferior vena cava (HIVC) plasma K^+, and on mean arterial blood pressure (MABP). Mean values $\pm$ SEM, n = 8.

% Haemorrhage	Aorta K^+ (mmol/l)	HIVC K^+ (mmol/l)	MABP (mm Hg)
Control	2.92 ± 0.12	3.00 ± 0.14	111 ± 11.0
5%	2.98 ± 0.06	3.23 ± 0.12	98 ± 6.0
10%	3.02 ± 0.10	3.59 ± 0.24	92 ± 6.0
15%	3.18 ± 0.15	3.62 ± 0.20	68 ± 5.0
20%	3.43 ± 0.16	4.13 ± 0.29	47 ± 5.0
25%	3.99 ± 0.49	4.17 ± 0.30	38 ± 4.0
30%	4.44 ± 0.55	5.36 ± 0.36	19 ± 3.0
35%	5.65 ± 0.23	7.94 ± 1.50	7 ± 3.0

Table 1.4: Showing the effects of withdrawal of various percentages of blood volume on MABP, and aortic and HIVC plasma K^+. Mean ± SEM, n = 8.
K^+ values shown were the peak values recorded over 5 minutes of observation.

Table 1.5: Comparative normo-kalaemic & MABP restoration effects of *shed blood reinfusion
*normal saline infusion
*dextraven 110 infusion.

Expt./ Electrode Site	MAP (mm Hg)	Plasma K^+ (mmol/l)	pH	PCO_2 (mm Hg)	PO_2 (mm Hg)	HCO_3^- (m.Eq/l)	CO_2 (mm/l)	BE (m.Eq/l)	SBE (m.Eq/l)	SBC (m.Eq/l)
CONTROL										
Aorta (A)	152±11	3.76±0.31	7.37±0.01	25.70±1.60	104±1.14	17.9±0.94	16.00±0.98	-5.96±0.27	-7.96±0.29	19.0± 0.48
HIVC (V)		4.78±0.44	7.31±0.02	31.32±3.00	40± 2.04	18.9±0.89	18.00±1.51	-7.80±0.66	-9.68±0.76	17.0± 0.54
35% v/w (A)	27±5.00	10.28± 1.21	7.49±0.02	19.98±3.40	123±6.72	12.57±1.39	13.20±1.50	-3.8 ±0.88	-6.00±1.19	17.38±0.74
Haemorrhage (V)		12.55±0.73	7.31±0.03	30.50±3.03	45.0±10.30	15.00±1.42	15.22±1.42	-4.6±1.44	-5.8±1.53	14.28±1.41
Shed blood Reinfusion (A)	142±9.0	3.33±0.40	7.29±0.04	27.40±3.16	115.30±6.40	13.38±0.83	14.27±0.9	-9.07±0.95	10.80±0.82	14.93±0.74
(V)		3.56±0.28	7.25±0.04	34.70±3.77	39.50±4.30	14.90±0.75	15.97±0.81	-10.08±0.8	11.75±0.81	14.18±0.69
Normal saline infusion (A)	61±4.0	2.54±0.18	7.28±0.03	23.1±2.27	135±15.40	10.95±1.22	11.70±1.26	-13.1±1.49	-15.95±1.54	14.73±1.15
(V)		4.03±0.60	7.19±0.04	36.6±1.86	37±7.67	14.20±0.94	15.30±0.94	-12.53±1.58	14.20±1.50	13.98±1.31
Dextran '110' Infusion (A)	128±13	4.94±1.02	7.15±0.07	32.72±3.71	119±15.29	11.32±0.92	12.30±0.89	-14.94±1.78	-16.53±1.75	12.85±1.39
(V)		4.89±1.13	7.08±0.09	31.38±6.57	30.83±5.17	16.32±2.33	17.30±2.16	-14.25±1.59	-14.30±1.11	15.73±2.76

*After haemorrhagic shock.

Table 1.6: Effects of sustained hypotension at MABP of 80 mm Hg on plasma K^+ and acid/base balance.

Expt/Site of electrode	MABP (mm Hg)	pH	PCO_2 (mm Hg)	PO_2 (mm^2Hg)	HCO_3^- (m.Eq/l)	CO_2 (mm/l)	BE (m.Eq/l)	SBE (m.Eq/l)	SBC (m.Eq/l)	Plasma K^+ (mmol/l)
Control (A)	151±4.0	7.398±0.2	26.0±1.50	106±1.8	17.00±0.98	18.3±0.78	-5.40±0.64	-6.80±0.22	20.7±0.58	2.90±0.32
(V)		7.395±0.01	28.6±2.60	39 ±2.08	18.36±1.38	19.7±1.21	-4.26±0.58	-5.90±0.84	21.4±0.74	3.15±0.46
MABP @ 80 mm Hg (A)	80±2.0	7.49 ±0.04	19.7±2.20	112±4.00	15.1±1.10	15.7±1.44	-5.1± 0.02	-6.10±0.28	21.0±0.82	4.80±0.48
0 min. (V)		7.44 ±0.05	28.7±3.60	28±6.00	19.7±0.99	20.6±0.98	-2.5± 0.88	-4.60±0.78	21.8±0.66	6.80±0.48
30 min. (A)	80±0.0	7.48 ±0.02	19.6±3.18	106±2.40	14.6±0.56	15.2±0.65	-5.7± 0.77	-9.1±0.44	20.5±0.56	2.85±0.33
(V)		7.47 ±0.04	23.4±2.66	38±3.11	17.5±0.89	18.2±0.99	-3.5±0.19	-6.3±0.62	21.6±0.85	4.00±0.49
60 min. (A)	82±2.0	7.51 ±0.05	16.5±1.88	107±1.88	13.3±0.48	13.8±0.66	-6.0±0.11	-9.9±0.12	20.3±0.28	3.00±0.38
(V)		7.48 ±0.04	19.8±2.00	33±2.22	15.0±0.88	15.6±1.36	-5.2±0.56	-8.6±0.24	20.1±0.44	4.00±0.28
90 min. (A)	80±0.0	7.52 ±0.06	13.8±2.55	105±4.20	11.4±0.66	11.8±0.99	-7.3±0.88	-11.7±0.66	19.2±0.64	3.25±0.31
(V)		7.46 ±0.05	20.0±3.40	28±6.10	14.4±0.64	15.0±0.28	-6.3±0.68	- 9.7±0.74	18.9±0.59	4.00±0.34
120 min. (A)	78±1.00	7.54 ±0.06	11.0±1.56	106±2.20	9.4±0.76	9.7±0.68	-8.6±0.55	-13.4±0.77	18.3±0.54	3.15±0.34
(V)		7.44 ±0.04	18.4±2.04	25±3.50	12.6±1.10	13.2±0.89	-8.2±0.82	-11.8±0.68	17.1±0.66	4.05±0.28
150 min. (A)	78±2.00	7.48 ±0.04	10.8±1.10	97±2.10	8.1±0.22	8.5±0.55	-10.8±0.87	-15.5±0.59	16.6±0.66	3.31±0.38
(V)		7.39 ±0.02	20.6±1.24	24±2.00	12.8±0.21	13.4±0.10	- 9.0±0.81	-12.2±0.88	16.4±0.28	4.00±0.32
180 min. (A)	80±2.00	7.36 ±0.01	14.9±1.60	105±1.98	8.5±0.34	9.0±0.12	-13.2±0.66	-17.1±0.98	14.6±0.48	3.31±0.42
(V)		7.22 ±0.04	31.7±3.20	23±2.86	13.0±0.41	13.9±0.44	-13.0±0.61	-15.0±0.86	12.9±0.52	4.00±0.32

Table 1.7(a): Peak effects of hyperventilation with a tidal volume of 125 ml of room air at 34 breaths/min. on the mean arterial blood pressure (MABP), and plasma potassium, before and after pretreatment with either prazosin, naloxone or propranolol (0.2 mg/kg) each.

Expt.	MABP (mm Hg)	pH	Plasma K^+(HIVC) (mmol/l)	End Tidal CO_2 (%)	ΔK^+ (mmol/l)
Control	146±6.6	7.36±0.02	3.04±0.06	5.73±0.25	-
Hyper-ventilation (Hv)	50.8±2.80	7.75±0.04	5.42±0.48	0.50±0.18	2.53±0.39
Prazosin pretreat-ment before Hv.	37.5±4.98	7.64±0.02	5.13±0.25	0.48±0.19	2.24±0.14
Naloxone pretreat-ment before Hv.	46.2±3.26	7.65±0.01	3.26±0.38	0.58±0.12	0.77±0.34
Propranolol pretreat-ment before Hv.	38.7±2.56	7.67±0.02	3.75±0.08	0.2 ±0.02	1.35±0.24

Results are expressed as means ± SEM. n = 6. Table contains values recorded at the maximum level of plasma K^+ by the 3rd or 4th min.

Table 1.7(b): Effects of 5-minute artificial hyperventilation with room air, 125 ml at 34 breaths/min. before and after pretreatment with 0.2 mg/kg. i.v. each, of Naloxone, Prazosin and Propranolol.

Experiment	MABP (mm Hg)	HIVC.K^+ (mmol/l)	End-Tidal CO_2 (%)
Control	168 ± 4.0	2.61 ± 0.08	5.98 ± 0.02
Hyperventilation (Time) mins.			
1	66.0 ± 2.0	3.47 ± 0.12	0.55 ± 0.11
2	72.0 ± 2.0	5.70 ± 0.61	0.50 ± 0.02
3	76.0 ± 2.8	7.50 ± 0.08	0.50 ± 0.01
4	83.0 ± 4.88	7.30 ± 0.06	0.45 ± 0.01
5	84.0 ± 2.88	7.10 ± 0.08	0.45 ± 0.01
Naloxone Pre-treatment before Hyperventilation			
Control	164 ± 4.0	2.54 ± 0.22	5.98 ± 0.10
Expt. Time mins.			
1	105 ± 2.0	3.47 ± 0.15	0.75 ± 0.11
2	109 ± 2.0	4.31 ± 0.17	0.65 ± 0.02
3	114 ± 4.0	4.93 ± 0.18	0.55 ± 0.01
4	121 ± 4.0	4.20 ± 0.10	0.50 ± 0.01
5	122 ± 4.0	4.0 ± 0.11	0.45 ± 0.02
Prazosin Pre-treatment before Hyperventilation			
Control	90.7 ± 2.0	3.47 ± 0.11	5.98 ± 0.20
Expt. Time mins.			
1	49.3 ± 0.98	3.88 ± 0.03	0.55 ± 0.01
2	45.3 ± 2.20	4.95 ± 0.12	0.45 ± 0.02
3	45.3 ± 1.0	5.51 ± 0.14	0.40 ± 0.11
4	46.7 ± 0.80	5.85 ± 0.21	0.40 ± 0.01
5	46.7 ± 0.88	6.0 ± 0.21	0.35 ± 0.11
Propranolol Pre-treatment before Hyperventilation			
Control	106 ± 4.0	2.76 ± 0.17	5.98 ± 0.02
Expt. Time mins.			
1	46 ± 4.8	3.10 ± 0.11	0.65 ± 0.01
2	41 ± 4.5	3.10 ± 0.11	0.65 ± 0.01
3	37 ± 2.5	4.10 ± 0.18	0.55 ± 0.07
4	38 ± 4.5	4.7 ± 0.09	0.45 ± 0.01
5	38 ± 4.5	4.3 ± 0.10	0.35 ± 0.01

Means ± SEM. n = 6. (Values continuously recorded for 5 minutes).

Table 1.8: Continuous monitoring of effects of haemorrhagic shock at MABP of 40 mm Hg on plasma K^+ and acid-base balance. Mean ± SEM. n = 6.

Expt. site of electrodes	MABP (mm Hg)	Plasma K^+ (mmol/l)	pH	PCO_2 (mm Hg)	PO_2 (mm^2Hg)	HCO_3^- (m.Eq/l)	CO_2 (mmol/l)	BE (m.Eq/l)	SBE (m.Eq/l)	SBC (m.Eq/l)
Control (A)	159±15	2.56±0.08	7.37±0.03	29.6±0.80	101±3.0	17.15±0.15	17.9±0.11	-5.96±0.12	-7.90±0.90	20.03±0.28
(V)		3.60±0.04	7.34±0.02	38.4±0.60	33±1.5	17.07±0.90	15.7±1.20	-7.00±0.80	-9.67±0.45	18.53±0.63
Post-Haem-orrhage (A)	38±2.0	4.75±0.02	7.49±0.05	20.6±0.65	121±2.50	19.1 ±1.44	13.8±1. 4	-2.20±0.40	-5.2 ±0.38	23.0 ±0.48
0 min. (V)		6.40±0.05	7.34±0.01	39.9±0.12	24±2.40	19.4 ±1.20	12.8±1.02	-4.20±1.80	-6.0±0.44	21.9 ±0.68
30 min (A)	40±0.00	4.80±0.44	7.33±0.02	29.8±0.88	102.8±1.60	12.8 ±0.88	13.4±0.62	-6.2 ±0.64	-8.5±0.28	15.1 ±0.52
(V)		6.64±0.60	7.08±0.06	48.0±0.24	26.7±2.40	17.8 ±0.98	17.3±0.44	-8.7 ±0.58	-10.6±0.36	14.1 ±0.48
60 min (A)	40±2.0	4.78±0.66	7.25±0.11	30.8±0.44	104.7±2.60	8.30±0.68	18.9±0.21	-9.8 ±0.48	-10.0±0.12	13.7 ±0.62
(V)		6.52±0.61	7.11±0.08	50.1±0.68	25.7±1.80	13.7± 0.56	25.2±0.41	-10.9±0.62	-13.0±0.22	12.5 ±0.52
90 min (A)	42±2.0	4.75±0.52	7.18±0.02	32 ±0.98	95 ± 2.44	11.0± 0.56	22.3±0.28	-14.9±0.44	-15.1±0.24	13.6 ±0.44
(V)		6.42±0.62	7.06±0.04	51 ±0.84	31 ± 2.21	16.0± 0.66	27.0±0.22	-16.8±0.4	+18.7±0.14	11.5 ±0.24
120 min (A)	38±2.0	6.68±0.48	7.02±0.04	33.8±1.44	84 ± 2.11	11.4±0.11	30.50±1.11	-19.9±2.11	-19.9±2.11	11.2 ±0.78
(V)		8.46±0.60	6.6 ±0.01	53.7±2.66	16 ± 4.60	14.1±1.22	46.10±2.10	-18.0±1.82	-18.0±1.82	10.3 ±0.33
At death	0±0.00	9.60±0.20 14.50±0.40								

APPENDIX II

Blood pH/Gas Analysis

To ensure that the data obtained corresponds directly with the actual state of the "in vivo" blood, proper collection and handling of the blood samples prior to analysis are extremely important.

Arterial and venous samples were collected in plastic syringes via indwelling catheters in the aorta and the high inferior vena cava as described in the Methods. To displace air and to act as an anticoagulant, the dead space of the syringe and needle were filled with 1000 units/ml sodium heparin. Bubbles which developed during sample collection were immediately removed and the syringes capped.

The Blood Gas Analyser (Model 1302) was calibrated using gas mixtures of 5% CO_2, 12% O_2 and 83% N_2 in one cylinder, and 10% CO_2 and 90% N_2 in the other and then allowed to run at standby overnight before the beginning of each day's experiment. All values for the blood samples withdrawn during experiments were determined within 5 min of sampling for optimal accuracy because blood pH and PO_2 fall and PCO_2 rises with time. Samples which could not be analysed within 5 min were stored anaerobically in ice-water. Under these conditions, even in leukaemic blood, pH will change less than 0.015 units in 2 hours (Manufacturer's Manual, Model 1302).

Blood gas analyses are usually performed at 37°C because human reference values are based on 37°C (body temperature).

The model 1302 has a temperature sensor which compares the samples with the normal ($37^{o}C$) for man and "reads out" the "temperature corrected" values for samples fed in at temperatures other than $37^{o}C$ for analysis. These values were then cross-checked with Siggard-Andersen's nomograms for accuracy. The values obtained are calculated by programmed data analyser in the Model 1302 on the assumption that pK_1' is 6.1 at $37^{o}C$. Values for pH, PCO_2 and PO_2 are directly determined by the gas analyser while HCO_3^-, CO_2 ct, SBC, SBE and BE are derived from the values of pH, PCO_2 and PO_2.

It is noteworthy that Flear et al. (1984) have suggested that the pK_1' value of 6.1 at $37^{o}C$ assumed for Gas Analysers is affected by changes in the bicarbonate concentration of the sample. The pK_1' falls with increasing bicarbonate ion concentration.

Definitions and Abbreviations

Definitions

Standard Bicarbonate (SBC) is defined as the bicarbonate concentration of plasma equilibrated at a PCO_2 of 40 mm Hg and PO_2 of 100 mm Hg (full oxygen saturation of available haemoglobin). Normal range in arterial plasma samples in man is 22-26 mEq/1.

Base Excess (BE) is defined as the number of mEq of strong acid or base required to titrate a sample to a pH of 7.40 at a PCO_2 of 40 mm Hg at $37^{o}C$. BE indicates the deviation, in mEq/L, of *Buffer Base from normal. Base deficit, a less frequently used term is the base excess with the sign reversed. The normal range for whole blood in man is 0 ± 2 mEq/L.

*Buffer Base is an indicator of tissue metabolism and is defined as the total equivalent concentration of all the anionic (basic) buffering components of the blood, i.e. haemoglobin, bicarbonate, plasma proteins, sulphates and phosphates. The total Buffer Base in normal man is about 48 mEq/L.

Base Excess of Extracellular Fluid (SBE) is differentiated from BE of blood because it represents the entire extracellular compartment of which blood is only 37%. Each of the extracellular fluid compartments has a different buffering capacity, hence the base excess of entire fluid compartment represents the best way to determine base excess in vivo.

ABBREVIATIONS

*Noradrenaline	= Nor
*adrenaline	= Adr
Hyperventilation	= h/vent, hv
Control	= Con, C, Cont, Contr
Prazosin	= Pra, Praz
Propranolol	= Pro, Prop
Naloxone	= Nal, Nalx
Electrocardiograph	= ECG
Intravenous	= iv
Inferior vena cava	= IVC
High inferior vena cava	= HIVC
Asphyxia	= Asph
Haemorrhage	= Haem
Phentolamine	= Phent
Mean arterial blood pressure	= MABP
Total CO_2 content of plasma	= CO_2 ct
Plasma Bicarbonate	= HCO_3^-
Standard Bicarbonate	= Std HCO_3^-, SBC
Base Excess	= BE
Standard base excess	= SBE

*Note that adrenaline and epinephrine are the same substances, likewise noradrenaline and norepinephrine. Both English and USA terms for these compounds have been used in this thesis.

Table showing the numbers of cats used in experiments.

Experiments		Cats	Total
<u>SECTION 1</u>			
	1	8	
	2	6	
	3	4	
haemorrhage 1.16	4)		
	5)	4 each	34
	6)		
	7)		
<u>SECTION 2</u>			
asphyxia	2.3.1	4	
haemorrhage	2.3.2)	4	16
adrenaline	2.3.3)		
morphine	2.3.4	4	
<u>SECTION [illegible]</u>			
	[illegible].1)	— 2 procedures in [illegible] cats	
	[illegible].2)		
	[illegible].3		
	[illegible].4)	— [illegible] procedures in [illegible] cats	
	[illegible].5)		
	[illegible] to [illegible]	[illegible] cats	[illegible]
	[illegible].11	—	
	[illegible].12	[illegible] [illegible]	

Lost [illegible] cats under deep anaesthesia.

Total: [illegible] cats.

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Docherty, G.E., Mackenna, B.R. and Sokari, A. (1987) Alkalosis and not acidosis accompanies early hyperkalaemia during haemorrhagic shock in cats. J. Physiol., 392: 95P.

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