PLASMA VISCOSITY CHANGES DURING HAEMODILUTION THERAPY OF CEREBRAL ISCHAEMIA

by

COLIN FARMAN

A thesis submitted for the degree of

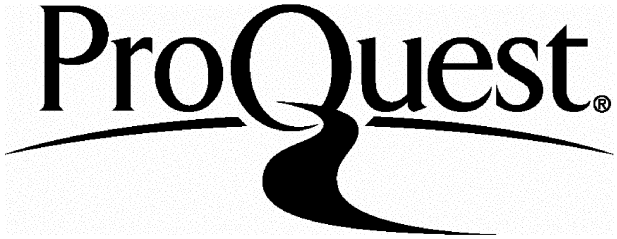
Doctor of Philosophy

in

University of London

from

Gough-Cooper Department of Neurological Surgery
Institute of Neurology
Queen Square
London, WC1N 3BG



ABSTRACT

The use of low molecular weight dextran (LMWD) as a haemodiluent can lead to a significant increase in blood plasma viscosity. This may be detrimental to the residual collateral blood flow through the ischaemic brain penumbra. In an experimental primate model of focal cerebral ischaemia, isovolaemic and hypervolaemic haemodilution with two infusates, LMWD and Haemaccel, were compared. Their influence on blood and plasma viscosity and haematocrit were studied along with their effect on regional cerebral blood flow (rCBF), oxygen delivery and tissue oxygen tension.

Plasma viscosity was significantly elevated following either isovolaemic or hypervolaemic haemodilution with LMWD, but was not increased following haemodilution with Haemaccel. The raised plasma viscosity was associated with elevated whole blood viscosity. Both infusates lowered erythrocyte aggregation, but the LMWD induced increase in plasma viscosity compromised any rheological benefit.

Hypervolaemic or isovolaemic haemodilution with either LMWD or Haemaccel significantly increased rCBF in all areas of the brain. The increase in blood flow to ischaemic areas with lost autoregulation to hypercapnia demonstrated the critical role of blood viscosity in determining blood flow, but these ischaemic regions also became more sensitive to hypercapnia induced intracerebral steal. The increases in rCBF were significantly correlated with changes in haematocrit and blood viscosity and an optimum haematocrit for maximal oxygen delivery to ischaemic brain was calculated (32%). Haemodilution induced changes in oxygen delivery were also directly linked with changes in brain tissue oxygen tension. The optimum haematocrit value for maximum oxygen delivery was found to be dependant upon the depth of ischaemia and could be influenced by changes in plasma viscosity.

It was concluded that haemodilution therapy during normocapnia could improve rCBF and oxygen delivery to ischaemic brain, and that the deleterious effects of LMWD elevated plasma viscosity could be avoided by the use of a comparable haemodiluent; Haemaccel.

CONTENTS PAGE

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS USED

BV	Whole Blood Viscosity
CBF	Cerebral Blood Flow
CMR	Cerebral Metabolic Rate
EEG	Electroencephalograph
HCT	Haematocrit
HES	Hetastarch
ICP	Intracranial Pressure
LMWD	Low Molecular Weight Dextran
MABP	Mean arterial blood pressure
MCA(O)	Middle Cerebral Artery (Occlusion)
Pa	Arterial partial pressure
Pt	Tissue partial pressure
PV	Plasma Viscosity
SD	Standard Deviation

This thesis is dedicated to my grandmother:

Mrs Eunice Morgan

ACKNOWLEDGEMENTS

The support, guidance and encouragement provided by Professor Lindsay Symon has been invaluable during this work and it has been a great privilege to learn from such an inspiring supervisor.

I would also like to thank Dr Neil Branston and Dr Tiho Obrenovitch for their guiding opinions and for continuous help with computer programming and technical developments.

A special thank you goes to Deanna Taylor, mainly for her support and companionship, but also for her technical assistance during the early stages of this work.

The success of this work relied on the skills of the visiting overseas research fellows and the technical staff within the department. My thanks therefore go to:

For Surgical Assistance

Minwoo Baik, Lorenzo Bordi, Zhen-Ping Chen, Marco Fonsenca, Masa Kawauchi, Yuki Ueda.

For Technical Assistance

Chris Bashford, Gerry Christofi, Liz Conway, Aiden Hardy, She-Yen Lok, Sean Maywood, Nicola Parkinson.

I would like to express my appreciation to a few people who played inspirational roles in the development of this work, namely; Robert Harris, Richard Whittington, Gordon Lowe and Nick Dorsch.

I am also grateful to the financial sponsors: the Medical Research Council, Hoechst UK and Behringwerke. I would also like to acknowledge the encouragement and advice from Dr Allan Lenox-Smith (Hoechst UK).

Last but no means least, I would like to thank Pip, family, and friends, for their unquestioning faith and support.

CHAPTER 1

INTRODUCTION

1.1 AIMS OF INVESTIGATION

Haemodilution therapy of cerebral ischaemia was subject to intensive review during the late 1980's, and saw the initiation of extensive clinical trials. Concern developed among researchers on one aspect of haemorheology for which little information had been gathered; the role of plasma viscosity in haemodilution therapy. Coincidental with these concerns were results demonstrating increases in plasma viscosity in patients receiving long term infusions of dextran-40 (Kroemer et al, 1987). Clinicians treating patients with sub-arachnoid haemorrhage or cerebral aneurysms, who are at risk of secondary vasospasm, favour the use of dextran-40 as a intravascular volume expander in an attempt to increase blood flow to affected brain regions. Concern therefore developed regarding the possible deleterious effects a dextran-40 induced increase in plasma viscosity could have on the cerebral blood flow.

This investigation was designed to help evaluate the effects of dextran-40 infusion on cerebral blood flow in focal ischaemia. In order to ascertain whether plasma viscosity influenced blood flow during haemodilution therapy, two infusates were compared: dextran-40, and a low viscosity plasma substitute Haemaccel (Hoechst). A preliminary clinical investigation of these two infusates confirmed the findings of Kroemer et al (1987) and demonstrated that plasma viscosity could be kept at a normal level through haemodilution with Haemaccel (Farman et al, 1991b; Appendix A). This thesis aims to describe the influence of these two infusates on measured systemic blood parameters and to demonstrate the effect these changes may have on cerebral blood flow.

The possible benefit of changes in cerebral blood flow following haemodilution has been under increasing speculation, especially following the recent negative trends of large clinical haemodilution trials. Concern about the

efficacy of haemodilution therapy includes :

Whether an improvement in blood flow following haemodilution is caused by changes in blood viscosity or through reactive vasodilation.

Whether increases in blood flow, induced by a reduction in haematocrit, are offset by the reduction in oxygen carrying capacity.

Whether haemodilution through blood volume expansion (hypervolaemia) has advantages over an isovolaemic infusion.

During the course of this investigation it was therefore necessary to address the general concerns about the clinical effectiveness of haemodilution therapy. Accordingly, this project aimed to determine how and where in the focally ischaemic brain, changes in blood and plasma viscosity may be of benefit. To achieve this, measurement of local cerebral blood flow changes were combined with determination of local vascular reactivity, and local brain tissue oxygen tension. By relating changes in systemic physiology with their influence on cortical haemodynamics and the subsequent effect on tissue oxygen availability, this thesis aims to describe the relationships between systemic blood manipulation and any therapeutic benefit in focal cerebral ischaemia.

1.2 FOCAL CEREBRAL ISCHAEMIA

1.2.1 Introduction

Cerebral ischaemia is a term used to describe a reduction in blood supply to the brain that arises from a variety of conditions. The sensitivity of the brain tissue to ischaemia and the reduction in available energy substrates can quickly lead to loss of neuronal function and cell death.

In the extreme case of cardiac arrest, circulatory insufficiency induces complete or diffuse cerebral ischaemia. Other conditions such as drowning or hypoxia secondary to carbon monoxide poisoning can lead to similar consequences. The clinical prognosis in diffuse ischaemia is generally poor, caused not only by the direct effect of hypoxic damage, but also through secondary cerebral swelling due to permeability changes at the vascular and cellular membrane level. Such disruption produces irreversible cellular changes and death follows within 5-10 minutes.

Focal cerebral ischaemia describes a localised reduction in cerebral blood flow secondary to conditions such as thrombosis, haemorrhage, embolism, and spasm. In all these examples, the clinical prognosis is better than following diffuse ischaemia because at least some regions of the brain have a sustained normal blood flow. This maintenance of blood supply is a consequence of both cerebral anatomy and the physiological response to ischaemia.

Blood supply to the brain arrives through three major arteries: the basilar, and the two internal carotids. These three are linked inside the skull via communicating arteries to form the Circle of Willis, before radiating out to each cerebral hemisphere through the anterior, middle and posterior cerebral arteries. This vascular arrangement ensures that in the event of reduced flow through any of the feed arteries, blood can be redirected around the Circle of

Willis to all cerebral arteries. Occlusion of the cerebral arteries themselves will lead to some degree of cerebral ischaemia, but the presence of three supply arteries to each hemisphere ensures some degree of circulatory overlap. This supply of blood from one cerebral artery to the territory of another is termed the collateral supply.

Following cerebral artery occlusion, blood flow falls rapidly in the territory supplied by that vessel. Lack of blood flow prevents the supply of oxygen and glucose and the removal of metabolic waste products such as CO_2. The rise in local tissue PCO_2 and reduction in PtO_2 induces vascular dilation in an attempt to improve blood flow. With no flow possible through the branches of the occluded vessel, it is the resulting increase in flow through the collateral supply that greatly influences the extent of ischaemia. Vascular dilation in the ischaemic region can reach a maximum, at which point vessels can not compensate for changes in $PaCO_2$ or systemic blood pressure (Symon et al, 1975, 1976a). This loss of "autoregulation" is potentially dangerous as any fall in systemic blood pressure can reduce blood flow to the ischaemic areas, and any increases in $PaCO_2$ that induce vasodilatation in normal cerebral vessels can induce intracerebral 'steal' and shunt blood away from the collateral supply.

Collateral circulation into ischaemic regions is rarely sufficient to maintain cellular requirements in all regions of the supply territory. Experimental middle cerebral artery occlusion has demonstrated that a gradation of blood flow occurs over the cerebral cortex with areas of lowest blood flow occurring where collateral supply is least and vice versa (Symon et al, 1974). A relationship between blood flow and cellular function has also been demonstrated. If blood flow falls below 10-12ml/100g brain tissue/min, cellular ion homeostasis can not be maintained which results in the breakdown of cell structure and leads to infarction within a few minutes (Symon and Brierley, 1976b). Blood flow above

this threshold can maintain cellular structure, but the electrical function of the cortical neurons remains severely reduced at flow levels below 16ml/100g/min and remains essentially normal only at a blood flow above 20ml/100g/min (Branston et al, 1984). Blood flows between 40-20ml/100g/min are not associated with any major pathological change, but there have been reports of increased extracellular acidosis (Harris and Symon, 1984; Harris et al, 1987), a slowing of the EEG (Suzuki, 1987) and a reduction in cellular K^+ re-uptake (Branston et al, 1977).

The gradation of ischaemic blood flow thresholds around an infarct zone has led to the description of an ischaemic 'penumbra' (Astrup et al, 1981). This describes a region of ischaemic brain that is electrically silent while still normal in terms of ionic homeostasis. The penumbra therefore encompasses a small area of tissue with a blood flow of between 10 and 20ml/100g/min. The extent and stability of the penumbra is a matter of current controversy, but recovery of cellular electrophysiological function on reperfusion of penumbral tissue has been demonstrated both clinically and experimentally. Secondary to removing the source of vascular occlusion, clinical management of focal ischaemia therefore aims to increase blood flow through the collateral supply and to raise the penumbral blood flow above the threshold for electrical activity (Symon, 1987).

The viability of cells in the penumbral region is thought to have a finite life span. Strong et al (1983) suggest that as little as two hours of ischaemia can irreversibly damage neuronal elements in the cortical penumbra. Harris et al (1987) also suggest that the pH changes seen even at relatively high blood flow levels can influence many metabolic processes and ultimately the loss of penumbral viability. Such information has added to the theory of a therapeutic time window for the successful recovery of penumbral tissue which is of the order of a few hours (Frackowiak, 1985).

1.2.2 Current Clinical Therapy

Treatment of cerebral ischaemia can be divided into two methods; surgical and medical. The latter is aimed mainly at the prevention of lesion progression and infarct formation while surgery aims to revascularise the ischaemic territories.

Medical treatment encompasses the physical and pharmacological control of the patient's condition to maintain or improve blood flow through the collateral supply and to prevent the formation of cerebral edema. Since focal ischaemia is associated with a loss of vascular autoregulation, maintenance of a normal blood pressure is desirable. Use of hypotensive drugs are therefore avoided and in some cases induced hypertension can be beneficial. Prevention of cerebral edema and the resultant rise in intracranial pressure is the main target of pharmacological intervention in the treatment of infarction. Administration of hypertonic solutions such as mannitol may dehydrate edematous tissue and lower intracranial pressure, but use of these drugs can only be temporary due to their effects on electrolyte balance. Corticosteroids have been used in a similar way and vasogenic edema can be reduced, but a lack of clear beneficial outcome has reduced administration of these drugs.

To prevent the formation of thrombi in the reduced blood circulation of the ischaemic territories, antithrombotic agents have been used. Antiplatelet drugs prevent platelet adherence and aggregation, therefore reducing the risk of further blood coagulation. This treatment is more common than direct anticoagulation therapy due to the additional risk of aggravating haemorrhagic infarction with anticoagulant administration. This risk is also present in the use of thrombolytic agents and the use of these drugs has been limited to prevention rather than revascularisation.

Many vasodilating drugs are available to increase blood flow to the brain. However their use is extremely limited due to the risks of : cerebral 'steal' from ischaemic regions; increased intracranial pressure and expansion of haemorrhagic infarction. The use of vasodilators is normally limited to treatment in the chronic stages of infarction and is without clearly proven benefit.

Increases in blood flow through the microcirculation by reducing blood viscosity and erythrocyte aggregation can be induced by intravascular infusions of low molecular weight dextran (LMWD). This is commonly performed hypervolaemically over a number of days and LMWD's additional dehydrating properties may also help prevent edema formation. As well as its use in stroke induced focal ischaemia, LMWD infusion has been used in the treatment of cerebral vasospasm, both for its blood flow increasing properties and on the basis that hypervolaemia, increased cardiac output, and raised blood pressure may help combat the narrowing of intracranial arteries. Such treatment is commonly restricted to the post surgical period when the cause and risk of further haemorrhage has been dealt with. It is the use of such drugs that forms the major part of this thesis and further details on their use are covered in later sections.

1.3 HAEMODILUTION

1.3.1 Introduction

This section on haemodilution will examine the theory behind the therapeutic benefit of reducing the red cell content of the blood. The theory encompasses many disciplines of physiology, physics and medicine which have been amalgamated to form the relatively new field of 'haemorheology'.

Simplified...

> "haemorheology is the study of how the blood and the blood vessels can function and interact as parts of the living organism."
>
> Al Copley, 1981.

Historical use of Haemodilution

It is worth remembering that...

> "History, of course, has been on the diluters side. Haemodilution by venesection was practised regularly by our ancestors, and we have to be very conceited to suggest that something practised for centuries by our professional predecessors must have been a universal disaster."
>
> John Dormandy, 1983

Both bleeding and cupping are amongst the oldest medical manipulations practised by our ancestors. Egyptian physicians in 400 BC extensively used such methods, as did Hippocrates in ancient Greece and Galen during the reign of the Roman Empire (Turk and Allen, 1983). The practice was continued in the middle ages by Arabs and became popular in

Europe during the Renaissance. During the 18th century, bloodletting became the principal remedy for all cases of inflammatory disease, fever, convulsions, pleural haemorrhage, whooping cough, congestion and many more (Haller, 1986). By diminishing the quantity of blood in the body, it was argued that congestion was relieved, diseased blood was removed, and blood quality was improved by allowing the production of fresh blood.

The decline of bloodletting as a medicinal panacea began in the early 19th century with the demonstration that fevers were self limiting without medication. Inflammations also proved to be unaffected by bleeding and serious debate on the efficacy of the lancet ensued. Many great physicians fought hard in the defence of bleeding, but the tide of opinion gradually shifted against history. Throughout the 19th century, medical therapeutics advanced rapidly, medicine transformed from an art to a science, and with it came the extinction of medicines most popular remedy.

Blood Flow Theory

In present day clinical therapy, haemodilution is used to improve blood flow. The theory behind the improvement in flow is based on the Hagen-Poiseuille equation. Hagen (1837), and particularly Poiseuille (1846), experimentally defined the factors which govern the volume flow of fluid flowing steadily through rigid tubes of capillary diameter <u>in vitro</u>:

$$Q = \frac{(P_1 - P_2).\ \pi r^4}{8L\eta}$$

Where:

Q = Volume flow per unit time (meters^3/sec).
$P_1 - P_2$ = Pressure difference (Pascals).
r = Radius of tube (meter).
L = Length of tube (meter).
η = Viscosity of fluid (Pascal.seconds).

The equation mimics that of Ohm whose electrical conductance work demonstrated that the flow of current was derived from the potential difference across a wire and its resistance:

$$I = E/R$$

$$\text{CURRENT} = \text{POTENTIAL DIFFERENCE/RESISTANCE}$$

Combining the two equations where I is represented by Q and E by (P_1-P_2), we see that the resistance to flow through a tube is represented by:

$$R = 8L\eta/\pi r^4$$

Resistance to flow therefore increases with reduced tube radius, increased tube length, or increased fluid viscosity. The most important factor in this equation is the radius which is raised to the fourth power. A doubling in tube radius therefore reduces resistance to flow sixteen fold. It is this factor that the human body uses to control blood flow though the vessels, increasing diameter to improve flow and reducing diameter to reduce flow.

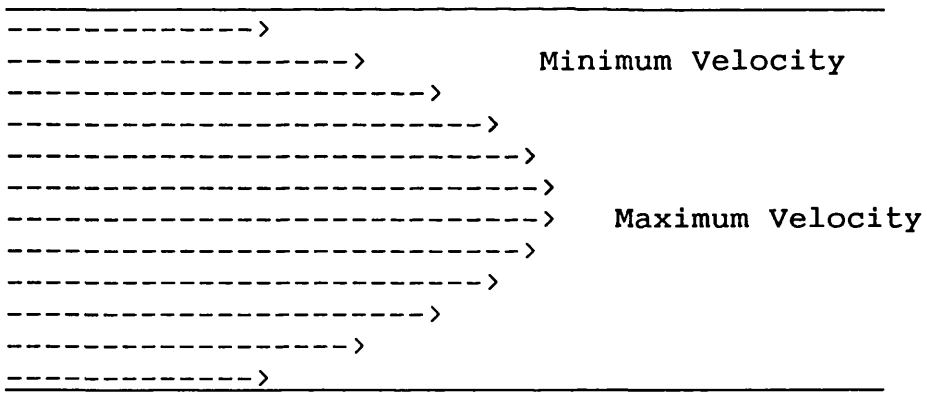
As outlined in the "Ischaemia" section (1.2), it is the flow to penumbral regions that interests clinicians. The blood vessels in these regions of the brain dilate to a maximum in an effort to restore blood flow. If the Poiseuille equation is applied to the human body, then with vessel radius (r) now constant, flow improvement can only be achieved by an increase in the pressure difference through the vessels, or a reduction in the blood viscosity. With the loss of autoregulatory capacity (Symon et al, 1976a), blood viscosity therefore becomes a major determinant of flow (Kee and Wood, 1987). However, applying the above flow equations to the human circulatory system involves some fundamental problems, namely that there is not a steady head of pressure and that blood is not a Newtonian fluid.

The heart, in pumping the blood, generates a pulsatile

change in pressure. If, as in Poiseuille's experiments, the blood flowed through rigid tubes, this would produce a pulsatile change in flow. In the body however, the lack of rigidity in blood vessels stores part of the potential energy produced during systole, and then releases it as kinetic energy during diastole. This 'dampening' effect helps reduce the pulsations and create a steadier flow of blood. The non-Newtonian property of blood however, is not so readily explained.

1.3.2 The Rheological Properties of Blood

The term 'Rheology' is used to define the study of the deformation and flow of matter. Haemorheology is a field of science which describes the flow and deformation of blood as it perfuses the blood vessels. The major focal point of haemorheology is blood viscosity. The viscosity of fluids was first described by Newton as their "lack of slipperiness". He demonstrated that the viscosity of a fluid remained constant irrespective of the flow conditions applied to it. Such fluids are termed Newtonian. The steady state flow of Newtonian fluids in a straight tube can be shown to exhibit a parabolic velocity profile:



At the wall of the tube the fluid is practically stationary, whereas in the centre, its velocity is maximal. The velocity gradient generated between the 'layers' of fluid is a property of the fluids internal friction or viscosity. The viscosity is an integral part of the shear stress and the

shear rate within the tube and is described by:

$$\text{Viscosity} = \text{Shear Stress} \;/\; \text{Shear Rate.}$$

Where:

Shear stress: Force per unit area applied to a layer of fluid to cause a movement relative to another layer.
Newtons.Meter^{-2}. Nm^{-2} or Pascals (Pa)

Shear rate : The velocity gradient between two layers of fluid.
Meters.Second^{-1}.Meter^{-1}. $m.s^{-1}.m^{-1}$ or s^{-1}

The units of viscosity are therefore: Nm^{-2}/s^{-1} or Pa.s.
With low viscosity fluids, most values for viscosity are expressed as: mPa.s.

The flow of a Newtonian fluid with constant viscosity therefore has a gradient of shear rate within a tube which is inversely proportional to the velocity: high velocity, low shear and vice versa.

The viscosity of a Newtonian fluid is not always constant and can be influenced by temperature, with a rise in temperature producing a reduction in viscosity. In the case of water, its viscosity of 1mPa.s at 20.3°C falls to 0.695mPa.s at 37°C, and rises to 1.8mPa.s at 0°C.

In haemorheology, the study of the viscosity of blood is more complicated as blood is a non-Newtonian fluid. This is due to blood consisting of corpuscles suspended in a plasma medium and this particulate nature makes the viscosity of the blood dependant on factors such as shear rate, haematocrit, erythrocyte deformation and plasma viscosity.

Shear Rate

At high rates of shear, blood viscosity possesses Newtonian properties with viscosity remaining constant with increased shear (figure 1.3.a). The high shear rates disperse red cell aggregates and red cells are deformed into ellipsoids in parallel with the flow streamlines. Cells also tend to occupy the central axis of the flow stream where shear rate is lowest and thus internal friction between cells and plasma is least. At such high shear rates ($200s^{-1}$) blood viscosity reaches a minimum value for a given red cell concentration. Such high rates of shear occur in the body circulation within the large arterial vessels.

As shear rate is reduced however, blood viscosity increases due to the changes in the particulate nature of the blood. The erythrocytes tend to lose their high shear deformation and form back into their circular shape thus increasing the resistance between fluid layers. The cells also have less tendency to occupy the central axis, thus increasing collisions with other cells. The increased interaction between cells allows them to aggregate, first into linear groups (rouleaux) and then into networks (Fähraeus, 1929). Aggregation tends to occur in the central axis of the flow where shear rate is least. The increase in viscosity with reduction in shear is termed 'thixotrophy' and is a property common to many fluids such as 'Non-Drip' paint.

Haematocrit

As described above, the rheology of blood is influenced by erythrocyte deformability and aggregation in response to changes in shear. It therefore follows that the number of red cells plays a vital role in changing viscosity. The standard human red cell concentration approximates to $6x10^{12}$ cells per litre (Dacie and Lewis, 1984). This value is best

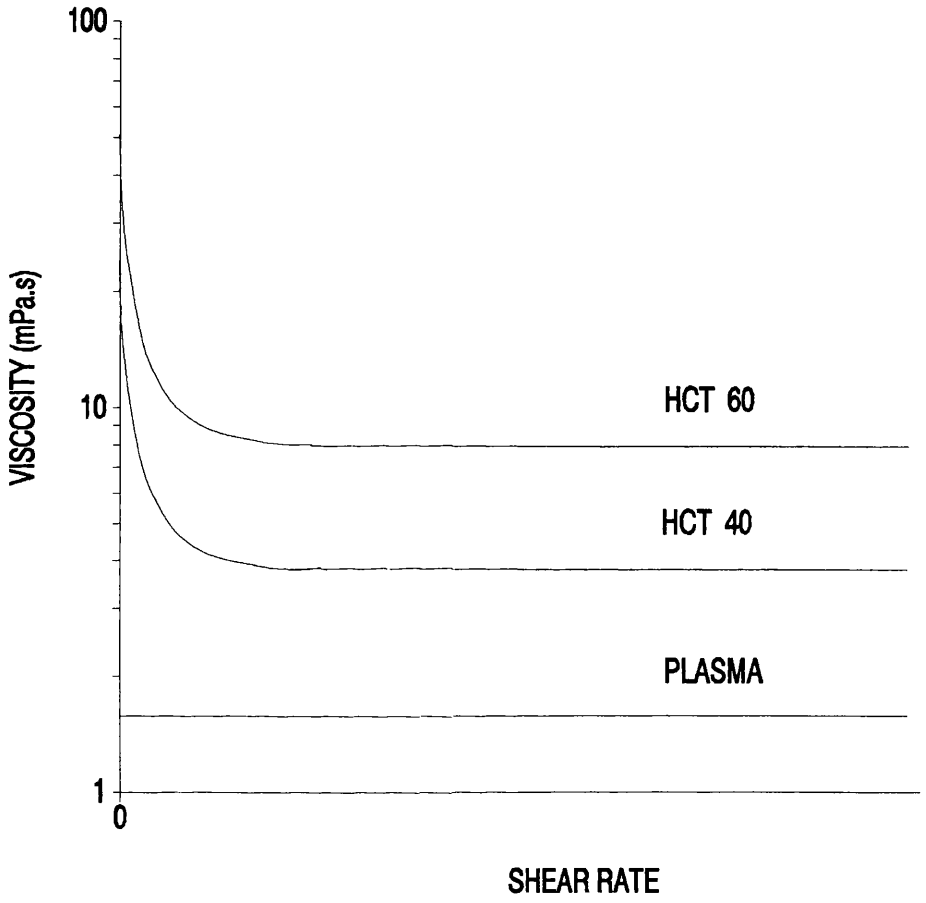


Figure 1.3.a Influence of shear rate on blood and plasma viscosity.

expressed as a ratio of red cell volume to whole blood volume, or the haematocrit; approximately 0.45. The haematocrit is the single most important factor influencing blood viscosity and its importance increases as shear rate decreases (Figure 1.3.b). It is evident from the figure that at low shear rates, even a slight reduction in haematocrit can produce a remarkable fall in blood viscosity. This is especially true around the normal physiological range where the slope of the correlation is steepest.

Erythrocyte Deformation

If erythrocytes were solid particles, then at an haematocrit of 0.65, blood would have the consistency of cement. However, blood remains fluid at an haematocrit of up to 0.99 due to the flexibility and low internal viscosity of the erythrocytes. As discussed above, high shear rates deform red cells into ellipsoids and reduce the intrinsic viscosity of the whole blood. Therefore, any changes in red cell deformability has an effect on whole blood viscosity. This can be observed in patients with sickle-cell anaemia where the sickling of the erythrocyte affects red-cell deformability and whole blood viscosity (Stuart and Johnson, 1987). Normally however, erythrocyte deformation is influenced by a number of factors including shear rate, plasma viscosity, haematocrit, pH, membrane flexibility and even the mean cell haemoglobin concentration.

Plasma Viscosity

Plasma is a Newtonian fluid and its viscosity is dependant only on temperature and plasma protein composition. In healthy adults, plasma viscosity remains relatively constant (1.35 mPa.s at 37 $^{\circ}$C) with less than 0.05 mPa.s variation (Harkness, 1971). Changes above this however, are usually linked with a pathological condition usually due to acute-

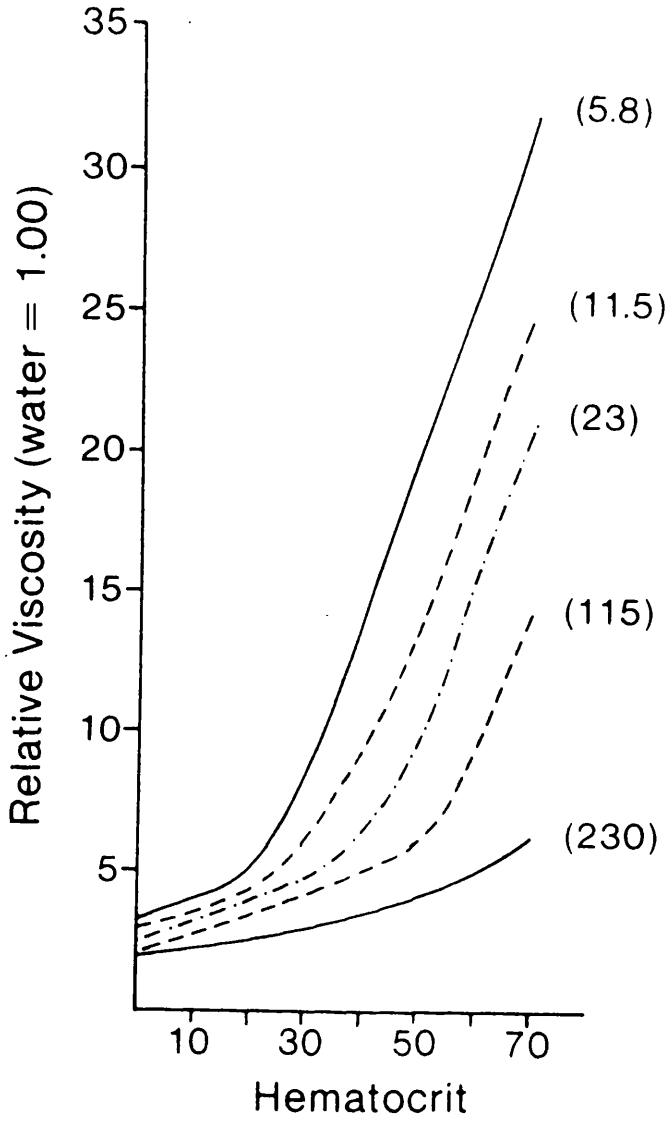


Figure 1.3.b Influence of haematocrit on viscosity at various rates of shear (within parentheses). (From Kee and Wood, 1987)

phase protein reactions. All forms of tissue injury such as surgery, infarction or acute infection, cause an increase in the plasma concentration of proteins involved in the inflammatory processes. Chronic infections and conditions such as paraproteinaemia also lead to elevated plasma viscosity. An increase in plasma viscosity produces a rise in blood viscosity, but the degree of rise is dependant on the shear rate applied. Under low shear, a rise in plasma viscosity increases the whole blood viscosity, but under high shear rates, a high plasma viscosity increases erythrocyte deformation and offsets the threatened rise in blood viscosity. The deformation is induced by the increased plasma viscosity and is analogous to pulling a balloon through treacle rather than through water (Lowe, 1987).

1.3.3 Haemodynamics

The factors outlined above refer to physical properties of the blood that influence its rheology both *in vivo* and *in vitro*. However, there are further physiological factors that affect the rheology of blood during its passage through the circulation.

Fähraeus-Lindqvist Effect

The Fähraeus-Lindqvist (1931) effect describes the effect of tube diameter on blood viscosity. If shear stress at the wall is kept constant but the diameter of the tube is narrowed, the relative viscosity of the blood is reduced. This is true for tube diameters less than 300μm: for example, relative viscosity in a 40μm tube is only 70% of that in a 150μm tube. Since neither plasma nor water viscosity are affected by changing vessel diameter the cause is obviously the presence of erythrocytes. It is suggested that the presence of erythrocytes affects the mathematical model of flow predicted by Poiseuille by breaking up the infinitely small laminae of fluid. The velocity gradient across the tube becomes discontinuous with layers of uniform velocity (occupied by corpuscles) and layers of shear (occupied by plasma). The effect is therefore greatest as the diameter of the tube approaches that of the erythrocyte.

Another theory to explain the Fähraeus-Lindqvist effect is that there is a cell free plasma layer at the wall of the blood vessels, and that this remains constant in size irrespective of tube diameter. If this was the case, as the vessel diameter was reduced the ratio of plasma to red cells would change, leading to a reduction in vessel haematocrit and blood viscosity. This phenomenon has been reported by a number of investigators and is thought to be produced by a process called 'plasma skimming' (see below).

Inversion Phenomenon

The 'inversion phenomenon' is the reversal of the Fähraeus effect at very low vessel diameters. As the capillary diameter approaches that of the erythrocyte (approx 6μm), the viscosity of the blood increases dramatically. This is determined by the ability of the erythrocytes to deform through a tube that is smaller than themselves and, under optimal conditions, they will pass through vessels as small as 2μm in radius. The 'critical radius' for the inversion phenomenon is dependant on the haematocrit, platelet and red cell aggregation and red cell deformability (Dintenfass, 1981). Reducing vessel radius below the critical radius produces further increases in blood viscosity.

Plasma Skimming

Normal blood flow has been shown to consist of an axial and more rapid movement of erythrocytes, with a plasma layer separating them from the vessel wall. As vessel branching occurs, the cell free zone at the periphery of the vessel is directed into branching vessels. The degree of plasma skimming is dependent on the geometry of the vessel lumen and the angle of vessel branching. The gradual reduction in haematocrit with repetitive vessel branching therefore reduces the blood viscosity in the microcirculation. As the cell free zone is only 5μm thick, it has little effect on the blood viscosity in large diameter vessels. In the microcirculation however, where this phenomenon is thought to be responsible for a reduction in haematocrit to two thirds that of the systemic value (Mchedlishvili and Varazashvili, 1981), the *in vivo* blood viscosity can fall to near that of the plasma (Keele et al, 1982).

1.4 HAEMODILUTION IN VIVO

1.4.1 Introduction

The rheology of blood, both *in vivo* and *in vitro*, is extremely complex in physical terms. The influence of haemorheology on blood flow in cerebral ischaemia, where normal blood flow is disrupted, is even more difficult to interpret. Probably the greatest influence on cerebral blood flow during cerebral ischaemia is the intrinsic thixotropy of the blood. As the blood flow is reduced in an ischaemic region, the shear rate also falls, both through the reduction in velocity and the reactive dilation of the cerebral vessels. This reduction in shear therefore produces an increase in viscosity and a further reduction in flow. This vicious circle further compromises perfusion and deepens the ischaemia (Grotta et al, 1982).

It has been the aim of many researchers to reduce the effect of increased blood viscosity on the remaining flow to ischaemic regions. As haematocrit is a major determinant of blood viscosity, reduction in red cell concentration or 'haemodilution' seems a probable way of improving flow. This can be achieved by either the removal of blood, the infusion of fluids or a combination of both. Exsanguination alone has not been common and most forms of haemodilution involve either infusion with exsanguination (Isovolaemic) or infusion alone (Hypervolaemic). However, in order to understand the arguments for and against the use of haemodilution therapy in cerebral ischaemia, one must first consider the normal but complex *in vivo* relationships between haematocrit, blood viscosity and oxygen delivery to the brain.

1.4.2 Haemodilution and Blood Flow in Normal Brain

Influence of Haematocrit

There have been relatively few clinical investigations into the effect of reduced systemic haematocrit on cerebral blood flow in non-ischaemic or 'normal' brain. Gottstein (1965) first observed that cerebral blood flow was high with low haematocrit and low with high haematocrit. This prompted therapeutic haemodilution with LMWD to reduce haematocrit and improve blood flow (Gottstein and Held, 1969). Similar results were observed during haemodilution of polycythaemics where reduction in haematocrit by exsanguination induced significant increases in cerebral blood flow (Thomas et al, 1977a). This was also the case in patients with high haematocrit and relative polycythemics (Humphrey et al, 1979). The latter two studies also demonstrated significant inverse correlations between cerebral blood flow and systemic haematocrit.

In non-ischaemic brain regions of stroke patients, Korosue et al (1988) recorded a significant 17% rise in cerebral blood flow after reducing haematocrit from 45% to 33% by isovolaemic infusion of plasma. Isovolaemic haemodilution with dextran 40 to reduce haematocrit from 46% to 39% was also associated with a significant 23% rise in blood flow to the non-ischaemic hemispheres of stroke patients. Significant correlations between changes in systemic haematocrit and associated changes in cerebral blood flow have also been demonstrated in both haemodiluted (Wood et al, 1984c) and non-haemodiluted patients (Grotta et al, 1982).

The most comprehensive non-clinical studies demonstrated an increase in blood flow to dog brain following hypervolaemic infusion of LMWD (Wood et al, 1982a) or autologous plasma (Wood et al, 1984a). In the latter study, mean cortical

blood flow rose by 15% and was significantly correlated with the 22% fall in haematocrit and the increase in cardiac output. However, in both these series of animals, the actual increase in blood flow in normal brain was not statistically significant. In the sham-clipped dogs used as controls for focal ischaemia studies, neither isovolaemic (Tu et al, 1988b) nor hypervolaemic (Wood et al, 1983) infusion of dextran produced any statistically significant change in cortical cerebral blood flow. However, the latter study (Wood et al, 1983) reported a non-significant 14% increase in cerebral blood flow following a reduction in haematocrit from 46 to 33%.

A similar correlation between haematocrit and blood flow to normal brain was demonstrated in anaesthetised dogs (Shimoji et al, 1979; same results published by Maruyama et al, 1985), but in this study, a 22% fall in haematocrit was accompanied by a significant 71% increase in cerebral blood flow.

In 'normal' cats, hypervolaemic infusions of HES or LMWD produced significant increases in cerebral blood flow, and these were significantly correlated with the degree of dilution (Scharf et al, 1989; vonKummer et al, 1988). Similar results were observed in normal baboons where hypervolaemic haemodilution with HES produced a significant rise in cerebral blood flow (Tsuda et al, 1987a , 1987b; Hartmann, 1989). In the latter studies however, similar infusions of dextran had no effect on the cerebral blood flow.

Isovolaemic haemodilutions of normal rabbits with either hypertonic or normal saline were associated with 60% increases in cerebral blood flow when haematocrit was reduced from 40% to 20% (Todd et al, 1985). The same group also demonstrated significant increases in cerebral blood flow in rats following isovolaemic haemodilution with hetastarch (Todd et al, 1992).

Further evidence into the effect of haemodilution on cerebral blood flow in normal tissue has been obtained from recordings from the contralateral hemisphere of animals with experimental focal cerebral ischaemia. Hypervolaemic infusion of dextran to reduce haematocrit by 30% and 45% in two separately reported investigations, both performed on a cat model of focal ischaemia, induced significant increases in flow in the non-ischaemic hemisphere (Coyer et al, 1987b and 1986, respectfully). However, using a similar haemodilution regime to reduce haematocrit by an unreported amount, the same researchers only observed "marginal increases in contralateral blood flow" (Coyer et al, 1987a).

In the normal brain regions of dogs with focal cerebral ischaemia, Tu et al (1988b) demonstrated small, but not significant, increases in cortical cerebral blood flow following reduction in systemic haematocrit from 45% to 33% using isovolaemic infusions of dextran 40.

In summary therefore, the majority of clinical and experimental studies have demonstrated that a reduction in systemic haematocrit is associated with an increase in blood flow through non-ischaemic brain tissue. However, some studies have failed to prove this statistically, while others have failed to find any increase at all. To this effect, work has continued on determining the mechanisms behind the relationship between haematocrit and the rise in cerebral blood flow. Influential candidates include cardiac output, blood volume, blood oxygen content, blood and plasma viscosity, blood pressure and vascular dilatation.

Influence of Blood Viscosity

As described in section 1.3.2, <u>in vitro</u> blood viscosity is strongly influenced by the red cell concentration. This finding led to the assumption that the increases in blood

flow seen following reductions in systemic haematocrit could be due to the inherent reduction in blood viscosity. Thomas et al (1977b) observed a strong correlation between haematocrit and both viscosity and cerebral blood flow, and suggested that "the observed improvement in CBF was predominantly due to a reduction in viscosity". Humphrey et al (1979) went on to demonstrate significant inverse correlations between cerebral blood flow and both high and low shear rate blood viscosity in venesected patients with high haematocrits. Again, it was suggested that "the rise in CBF is due mainly to the fall in whole-blood viscosity".

In normal dog brain, a significant inverse correlation was found between blood viscosity and cerebral blood flow during hypervolaemic haemodilution with dextran (Wood et al, 1982a) or plasma (Wood et al, 1984a). Although the increases in flow observed following haemodilution were not statistically significant, these investigations supported the hypothesis that a reduction in viscosity, as well as reduced haematocrit, was a significant "factor responsible for improvement in the cerebral blood flow during hypervolaemic hemodilution".

However, Muizelaar et al (1986) demonstrated that reducing haematocrit and blood viscosity in cats by mannitol infusion, did not improve cortical CBF, and that previously reported vasoconstriction was responsible for maintaining a constant blood flow (Muizelaar et al, 1983). The authors explained that at high haematocrits, such as those reported by Thomas et al (1977a), CBF would be reduced despite maximal vascular dilatation, whereupon CBF would become dependant upon the blood viscosity. However, Wade (1981) had clearly demonstrated that vascular reactivity to CO_2 was not compromised in patients with low cerebral blood flow secondary to elevated haematocrit.

A significant publication by Brown and Marshall (1982) presented data from plasma exchange patients, where blood

and plasma viscosity were reduced, but haematocrit was not affected. Cerebral blood flow did not change significantly and led the authors to conclude that compensatory vasoconstriction was responsible for maintaining constant oxygen transport to the brain. However, in a further group of patients with wide ranging blood haemoglobin concentrations, the same investigators found a significant correlation between blood viscosity and cerebral blood flow. They concluded that, in normal brain at least, the viscosity/blood flow relationship was coincidental to a dominant cerebral autoregulation to oxygen content (Brown et al, 1985b). This was further supported by measurements of cerebral blood flow in patients with raised blood and plasma viscosity where, in two patient groups, blood oxygen content, haemoglobin concentration and cerebral blood flow were similar, despite significant differences in blood viscosity (Brown and Marshall, 1985a).

In summary, there is clear evidence of a blood viscosity / cerebral blood flow correlation when haematocrit is manipulated, but that this relationship may be secondary to cerebral autoregulation in response to alterations in systemic oxygen content in an attempt to maintain constant cerebral oxygenation.

Influence of Oxygen Content

The results of Brown and Marshall (1982, 1985a) induced much debate on the cause of the rise in cerebral blood flow following haemodilution. A significant correlation between arterial oxygen content and cerebral blood flow was demonstrated and multiple regression analysis indicated that oxygen content rather than blood viscosity was the major determinant of blood flow (Brown et al, 1985b). These findings were in line with the results of Paulson et al (1973) who demonstrated that a reduction in arterial oxygen content through inhalation of carbon monoxide resulted in

increases in cerebral blood flow whilst blood viscosity remained constant. In normal brains, it seems likely that vascular reactivity to brain metabolic needs is the controlling mechanism of blood flow. Todd et al (1992) recently demonstrated this in rats, where isovolaemic haemodilution with hetastarch was accompanied by vasodilation induced increases in cerebral blood volume and blood flow, but that calculated oxygen delivery remained relatively constant. This was in line with Henriksen's views (1981); he showed that the jugular venous PO_2 was normal in patients with high haematocrit and low CBF, implying that low flow was not accompanied by any tissue hypoxia. Wade (1981) also found that vasoreactivity was normal in polycythaemics both before and after venesection, indicating that there was no vasodilation to viscosity induced low flow because the increased haematocrit carries sufficient oxygen to prevent hypoxia.

It has been difficult, however, for scientists to ignore the possible benefit of the fall in blood viscosity with reduced haematocrit. In order to assess the possible role of viscosity in controlling oxygen delivery, theoretical works have incorporated viscosity in the calculation of an optimal haematocrit for maximal oxygen delivery. If a compromise between these two physical properties of the blood exists, it is possible there is an optimal haematocrit for maximal oxygen delivery to the brain. This was initially calculated as being 0.30 (Hint, 1968) and more recently as 0.40 (Gaehtgens and Marx, 1987).

A plateau of maximal oxygen delivery at haematocrits between 0.3 and 0.43 was demonstrated by Schmid-Schönbein (1988) in amalgamated data of humans and at either end of this haematocrit plateau, the oxygen delivery was reduced. Wade (1983) also found that a reduction in human haematocrit from 0.54 to 0.44 increased blood flow by 30% and resulted in an 8% increase in oxygen delivery to the brain. More significant results were obtained in baboons (Tsuda et al,

1987a) where hypervolaemic haemodilution with HES reduced haematocrit from 0.42 to 0.36 and the associated rise in cerebral blood flow increased erythrocyte flow by almost 25%.

Experimental investigations clearly indicate that a reduction in systemic haematocrit can improve oxygen delivery to the normal brain. Whether this is induced by vascular dilatation in response to reduced oxygen content of the blood (Brown et al, 1985a), reduced blood viscosity through the reduction in erythrocyte concentration (Thomas et al, 1977a) or a combination of both remains debateable. However, the fact that increases in oxygen delivery have been demonstrated, infers that viscosity reduction must play at least some part in this increase.

If haematocrit is further reduced, the haematocrit has less of an effect on blood viscosity and a larger influence on blood oxygen content. At some point, reduction in haematocrit leads to a reduction in oxygen delivery, and one might think that reducing haematocrit to below the calculated optimum level for maximal oxygen delivery would be detrimental. However, in normal dog brain, Maruyama (1985) demonstrated that by systematically reducing the haematocrit to 5%, brain function was not affected despite the remarkable fall in blood oxygen content. Chan et al (1983) also found that drastic reduction in the haematocrit of cats to 20% produced no significant alteration in the brain PtO_2. Messmer et al (1973) demonstrated that reducing the haematocrit of dogs from 0.42 to 0.20 with normovolaemic haemodilution produced a rise in blood flow but that tissue PO_2 did not fall. The reduction in haematocrit to below the theoretical optimal level in all these cases, indicates a possible increased tolerance of the brain to reduced oxygen delivery.

Gaehtgens and Marx (1987) suggested that a compensatory increase in brain O_2 extraction helps preserve brain

function, but using their calculations, the theoretical delivery curve of Hint (1968), and the delivery curve of Bruckner and Messmer (1990), relative oxygen transport falls to only 50% of control at an haematocrit as low as 15%. This is not a critical reduction if we consider that normal oxygen delivery to the brain is about 10ml/100g/min and oxygen consumption approximates to around 3.3ml/100g/min. Brain tissue should therefore tolerate a reduction in oxygen delivery of over 60% before any detrimental effect on cerebral metabolism would be seen. This was confirmed in part by Harris et al (1987) where brain PtO_2 was reduced to around 30% of the control without any apparent pathological change.

In summary, oxygen content appears to be a major determinant of blood flow to the brain and increases in flow to normal brain following haemodilution are due primarily to compensatory vasodilation. However, increases in oxygen delivery to normal brain following haemodilution have been observed and these can be explained by the concomitant fall in blood viscosity. Where these findings are of particular interest is in patients with cerebrovascular disease, where compensatory vasodilatation is impaired and blood flow is more likely to be influenced by changes in blood viscosity.

1.4.3 Haemodilution and Blood Flow in Ischaemic Brain

Haematocrit and Blood Viscosity

One of the earliest investigations into the effect of haemodilution on cerebral blood during experimental focal ischaemia demonstrated increased blood velocity following hypervolaemic infusions of dextran (Sundt et al, 1967).

Wood et al have been responsible for a lot of the published data on the effects of haemodilution on cerebral blood flow and inevitably, they also investigated the haemodilution induced changes in flow during cerebral ischaemia (Wood et al, 1984b, 1983). They clearly demonstrated that hypervolaemic infusions of LMWD improved cerebral blood flow to the ischaemic brain of dogs. The increase in flow was twice that in sham-clipped animals, which indicated that LMWD was more effective at improving flow to ischaemic rather than normal brain. An inverse correlation between blood flow to ischaemic regions and systemic haematocrit confirmed a direct relationship between blood flow and blood viscosity that could explain the "remarkable increased collateral perfusion within the territory of the occluded MCA" (Wood et al, 1984b).

These results were augmented by an investigation into the relationship between CBF and mannitol induced haemodilution in cats (Muizelaar et al, 1986). The observed reduction in blood viscosity and haematocrit was accompanied by significant increases in blood flow to ischaemic tissues. A lack of vascular reactivity in the ischaemic regions was confirmed by a reduction in cerebral blood flow during a period of systemic hypotension and confirmed that "CBF passively rises with decreased viscosity".

An increase in cerebral blood flow to ischaemic cat brain following hypervolaemic haemodilution was reported by Coyer

et al (1986). However, this increase was reported as not statistically significant. No data was presented on the haematocrit, and haemodilution was with either saline or dextran and so it is impossible to determine the true effect of haematocrit reduction on blood flow in this series of experiments.

It could be argued that the increase in blood flow seen following hypervolaemic infusion might be attributed to the resultant increase in blood volume and raised cardiac output. However, Wood et al (1982b) demonstrated that the blood flow improvement to ischaemic tissue following hypervolaemic haemodilution was a result of the dilution effect and not hypervolaemia: intravascular volume expansion with whole blood did not result in an increase in blood flow to ischaemic territories even though CO was elevated.

As haemodilution and not hypervolaemia may be the likely cause of blood flow changes in ischaemic territories, further experimental investigations have been performed to examine the effects of isovolaemic haemodilution. This became especially important with the observation that hypervolaemic haemodilution increased intracranial pressure (Wood et al, 1984a). Tu et al (1988b) found that isovolaemic haemodilution of dogs with LMWD improved cerebral blood flow to ischaemic regions without additional increases in intracranial pressure. The within-animal increases in blood flow were not statistically significant, but compared to non-haemodiluted controls, cerebral blood flow was significantly higher. The significant haematocrit/viscosity correlation reported previously (Tu et al, 1988a) led the authors to conclude that the increase in cerebral blood flow "appeared to be related to the decrease in viscosity".

Similar increases in cerebral blood flow were reported by Yamashita et al (1989) where isovolaemic haemodilution with dextran produced a higher cerebral blood flow through focally ischaemic cat brains than found in non-diluted

animals. An inverse correlation between haematocrit and blood flow was also reported.

In patients with cerebrovascular disease, cerebral blood flow has been shown to increase in both ischaemic and non-ischaemic territories following isovolaemic haemodilution with LMWD (Wood et al, 1984c). In these stroke patients, the change in cerebral blood flow was inversely related to the reduction in haematocrit. Similar results were found in a separate group of stroke patients following hypervolaemic haemodilution (Vorstrup et al, 1989). Cerebral blood flow was improved to ischaemic territories by around 20% with a 16% reduction in haematocrit.

In general, haemodilution has been shown to increase cerebral blood flow to ischaemic regions but, as in normal brain, some researchers have failed to demonstrate that the increase is statistically significant and others have not found any rise in flow. Also, none of the investigations have clearly demonstrated whether blood viscosity or vasoreactivity to reduced oxygen content is responsible for the changes in blood flow. The work of von Kummers group (1988), demonstrated that isovolaemic haemodilution with dextran did not increase cerebral blood flow in non-ischaemic cats with severe hypotension. The authors concluded that in cerebral areas where autoregulatory capacity had been lost, haematocrit and thus viscosity could not influence cerebral blood flow. Such an argument supports the theory that blood flow in the brain following haemodilution is dependant upon vascular dilatation in response to reduced oxygen content and not the reduction in blood viscosity. Haemodilution, which reduces oxygen content, may not therefore improve oxygen delivery to vasodilated ischaemic tissue, but a number of investigations have aimed to determine how oxygen delivery to ischaemic brain is affected by reductions in haematocrit.

Oxygen Delivery

Kusunoki et al (1981), determined oxygen delivery in stroke patients and found that CBF was elevated with reduced haematocrit, but calculated that oxygen delivery was maximum at an haematocrit around 40%. Haematocrit values above or below this level produced a fall in oxygen delivery. A similar investigation performed by Cavestri et al (1986) found that oxygen delivery was not reduced when isovolaemic haemodilution decreased the haematocrit from 45% to 35% and demonstrated a possible benefit to clinical outcome. However, in both of these studies, blood flow measurements were recorded as hemispherical means and therefore may not be indicative of changes occurring only in ischaemic regions.

There are practical difficulties in assessing the changes in oxygen delivery to ischaemic regions following haemodilution in patients, and therefore most information has come from animal models. In MCA occluded cats, Chan et al (1983) failed to find any change in the tissue oxygen tension in the ischaemic brain tissue following isovolaemic haemodilution with dextran. By lowering haematocrit from 0.39 to 0.20, arterial oxygen content was reduced, but as blood flow was not monitored and no indication of vascular reactivity was available, it is impossible to determine whether vasodilation in response to reduced oxygen content or the fall in viscosity was responsible for maintaining PtO_2.

Recent data has been presented to demonstrate that haemodilution with LMWD significantly reduces oxygen delivery to maximally dilated brain regions (Back and vonKummer, 1991). As with most studies of this kind, oxygen delivery was calculated from systemic blood haematocrit and local blood flow. Although the calculations included adjustments for the differences in systemic and capillary haematocrit, it was inevitable that calculated oxygen

delivery would be reduced due to the lack of blood flow increase with haemodilution.

In focally ischaemic cats (Coyer et al, 1987a), brain tissue PO_2 in the ischaemic hemisphere was not significantly affected by a 30% reduction in haematocrit by hypervolaemic haemodilution with saline or dextran. This investigation highlights the difficulties in recording tissue PO_2 : a blood flow reduction from 52 to 13ml/100g/min was associated with a 50% fall in PtO_2, whereas in focally ischaemic baboons (Harris et al, 1987), PtO_2 at 50% of control was associated with blood flows of around 40ml/100g/min, and at a blood flow of 13ml/100g/min PtO_2 was approximately 15% of control.

None of the various models or haemodilution regimes used in the animal investigations have yet demonstrated an increase in oxygen delivery to ischaemic tissue, even though most have demonstrated an increase in blood flow. Such results tend to indicate that there is no benefit in haemodilution therapy due to the conflicting influences of oxygen content and cerebral blood flow. However, none of these investigations clearly determined the depth of induced cerebral ischaemia or the vaso-reactive capacity of the brain regions they were investigating. As demonstrated in normal brain, the presence of compensatory vasodilation will contribute to increases in flow and maintain oxygen delivery to the brain when haematocrit is reduced. This fact emphasises the need for detailed assessment of preserved vasodilatory capacity during haemodilution investigations in cerebral ischaemia.

There remains a lack of data on the effect of haemodilution on oxygen delivery to ischaemic brain, and determination of an 'optimal haematocrit' in pathological states is still not possible (Grotta, 1987b). Such a task seems difficult mainly due to problems in assessing oxygen delivery, and the unknown degree of change in CMR_{02} and oxygen extraction

fraction (OEF) that can occur in ischaemic regions (Hino et al, 1989). With such difficulties, most research on haemodilution therapy has moved away from the direct effect on physiology, to look at the consequences of these changes. Monitoring changes in brain function and clinical outcome may be used to determine the efficacy of haemodilution therapy without necessarily knowing the means through which this is achieved.

1.4.4 Evidence of Possible Benefit of Haemodilution

Cerebrovascular research on changes in blood flow and metabolism in ischaemic brain demands extensive resources. Patient monitoring requires both staff and expensive equipment, and experimental investigations are also costly and technically demanding. For these reasons, evaluating the possible mode of action of haemodilution therapy has been a slow process and generally inconclusive. Most researchers have therefore directed their investigations towards the possible benefit of haemodilution rather than the mechanism of such benefit.

Experimental Investigations

Ischaemic infarct size was the first outcome parameter to be monitored for changes following haemodilution, and was found to be significantly lower in focally ischaemic cats following LMWD haemodilution compared to non-haemodiluted controls (Sundt et al, 1967). The same paper examined the infusion of red cells to increase volume without haemodilution, and found an increase in infarct size. Haemodilution by hypervolaemic infusion of LMWD increased cerebral blood flow in focally ischaemic dog brain and reduced infarct volume from 10% in controls to 4% (Wood, 1984b), although this reduction was not significant. Tu (1987, 1988b) found a significant reduction in infarct size in dogs isovolaemically haemodiluted with LMWD concomitant with a rise in CBF. The infarct volume was 11% in control animals and 1.4% in haemodiluted animals. The same group later produced a similar reduction in infarct size to 2.2% following isovolaemic haemodilution in comparison to non haemodiluted animals where 12% of the ischaemic hemisphere was infarcted (Korosue et al, 1990).

A reduction in infarct size following haemodilution has been demonstrated consistently throughout the last 20 years. Such

data demonstrates the ability of reduced haematocrit to prevent cell death in an area around the infarct core. As detailed in section 1.2.1, this is likely to be the penumbral area, where cells are in a reduced functional state but remain viable for recovery. However, there has been no experimental data to prove such a theory.

Clinical Investigations

Ischaemic infarct size is a difficult outcome parameter to monitor in patients. Harrison et al (1981) however, have demonstrated a good correlation between infarct size and haematocrit using data from CT scans. Such data however, gives only limited information on the role of high haematocrit as a risk factor for ischaemic infarction (Tohgi et al, 1978) and not the possible benefits of haemodilution. Easier and more practical methods have therefore been employed with the most common, and the most relevant, being the influence of treatment on clinical outcome. The outcome for a patient can be 'scored' to encompass many variables such as mortality, severity of disability or length of stay in hospital.

An early trial on the use of LMWD to reduce haematocrit in stroke patients found a significant improvement in neurological status when compared with a group infused with dextrose solution (Gilroy et al, 1969). This early study did not monitor haematocrit changes, and the possibility of dextrose infusion affecting metabolism in ischaemia has led to caution on its use in ischaemic conditions (Heros and Korosue, 1988). A more recent non-blind trial in 1984 showed that rapid post stroke haemodilution with LMWD led to an improvement in clinical outcome (Strand et al, 1984). After 10 days, 85% of haemodiluted patients showed an improvement in neurological score compared with 64% of a randomly chosen control group. After 3 months, 31% of surviving undiluted patients were unable to walk compared to 8% of those

haemodiluted, however, the mortality rate at 3 months was not altered by the treatment. A single blind trial in China also found that isovolaemic haemodilution with LMWD over 14 days reduced the haematocrit from 45% to 36% and improved curative rate and mortality rate after one month when compared with non-haemodiluted controls (Wang and Sun, 1988). The benefit to the haemodiluted patients was still noticeable after one year.

The positive results of Strand's et al (1984) controlled study prompted a much larger haemodilution trial (Scandinavian Stroke Study Group (SSSG), 1985). This multi-centre trial included 373 patients half of which were haemodiluted with LMWD within 48 hours of stroke. However, the first results were negative, with no significant improvement in mortality, neurological score or length of required treatment (SSSG, 1987). Further analysis of the same data was performed later in an attempt to define possible sub-sets of patients that might find benefit from haemodilution, but no such sub-set could be found (SSSG, 1988). The results of these trials were summarised by Asplund (1989), who concluded that "the present haemodilution regime cannot be recommended for general use in patients with ischaemic stroke".

Similar conclusions were drawn from a further multi-centre trial set up in Italy (Italian Acute Stroke Study Group (IASSG), 1987). Patients were haemodiluted within 12 hours of stroke, by a combination of venesection and dextran-40 infusion resulting in a moderate reduction in haematocrit by approx 13% to 0.37. No difference in outcome or death rate was seen between haemodiluted and non haemodiluted patients at either discharge or after six months (IASSG, 1988).

The negative outcome of these trials did not stem the interest in haemodilution therapy and Grotta (1987b) raised a number of points about the trials that could explain the negative results. These included the relatively long delay

from stroke to haemodilution, the use of isovolaemic haemodilution, and the small reduction in haematocrit. Harrison (1989) suggested explanations for failure which included; the lack of haemodilution within a few hours of stroke, a statistically small sample size, the possibility that blood flow did not increase, or that hypervolaemia was critical to haemodilution therapy of stroke patients.

The results of a large multi-centre trial in the USA (The Hemodilution in Stroke Study Group (HSSG), 1989) aimed to answer some of the questions posed by the negative results of the SSSG and the IASSG. This trial reduced the time from stroke to haemodilution to less than 24 hours and used a hypervolaemic haemodilution regime with HES to reduce haematocrit to 33% for 3 days. Compared with non haemodiluted patients, this haemodilution therapy improved outcome assessed by neurological score. However, no difference was seen in the mortality rate between the two groups of patients and a fear of increased cerebral edema in the haemodilution group led to an early termination of the trial. The outcome of this trial came under strong criticism by von Kummer et al (1989) with a reply by Grotta (1989) highlighting the division within the scientific and clinical communities on the efficacy of haemodilution therapy.

Further negative results have recently been published by Mast and Marx (1991) where, in a randomised trial, 33 patients were hypervolaemically haemodiluted with HES and 37 received standard treatment. Haematocrit was reduced from 44% to 38% within 5 hours of admission, but the neurological score recorded at 14 days was found to be worse than the undiluted patients. Even in a subgroup of patients treated within 12 hours of stroke, no improvement was seen and the significant deterioration of eight haemodiluted patients led to a termination of the trial and a conclusion that "both isovolaemic and hypervolaemic haemodilution below a hematocrit of approximately 45% is a potentially dangerous stroke therapy".

Another recent trial of stroke patients treated with haemodilution within 24 hours of ischaemia has, however, reported positive benefits (Koller et al, 1990). Using hypervolaemic haemodilution with LMWD, clinical outcome was improved over a non haemodiluted group, and this continued for at least 3 months. Mortality rate was not altered. The authors concluded that "hypervolaemic hemodilution in ... stroke is well tolerated and improves early neurologic outcome".

The only major difference between the last two trials described was the infusate used; one employing HES, the other LMWD. One may question therefore, whether the type of haemodiluent may influence the effects of such therapy.

1.4.5 Type of Haemodiluent

Colloids have been the preferred infusate for haemodilution, mainly due to their long half life in the body and their relative safety with regard to the risk of cerebral edema. In ischaemic brain injury, the blood-brain barrier can be disrupted and the alternative use of crystalloids for haemodilution presents a risk of fluid leakage from the vascular compartment leading to brain edema and intracranial hypertension.

Early experimental investigations into haemodilution conclusively showed that infarct size and edema formation was increased in ischaemic dog brain following haemodilution with saline, but was significantly reduced following dilution with serum albumin and LMWD (Sundt et al, 1967). Following more recent suggestions (Poole, 1982) of using balanced salt solutions for restoring circulatory dynamics after severe blood loss, Korosue et al (1990) compared crystalloids and colloids as haemodiluents in ischaemic brain. In isovolaemically infused dogs with experimental focal ischaemia, the use of Ringer's solution reduced osmotic and oncotic pressure and led to a significantly larger infarct size than if LMWD was used. In similar data from the same group, isovolaemic haemodilution with Ringer's solution significantly increased the accumulation of water in the ischaemic hemisphere (Hyodo et al, 1989). In comparing the hypervolaemic infusion of colloids or crystalloids into dogs with cold brain lesions, Tranmer et al (1989) found an increase in ICP and reduced EEG power following crystalloid infusion, whereas colloids had no demonstrable effect.

All these recent investigations conclude that the use of a crystalloid infusion for haemodilution therapy of ischaemic stroke should be avoided, due mainly to the risk of cerebral edema. However, the use of hypertonic crystalloids can reduce brain water content, increase CBF and prevent the

rise in ICP seen with isotonic saline (Todd, 1985). These results from neurologically normal animals may indicate a future use of hypertonic crystalloids. However, published data so far indicates colloids as the preferred haemodiluent. Colloid infusions are designed to imitate the function of plasma proteins and to exert an colloid-osmotic pressure that will bind water and maintain haemodynamic conditions (Rudowski, 1980). Initial studies on LMWD as a plasma substitute were extremely encouraging and its use as a haemodiluent was a natural progression. Being cheaper and more easily obtainable than plasma, LMWD became the popular infusate.

Low Molecular Weight Dextran (LMWD) is the term generally used for a 10% saline solution of dextran with an average molecular weight of 40,000. The solution is hyperoncotic and an intravenous infusion therefore leads to dehydration of the extravascular space and an increase in blood volume. Expansion can be up to 100% of the infused volume, and it is necessary to compensate for extravascular fluid loss by an additional infusion of electrolyte. The half life of LMWD is approximately 3 hours from a single infusion, but long term infusion can be associated with the accumulation of high molecular weight molecules and extend the half life to up to 3 days (Kroemer et al, 1987).

Hydroxyethyl Starch (HES) is similar to LMWD in that it is hyperoncotic and expands plasma volume by around 100-172%. Infusions are normally of 6% or 10% in saline which contain a large range of molecular weight starch molecules. Metabolism involves degradation by alpha-amylase in the body, which reduces the molecular weights to allow excretion via the kidneys. While smaller molecules are excreted rapidly, the slow degradation of the larger molecular weight fraction increases the average half life to 17 days (Hulse and Yacobi, 1983).

Replacement of LMWD as the conventional haemodiluent for

treatment of stroke was first examined by Grotta et al (1985). This group recognised the negative aspects of dextran as a long term haemodiluent and aimed to determine the role of HES as a haemodiluting infusate. Their major concern was the rise in plasma viscosity seen in patients infused with LMWD.

1.4.6 Plasma Viscosity

In vitro, haematocrit is the dominant determining factor of whole blood viscosity. In vivo however, plasma skimming and the associated reduction in haematocrit at capillary level can reduce the viscosity of the blood to a level similar to that of the plasma (section 1.3.3). In cerebral ischaemia, the reduction in haematocrit at the capillary level is still present, and may be even further reduced (Mchedlishvili and Varazashvili, 1987). Thus, blood viscosity in the capillary bed of focally ischaemic brain may in fact be more dependant on plasma viscosity than on systemic haematocrit (Grotta, 1987b).

If plasma viscosity plays an important role in blood flow at the capillary level, then an increase in viscosity could be detrimental to clinical outcome. Plasma viscosity has not been a commonly measured parameter in haemodilution studies, but in those that have studied the changes, there have been considerable increases in viscosity (Schievink, 1988; Kroemer et al, 1987; Tsuda et al, 1987a and b; Farman et al, 1991b). The cause of these increases in viscosity was the use of LMWD as the haemodiluent. Original data on the viscosity of this fluid, suggested that the in vivo plasma viscosity was not affected by intravenous infusion (Groth, 1966). However, recent reports suggest that long term use of this drug, as is normally the case in haemodilution therapy, can lead to a substantial increase in plasma viscosity.

In patients suffering from all clinical manifestations of

cerebral ischaemia, there is a significant increase in plasma viscosity, usually due to raised fibrinogen levels (Fisher and Meiselman, 1987). This tends to increase red cell aggregation, especially at low shear rates, and can lead to a further reduction in flow. In patients infused with dextran 40, the concentration of dextran molecules can rise to 5 times higher than the fibrinogen level (Haass et al, 1987). The long term accumulation of higher molecular weight molecules of dextran and the increase in aggregation by so called 'bridging effects' could therefore further compromise blood flow in the microcirculation (Haass et al, 1986)

In vitro studies have demonstrated that increases in plasma viscosity are associated with increases in blood viscosity (Rand et al, 1970). Theoretical modelling of vascular networks predicts that a 50% increase in plasma viscosity (1.2mPa.s to 1.8mPa.s) also increases whole-blood viscosity in both large and small blood vessels by 50% and increases total peripheral resistance by 75% (Schmid-Schönbein, 1988). Normal circulation should however, cope with changes in plasma viscosity through compensatory changes in vascular tone. Such a response has been demonstrated in patients following plasmapheresis, where plasma viscosity fell significantly but cerebral blood flow remained constant (Brown and Marshall, 1982). In dogs, dextran induced increases in plasma viscosity were also compensated for by compensatory vasodilation in many organs (Chen et al, 1989). This was confirmed by Brückner and Messmer (1990) who also demonstrated that elevated plasma viscosity following HES infusion did not jeopardise regional blood flow or local tissue oxygenation. These two investigations also emphasised that plasma viscosity and not red cell aggregation was responsible for the apparent increases in blood viscosity.

In ischaemic conditions, where vascular regulatory capacity has been reduced, the effect of plasma viscosity is still unclear. Sakaki et al (1991) attempted to evaluate the

effect that reduced plasma viscosity had on focal ischaemia in cats, but found no therapeutic effect due to the presence of autoregulation. However, in plasmapheresis treatment of Raynaud's phenomenon, a reduction in plasma viscosity has been associated with an increase in skin capillary blood flow and an improvement in clinical condition (Jacobs et al, 1991). Raynaud's phenomenon is associated with impaired circulation of the digits, which indicates that changes in plasma viscosity may be relevant to blood flow in regions of impaired vascular reactivity. However there is still little experimental evidence that raised plasma viscosity can affect the blood flow to ischaemic regions.

Clinically, there is increasing evidence of a correlation between raised plasma viscosity and clinical manifestations. In epidemiological studies, raised plasma viscosity has been strongly correlated with serum fibrinogen, cholesterol and triglyceride levels and, therefore, not surprisingly, correlated with hypertension and the incidence of ischaemic heart disease. High plasma viscosity has also been found in a non-exercising sample population, and in a sample population of smokers, the later being associated with high levels of serum fibrinogen (Lowe and Barbenel, 1988).

Polygeline as a haemodiluent

With mounting concern about the influence of high plasma viscosity on blood flow to ischaemic regions, this investigation was designed to evaluate the influence of LMWD on blood rheology in ischaemia. In doing so, comparison with infusions of polygeline have been made. The following review outlines the additional negative characteristics of LMWD and the reasons for the use of polygeline as a haemodiluent.

LMWD is hypertonic and draws fluid from the extravascular space to increase blood volume. It is therefore critical that the state of hydration of the patient is monitored, to

prevent dehydration and renal impairment. Volume expansion of the blood is dependant on body hydration and further crystalloid infusion and can therefore produce a risk of heart failure through vascular overload. With a short half life, the excretion of dextran can lead to a state of hypovolaemia if the colloid-oncotic pressure is not maintained by other plasma proteins (Thomas, 1989).

Polygeline in the form of Haemaccel (Hoechst) has a neutral oncotic pressure compared with plasma. Intravenous infusion leads to an increase in blood volume by the amount infused and does not require the state of hydration to be adjusted. Cardiovascular overload is therefore less likely and there is no deleterious effect on renal function. Oncotic pressure can be maintained for longer due to a half life of approximately 5 hours with hypervolaemic infusion, and 8 hours with isovolaemic infusion. It is unknown whether long term use of polygeline can lead to accumulation of the product as found with dextran, but the efficient excretion, even in patients with renal insufficiency, suggests that this is unlikely. Twice weekly infusions (500ml) over six weeks did not cause significant accumulation even in patients with terminal renal insufficiency (Köhler et al, 1978).

The in vitro rheology of both the infusates is remarkably different, with Haemaccel having a viscosity similar to plasma of 1.28mP.s at 37°C (Barras, 1969), compared with that of LMWD at 3.5mPa.s (Groth, 1966). In vitro mixing of blood and LMWD produced higher blood viscosities than Haemaccel blood mixtures due to this difference in plasma viscosity (Ehrly, 1969). This does not reflect the viscosity of the two fluids in vivo because of the differing oncotic properties, but does highlight the 'artificial' nature of LMWD compared to the more plasma like properties of Haemaccel. This is particularly noticeable in the pH of the solutions, with Haemaccel having a pH of 7.2-7.3 and with some degree of buffering capacity, compared to LMWD with an

acidic pH of 4.4-5.6.

The dehydrating property of plasma expanding infusates used for haemodilution of ischaemic stroke, can be of benefit in prevention of edema formation, raised intracranial pressure and expansion of infarct size. Dehydration is not possible with iso-oncotic solution infusions, but by maintaining oncotic pressure, it is unlikely that extravascular water accumulation will increase.

Additional benefits of Haemaccel, include the cost, shelf life and stability. The cost is half that of LMWD infusions, it can be stored at room temperature for 8 years as opposed to 5, and is unaffected by wide temperature fluctuations.

CHAPTER 2

MATERIALS AND METHODS

2.1 ISCHAEMIC MODEL

Introduction

The baboon model of focal ischaemia used in this study was adapted from that developed by Symon et al (1974; Symon, 1975). Models of focal ischaemia have certain advantages over other ischaemic models due to the large portion of the brain that is left intact. Having normal and ischaemic brain in the same animal eliminates the need for, and cost of, control studies and also reduces the effect of inter-animal variation. A further advantage of this model is the similarity of primate and human cerebral circulations, increasing the relevance of experimental results to the problems of the clinic. The large animal size provides other advantages including: ease and stability of anaesthesia over long periods, the availability of a large brain area for numerous electrode insertions, and the ability to draw a large number of blood samples without undue effect on blood volume.

Occlusion of the middle cerebral artery produces graded ischaemia across the cortex with the densest region in the sylvian opercula. Each animal responds differently to the MCAO due to variations in collateral circulation. In some animals, the cortical blood flow following MCAO may not fall to a level where major pathological events occur. Previous workers have aided the formation of dense ischaemia in such animals by a process of exsanguination. In this investigation however, such interference with the circulation would have influenced the effects of haemodilution. To encourage a sufficiently dense area of ischaemia, additional occlusion of the anterior cerebral artery was performed in some of the animals. This was only necessary in smaller, younger animals which usually had better collateral circulation.

The animals used in the study were all male baboons (Papio anubis or Papio cynocephalus).

Preparation

On the morning of an experiment, fasted animals were sedated with an intramuscular dose of ketamine (7mg kg^{-1}; Ketalar, Parke-Davis) and atropine sulphate (1.2µg kg^{-1}; Antigen Pharmaceuticals). The head, chest, groin and calf were shaved and a Venflon 2 catheter (Viggo) placed in the saphenous vein for administration of maintenance doses of sodium thiopentone (5mg kg^{-1} IV; Intraval, May and Baker). Intubation and ventilation on pure oxygen using a Starling pump (20 strokes min^{-1}; CF Palmer) was followed by a paralysing dose of gallamine triethiodide (1mg kg^{-1} hr^{-1} IV; Flaxedil, May and Baker). The femoral arteries and veins of each leg were catheterised. One arterial catheter was connected to a blood sampling set, and the other to a pressure transducer (Bell and Howell) for continuous monitoring of blood pressure. The venous catheters were used for drug and drip infusion of saline (0.5ml min^{-1}, Hartmanns, Baxter), and for later infusion of the haemodilutent. The animals were permanently anaesthetised with an intravenous dose of alpha-chloralose (60mg kg^{-1}; BDH Chemicals Ltd). External skin electrodes were arranged on the chest for ECG monitoring. Blood gases were maintained in the normal range through repetitive arterial blood sampling, blood gas analysis (AVL Medical, Model 995) and subsequent adjustment of the ventilatory stroke volume. Body temperature was maintained using a electric heating blanket with feedback control from a rectal temperature probe. Blood glucose was monitored using glucosticks read by a glucometer (Ames).

The animals were positioned on their chests and the scalp and temporal muscle removed with monopolar diathermy. Four craniectomies of approximately $4cm^2$ were made in the skull, three over the right hemisphere exposing the base of the operculum and sylvian fissure, the post-central region, and the parasaggital region. The other opening was over the left hemisphere. The dura was reflected and, following haemostasis, the brain was kept moist with patties and warmed

saline irrigation.

The recording electrodes for monitoring blood flow, oxygen tension and direct cortical response were placed within or on the cortex of the exposed brain (details in relevant electrode chapters). Positioning of up to ten electrodes in each area was achieved using an array of small electrode manipulators. After drawing a plan of the electrode positions, the electrodes were cemented in place using bone acrylic (Surgical Simplex, How Medica).

When all the electrodes were in place, the animals were turned onto their backs and the contents of the right orbit were removed. Using a air drill and burr, the base of the orbit was drilled through and the dura reflected to expose the origin of the middle cerebral artery.

In a number of smaller animals, the proximal anterior cerebral and common anterior cerebral arteries were also exposed. The vessels were dissected sufficiently for later occlusion with one or more Scoville Lewis clips (10x1mm, Downs Surgical).

Experimental procedure

A 30 minute stabilisation period was left between the completion of surgical preparations and the start of recording. Figure 2.1.c outlines the series of recordings made during each experiment and the following sections give a detailed account of the methods used. Brain PtO_2 was recorded continuously throughout the recording period.

Figure 2.1.a Schematic diagram of recordings

	ACTION		RECORDING
CONTROL PERIOD	HYDROGEN ON		
	HYDROGEN OFF	->	BLOOD FLOW 1
	CO_2 ON AND HYDROGEN ON		
	HYDROGEN OFF	->	BLOOD FLOW 2
	CO_2 OFF		
	HYDROGEN ON		BLOOD/PLASMA VISCOSITY
	HYDROGEN OFF	->	BLOOD FLOW 3
	HYDROGEN ON		
	MIDDLE CEREBRAL ARTERY CLIPPED		
ISCHAEMIA	HYDROGEN OFF	->	BLOOD FLOW 4
	HYDROGEN ON		
	HYDROGEN OFF	->	BLOOD FLOW 5
	CO_2 ON AND HYDROGEN ON		
	HYDROGEN OFF	->	BLOOD FLOW 6
	CO_2 OFF		
	HYDROGEN ON		BLOOD/PLASMA VISCOSITY
	HYDROGEN OFF	->	BLOOD FLOW 7
	HAEMODILUTION		
DILUTION	HYDROGEN ON		
	HYDROGEN OFF	->	BLOOD FLOW 8
	CO_2 ON AND HYDROGEN ON		
	HYDROGEN OFF	->	BLOOD FLOW 9
	CO_2 OFF		
	HYDROGEN ON		BLOOD/PLASMA VISCOSITY
	HYDROGEN OFF	->	BLOOD FLOW 10
	SACRIFICE		

Control period

Blood flow measurements were performed after saturation with 5% hydrogen at normal blood gas levels with continuous cortical oxygen tension recording throughout. Control blood samples for blood and plasma viscosity measurements were taken. The arterial PCO_2 was then raised to approx 50 mmHg by inhalation of 5% CO_2, and blood flow measured to determine the degree of vessel reactivity to CO_2 change in normal brain. Following normalisation of the arterial blood gases, a further blood flow determination was carried out to establish the pre-ischaemic control flow.

Ischaemic period

Following saturation with hydrogen the middle cerebral artery and, where applicable, the anterior cerebral artery were occluded using Scoville clips. The blood flow was measured immediately after vessel occlusion. The animal was left to stabilise for 15 minutes following MCAO and a second series of blood flows were recorded. Blood samples were also taken at this point to check the effect of ischaemia on systemic blood rheology. Vessel reactivity was again determined by flow measurement at elevated arterial PCO_2. Having restored normal blood gases, further blood flow measurements established the ischaemic baseline prior to haemodilution.

Dilution:

Prior to the haemodilution stage, all animals were treated in an identical fashion. Each animal was then haemodiluted with one of four haemodilution regimes :

Isovolaemic haemodilution with Haemaccel
Hypervolaemic haemodilution with Haemaccel
Isovolaemic haemodilution with LMWD
Hypervolaemic haemodilution with LMWD.

Hypervolaemic Infusion.

Animals in the hypervolaemic series received infusions of either Haemaccel or LMWD into the right femoral vein at a rate of 10ml min^{-1}. The volume infused was 15ml kg^{-1} of Haemaccel or 10ml kg^{-1} of LMWD. The difference in the infusion volumes compensated for the intravascular expansion due to the hyperoncotic action of LMWD (Tu et al, 1988). A maintenance dose of fluid was administered following infusion via a drip set.

Isovolaemic Infusion.

For isovolaemic haemodilution, blood was withdrawn from the left femoral artery as fluid was infused into the right femoral vein. Infusion of Haemaccel and withdrawal of blood was at a rate of 10ml min^{-1} and in volumes of 10ml kg^{-1}. For LMWD, blood was withdrawn at the same rate and volume as for Haemaccel, but the rate and volume of infusion was reduced by 35% (6.5ml kg^{-1} at 6.5ml min^{-1}).

Post dilution Period

Post dilution blood samples were taken to assess the changes in blood and plasma viscosity. Blood flow measurements were made to establish the effect of haemodilution on cerebral blood flow. As with the pre-dilution recordings, three blood flow measurements were made, two at normal $PaCO_2$ and one during hypercapnia.

2.2 CEREBRAL BLOOD FLOW

Introduction

Numerous methods have been developed for the measurement of cerebral blood flow. The most versatile and probably the most accurate of these is the hydrogen clearance technique. Developed in the 1960's (Aukland, 1965), this method has become popular among many research groups. The technique does however have a number of limitations which have been extensively reviewed elsewhere (Young, 1980; von Kummer, 1986a and b). Having been well tried and tested within this department (Pasztor et al, 1973) the hydrogen clearance method was used to make blood flow measurements in this series of investigations.

Electrode Production

Blood flow electrodes were constructed from 125 µm diameter teflon coated platinum/iridium wire (Pt/Ir 90:10 w/w; Clark Electromedical). The coating was stripped back 2mm from one end, and the exposed platinum soldered to a length of PVC insulated multistranded wire. A hot soldering iron (370 °C) was used to make this connection to prevent dry joint formation. The platinum wire was cut to a total length of 10mm. The cut was made at a 45 degree angle to aid insertion of the tip into the cortex. The teflon coating was removed 1mm back from the tip and the exposed wire surface cleaned by scratching with a sharp blade.

It was not necessary to insulate the solder junction to the connecting wire as, when cemented into the skull of the animal, the bone acrylic formed an insulating enclosure of the joint (figure 2.2.a). The figure also shows the oblique insertion of the electrode at approximately 45 degrees to the brain surface. This minimised changes in electrode depth that could be produced during brain pulsations and ensured that the exposed area of the wire was placed in the grey matter of

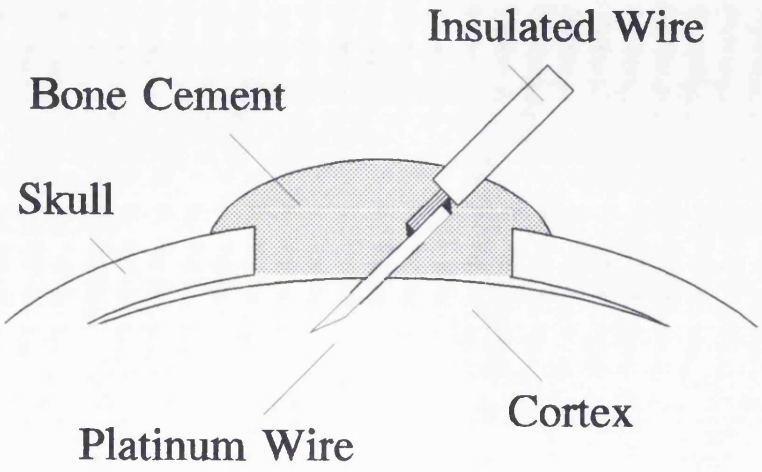


Figure 2.2.a

Diagram of hydrogen clearance blood flow electrode inserted into the cortex and sealed in with bone cement.

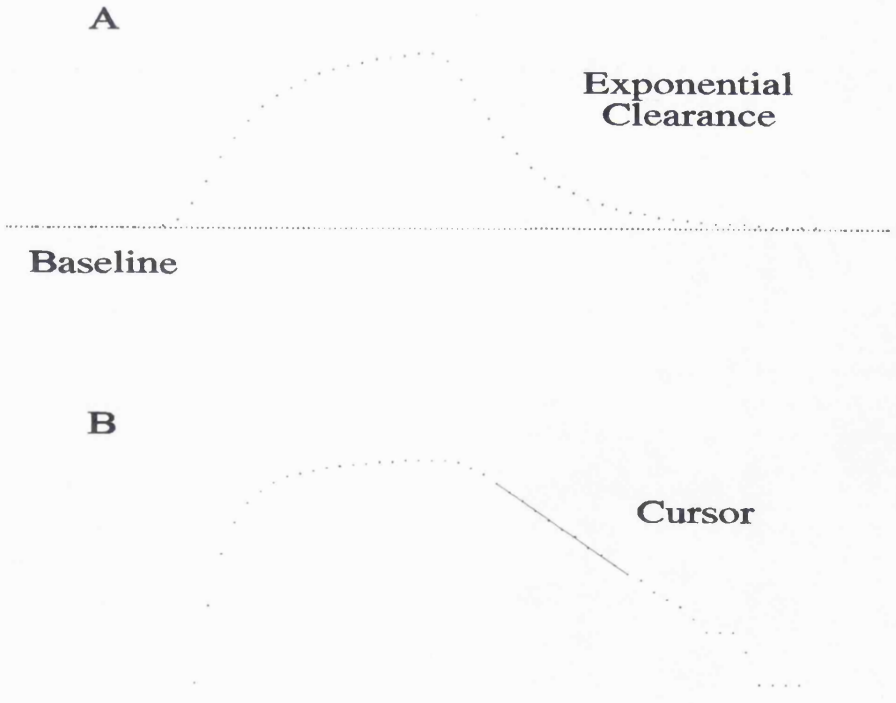


Figure 2.2b

An example of a blood flow recording:
A: Exponential saturation with hydrogen followed by clearance from the brain.
B: Logarithmic transformation of trace A, with measurement cursor positioned on linear portion of clearance slope.

the cortex so as to produce monoexponential clearance curves. The flow electrodes were arranged within the cortex in groups around the oxygen tension electrodes, thus enabling direct comparison between the measured parameters. Generally, 5 or 6 flow electrodes were placed within each craniectomy.

Recording System

The polarising voltage required for the reduction of hydrogen at the surface of the electrode was produced using a large silver/silver chloride electrode (Clark Electromedical), encased in a saline soaked swab and placed in the animals mouth. This electrode was polarised to -400mv which, because the animal was electrically floating, produced a potential difference of +400mv at the surface of the flow electrodes. The current generated through the polarisation of hydrogen at this voltage was amplified using a similar circuit to that described by Pasztor et al (1973). The electronic circuitry is shown in figure 2.2.c along with that used for monitoring oxygen tension. The balance control was used to set the amplifier output to zero when no hydrogen was present. This both eliminated the residual current produced by the polarisation of other molecules around the electrode tip, and permitted changes in gain without affecting the baseline.

The outputs from all the amplifiers were passed to a multiplexer through which each channel was sampled once every ten seconds by a computer controlled Analogue/Digital converter. The digital signals were then displayed on a computer monitor and stored on disk for subsequent analysis.

Blood flow recording began by switching a gas tap to release hydrogen gas from a fine bore tubing inserted into the ventilation tube of the animal. The gas supply from a storage cylinder was humidified and the rate and volume of delivery was calculated to give a 4% inhalation mixture. This led to saturation of the normal cortex within 5 minutes, at which

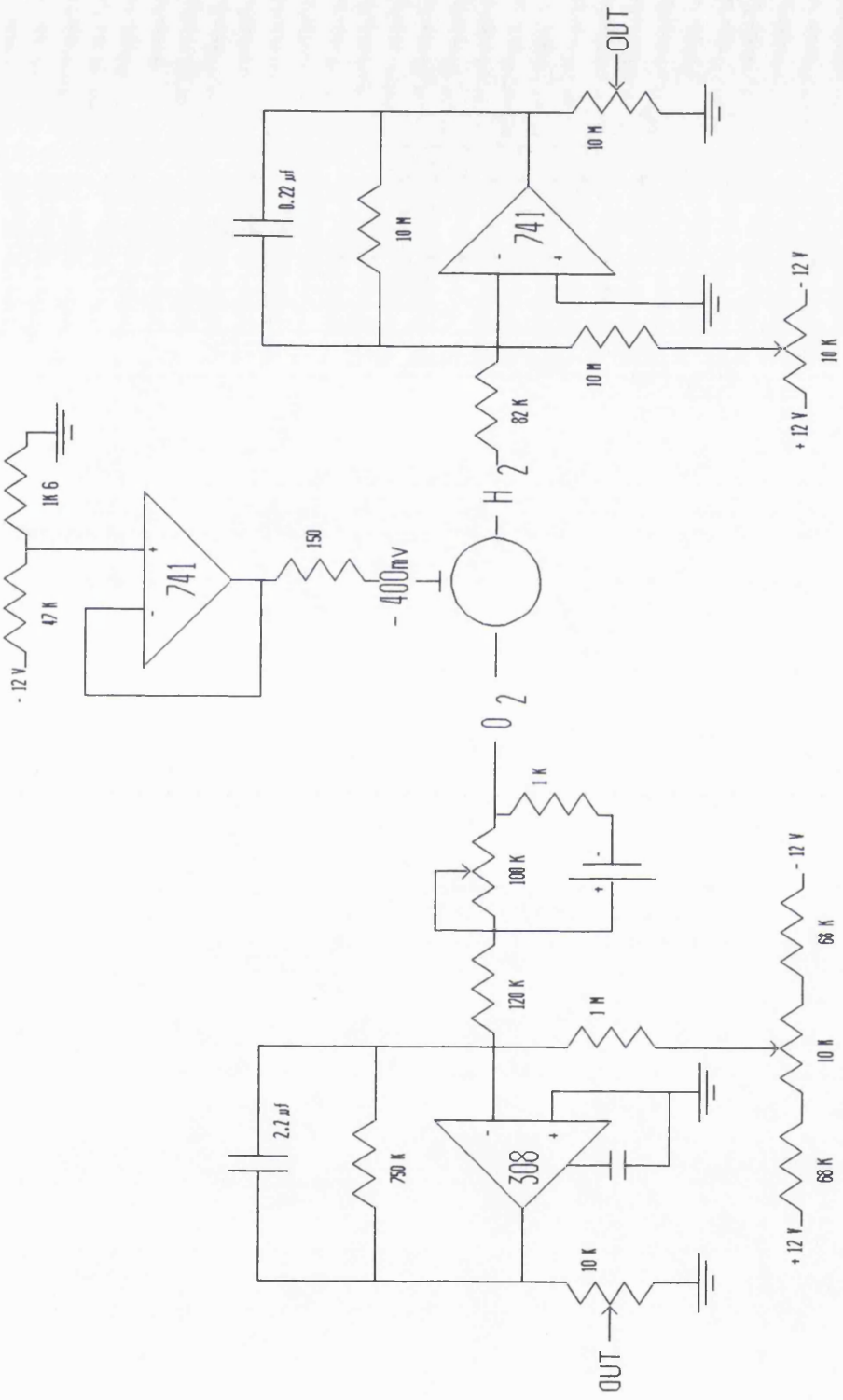


Figure 2.2.c

Circuit diagram of amplifier system for polarisation of recording electrodes and measurement of cerebral blood flow and tissue oxygen tension. Central circle represents electrically floating animal or other recording medium.

point the gas supply was shut off. The saturation and clearance of hydrogen from the tissue could be followed continuously on the computer (Figure 2.2.b).

Analysis of the signal on the computer was performed in three stages. First the baseline of the curve was selected. Second, the display was frozen and a semi-logarithmic plot of the curve was produced. Finally, the flow could be measured using a variable length cursor which could be positioned on a linear portion of the slope.

Eliminating Sources of Error

The technique of hydrogen clearance for recording blood flow is not without errors (Young, 1980). Methods employed in this department to reduce error include meticulous attention to baseline setting. Taking the logarithm of the clearance curve from anywhere but the true level of zero hydrogen concentration leads to a significant error in the measured flow. At specific periods during the recording protocol, sufficient time was therefore left for complete elimination of hydrogen from the brain tissue. At such time, baselines were noted and electrodes rebalanced to electrical zero before further recordings were taken.

Another source of error was the influence of slower clearing tissue, such as white matter, on the cortical clearance rate. Diffusion of hydrogen from slowly clearing tissue could lead to bi-exponential clearance curves, but the occurrence of this phenomenon was virtually eliminated by careful electrode positioning. In those cases where electrodes did produce a biexponential clearance, the initial 1 to 2 minutes of clearance tended to be monoexponential, and a flow value taken at this point was deemed to be indicative of the flow through the grey matter. Logarithmic signals that were not linear over the first 2 minutes, were not analysed.

Recirculation of hydrogen due to incomplete elimination through the lungs was a likely source of error at the beginning of desaturation. Signals were therefore not analysed from the point at which the hydrogen delivery ceased, but from the point at which the logarithm of the desaturation curve became linear, approximately 40 seconds later (Pasztor et al, 1973).

2.3 OXYGEN TENSION

Introduction

The technique of oxygen tension measurement developed rapidly following the production of the Clark electrode (Clark, 1956). This electrode incorporated both the cathode and anode for the polarisation of oxygen behind an oxygen permeable membrane and was termed a 'bipolar' electrode. Prior to Clark, oxygen tension was recorded using 'monopolar' systems using a single wire anode and a remote cathode. Both types of electrode have been used for recording oxygen tension from the brain, but technical difficulties in the miniaturisation of the Clark electrode have restricted its use to recording from the surface of the cortex (Leniger-Follert et al, 1975). Monopolar electrodes were the first to be used for recording oxygen tension from within the brain (Davies and Brink, 1942) and have remained popular due to the ease of miniaturisation.

The technique became popular in the 1970's, and was adapted for use in the primate model of focal ischaemia (Crockard et al, 1976a and b). This department also developed a method for recording oxygen tension continuously throughout hydrogen clearance measurement of cerebral blood flow (unpublished work). The technique was therefore ideal for augmenting the measurement of blood flow during haemodilution, by giving an index of the changes in oxygen availability in the ischaemic tissue.

Electrode Production

Electrodes for cortical oxygen tension measurement were constructed two days prior to their use in an experiment. A set of twelve were made to ensure enough spares should calibration of any prove difficult. A diagram of a completed oxygen electrode is shown in figure 2.3.a.

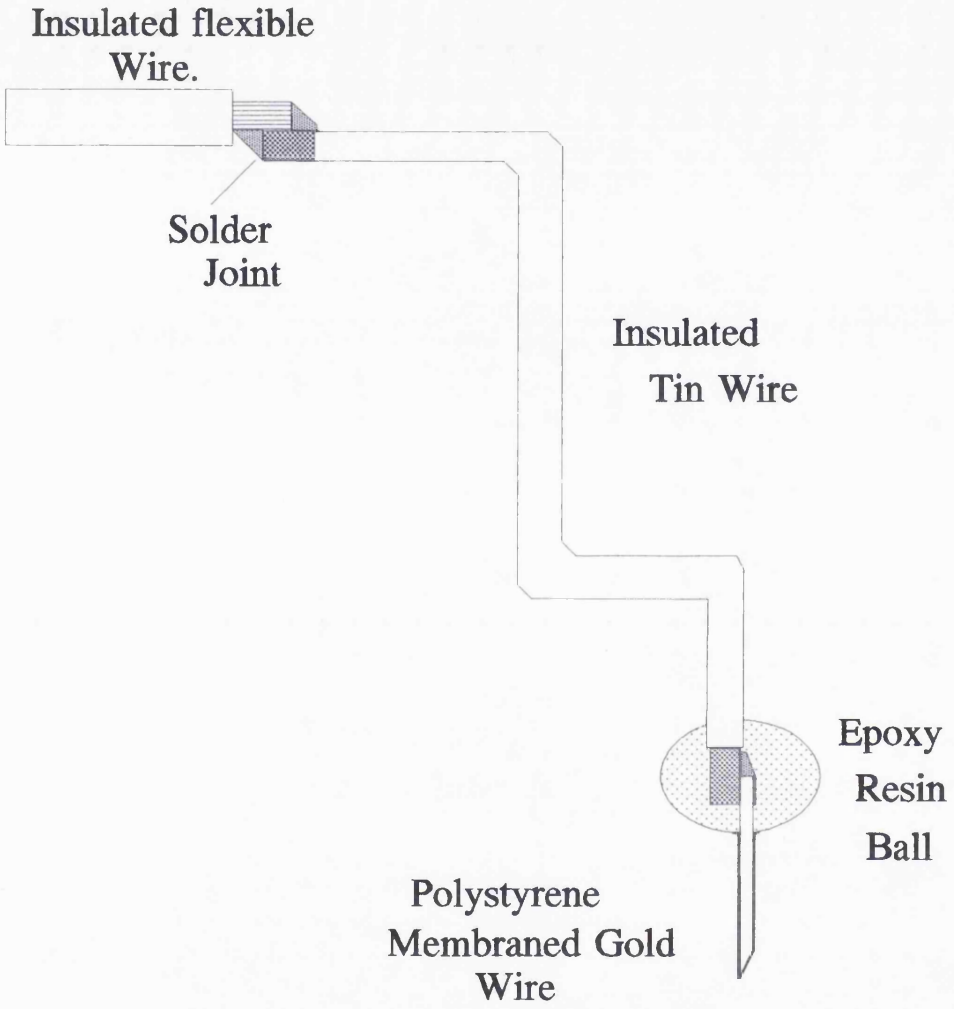


Figure 2.3.a
Oxygen tension electrode, showing bends in the tin wire which acted as a small "spring" to enable the electrode to follow the pulsatile movements of the brain cortex.

The electrodes consisted of a short length of 125 micron diameter pure gold wire (Goodfellow Metals) soldered to an insulated tin wire of 500 micron diameter (RS Components). A low temperature soldering bit (260°C) and solder (60% tin 40% lead, 183°C melting point) was used to prevent vaporisation of the fine gold wire. Care was taken during the soldering process to produce a very small junction of the two wires with the distance from the insulation to pure gold being no more than one millimetre. Epoxy resin (Araldite Rapide; Ciba Geigy) was applied to this junction and moulded into a small ball. By leaving the resin to cure slightly before application, it was possible to prevent it spreading over the gold wire. After drying overnight, the exposed gold wire was trimmed back to 1.5mm using a scalpel blade. The cut was made obliquely so as to provide a sharp point for smoother insertion into the brain. A microscope was required for the cutting process, and at this point a check of the gold surface was made to ensure cleanliness and straightness, and the epoxy was inspected to ensure complete insulation of the junction.

The diffusion membrane was produced using a 10% w/v solution of polystyrene (BDH) in CCl_4 (AnalaR, BDH)(Naylor and Evans, 1963). This was stored in a tightly capped vessel with minimal air space to prevent CCl_4 evaporation from altering the solution concentration. The solution could be stored in this way for several months. On the day prior to an experiment, the electrodes were dipped quickly into the solution up to the epoxy ball and then withdrawn at a slow even pace. This was conducted smoothly so as to prevent drips of the solution forming on the wire and creating an uneven diffusion barrier. After drying for ten minutes in the upright position, the process was repeated. The electrodes were left to dry for 6 to 8 hours, and then immersed in distilled water overnight.

Electronic Circuitry

A polarisation voltage of -800mv was required to reduce oxygen at the surface of the electrode. However, the use of hydrogen clearance for the measurement of blood flow required an electrode polarisation of +400mv. To combine the two recording techniques in the same animal, the following circuitry was developed (figure 2.2.b). Figure 2.3.b is a schematic representation of the polarising voltages achieved by this circuitry.

The reference electrode polarised the electrically floating animal or recording medium to -400mV. When the oxygen or blood flow electrodes were placed in the animal, they became polarised to +400mV with respect to the reference voltage. In order to polarise the oxygen electrodes to -800mv in the +400mV recording environment, each electrode was fitted with a floating nickel/cadmium battery to polarise the electrode to -1.2V. With respect to the reference voltage of -400mV the electrode surface charge became -800mV, the optimum for oxygen reduction (Fatt, 1976).

The amplifier system operated in the same way as the blood flow system with simple balance and gain controls, except that the response time of the circuit to a square wave input was reduced to approximately 1.5 seconds.

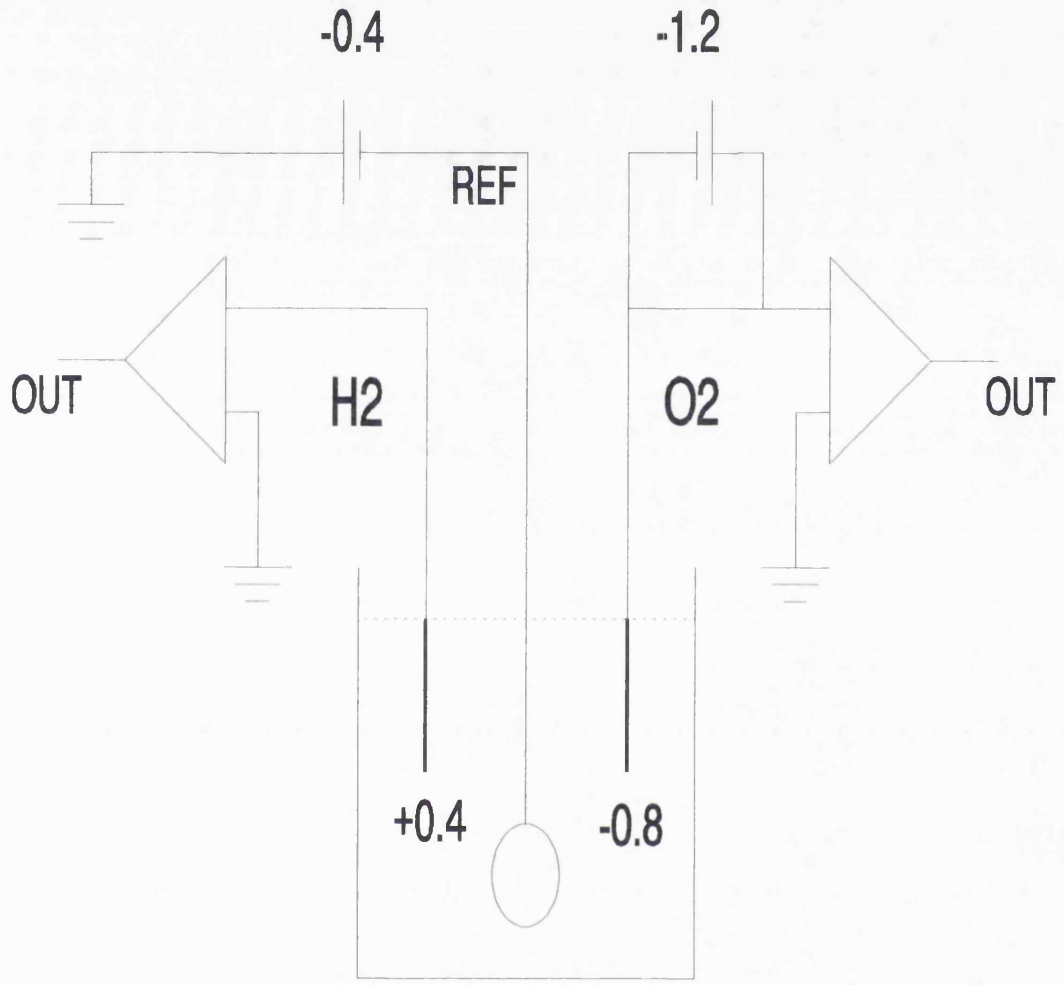


Figure 2.3.b
Schematic diagram of the polarisation voltages induced by the electronic circuitry used to measure blood flow and oxygen tension.

In Vitro Calibration

Calibration of the oxygen electrodes was performed in a system that mimicked the electronic and physiological environment of the animal model. A glass vessel was designed and manufactured (Radleys) to provide a constant temperature and controlled distribution of calibrating gases (Figures 2.3.c). The water jacket was fed from a circulating water bath (Julabo) to maintain a temperature of 37°C. The flat base of the vessel allowed it to stand on a magnetic stirrer, and a large 'flea' was used to swirl the gas that bubbled from the scintillated base. The gases were prewarmed by delivering them though a tube that passed inside the water jacket. The perspex lid was a good fit on the flat edges of the vessel and reduced the interference of the atmosphere on the calibrating solution. The opening for the electrodes was small and the flow of gas from the chamber prevented the entry of the surrounding air.

The calibrating gases were ready mixed at about 6, 3 and zero percent oxygen in nitrogen (BOC Special Gases). All the gases were supplied with calibration certificates for accurate determination of the oxygen tension they produced. The gases were delivered to the calibration chamber though a manifold of taps to allow rapid switching from one gas to another.

The electrodes were held in the chamber by securing them to glass microscope slides and then lowering the slide through the opening in the perspex top. The chamber was filled with 400ml of 150mM sodium chloride. Another small hole in the lid allowed access of a polarising silver/silver chloride plate electrode (Clark Electromedical). Testing of the electrodes was carried out after soldering multistranded wires to the stripped ends of the tinned wire, allowing connection to a total of eight amplifier units.

Before the electrodes were connected to the amplifiers, the balance of the circuit was adjusted to set the output to

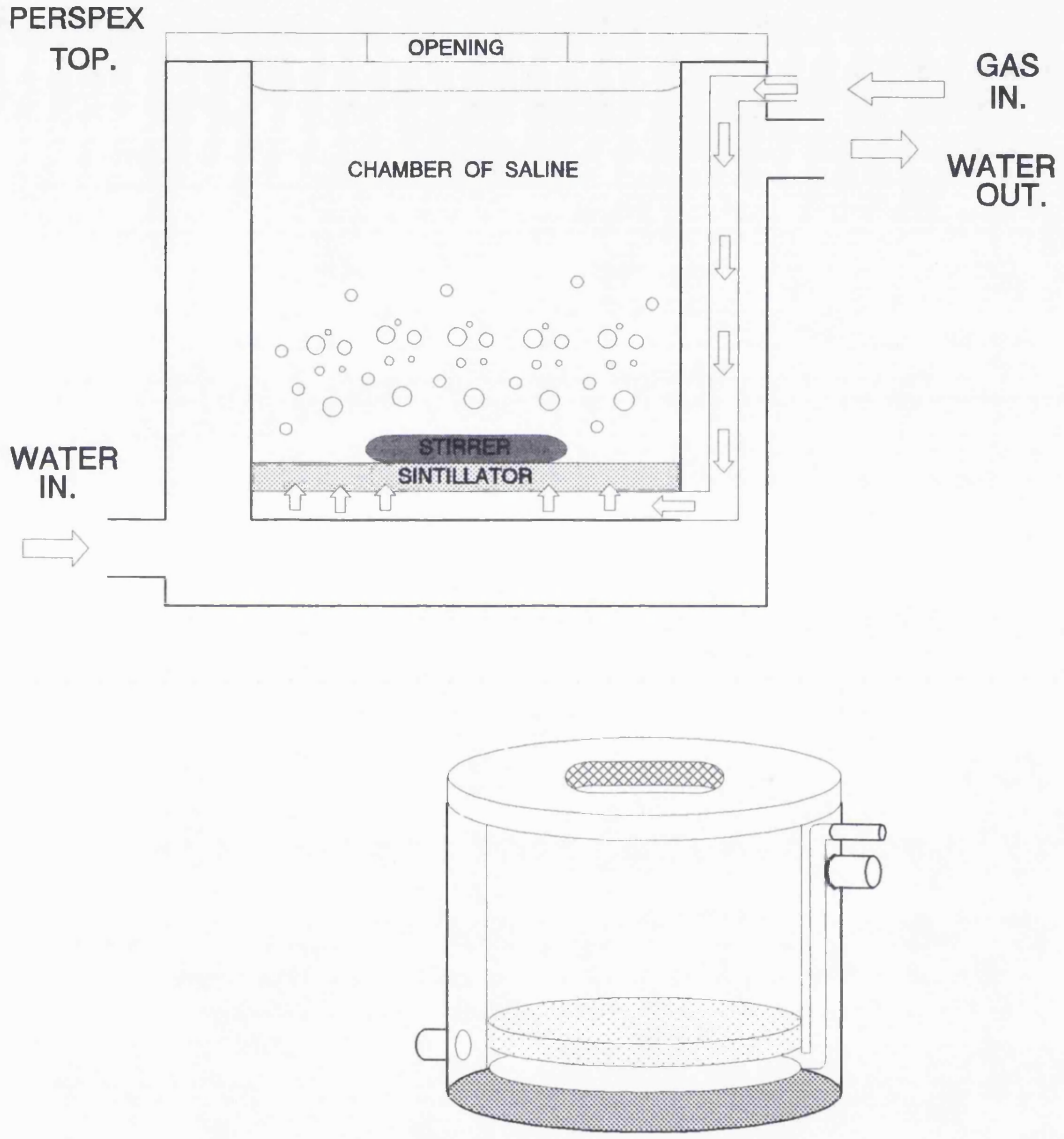


Figure 2.3.c
2D and 3D representations of the glass blown oxygen electrode calibration chamber.

zero. On connection to the amplifiers, the electrodes immediately registered an output corresponding to the tension of oxygen in the calibrating vessel. The 6% gas was bubbled though the chamber first and the gain of the amplifier set to give a near maximal output on the chart recorder. Switching to the zero oxygen gas, the output of the amplifiers fell near to the baseline set by the balance prior to connection. The output above baseline represented the residual current produced by the polarisation of other species besides oxygen and by leakage current though poor electrode insulation. Electrodes with a large residual current did not calibrate linearly and those with a residual current of over 50nA were not used. Calibration was completed by monitoring the response to 3% oxygen gas and again to 6%, the latter being to check the stability of the original 6% measurement. The complete cycle of calibration was repeated if necessary.

Electrode Characteristics

A calibration curve of a typical oxygen electrode is shown in figure 2.3.d. The output of the amplifiers was usually measured in millimetres deflection from electrical zero, but in this figure, the actual current output is shown. The current output and the size of the residual current were determined by disconnecting the electrode from the amplifier and replacing the input with a known current source. The calibration data was fed into a mathematical computer program to calculate the regression of the response and the correlation coefficient. Only electrodes with a correlation of over 0.99 between oxygen tension and current output were used.

The polarising voltage used in this study was -800mv. Figure 2.3.d also shows the response of an electrode to changes in the polarising voltage at different oxygen tensions. This demonstrates the plateau of the polarisation curve described by other workers. By utilising a polarising voltage on this

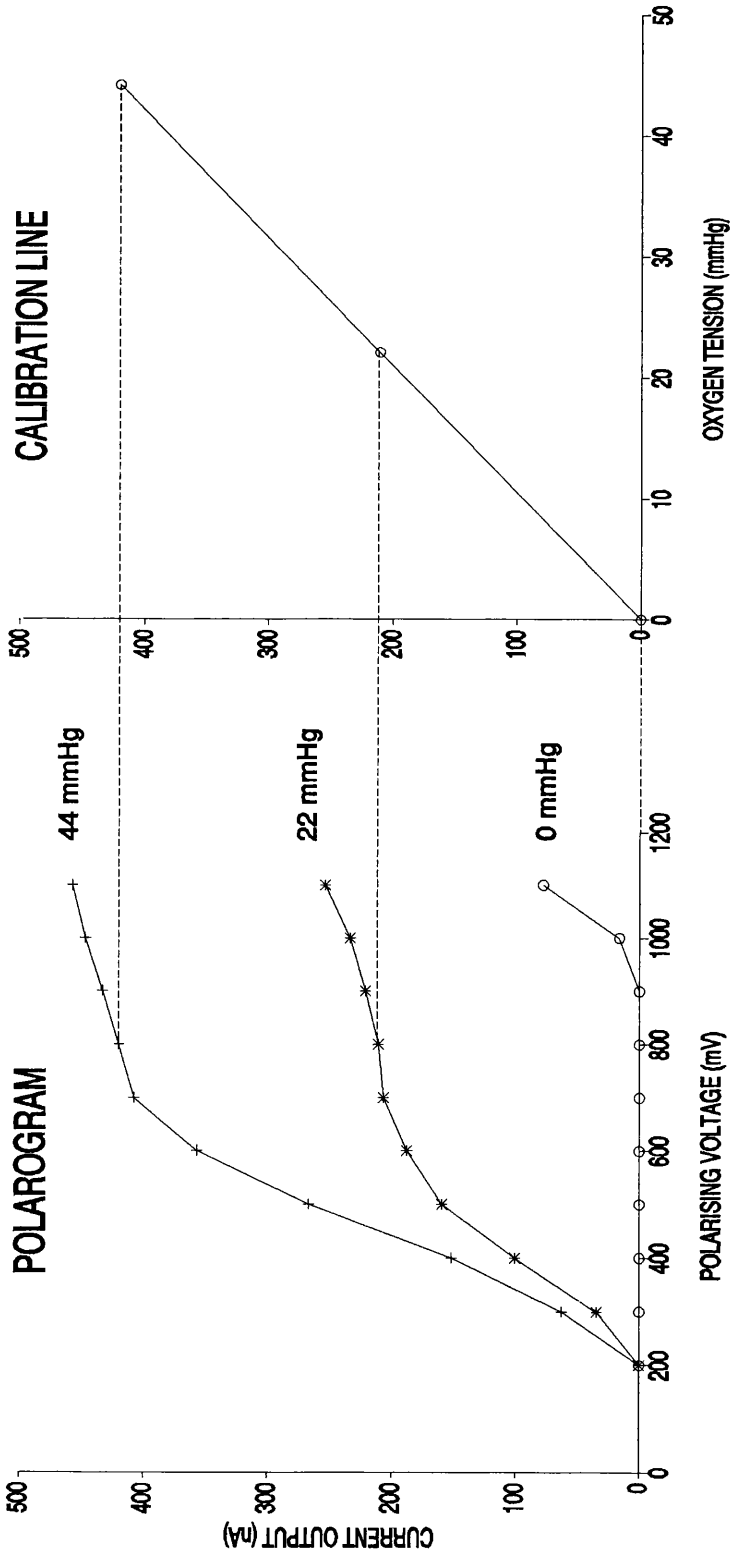


Figure 2.3.d

Polarogram from a single oxygen electrode demonstrating the changes in electrode output at various polarising voltages and oxygen tensions. The calibration curve generated at the plateau polarisation voltage of -800mv is also shown.

plateau, any small changes in polarising voltage would not effect the current output of the electrode at a given oxygen tension and ensured that membrane diffusion was the limiting factor in the reduction of oxygen at the surface of the electrode (For review see: Fatt, 1976; Linek et al, 1988).

Selectivity

Oxygen tension electrodes have been shown to be sensitive to other electro-active molecules in the recording medium, for example, the reduction of halothane anaesthetic (Dent and Netter, 1976). In the experimental model described, there were a number of induced changes in the contents of the circulating blood. These included hydrogen, for blood flow recording; carbon dioxide, for vascular reactivity testing, and the two infusates used in haemodilution.

The selectivity of the electrodes for oxygen rather than hydrogen was achieved by the use of gold wire instead of the platinum favoured by other investigators. Platinum oxygen electrodes respond to changes in hydrogen concentration in the recording medium even when the polarising voltage is -800mv. Platinum metal has a certain degree of catalytic reaction with oxygen and hydrogen at the surface of an electrode, the mechanism of which is very complicated. To avoid the influence of hydrogen, a cathode of gold was ideal due to its inert properties (Linek et al, 1988). Oxygen electrode calibration in the presence of hydrogen had no effect on the electrode response (figure 2.3.e).

To test the selectivity of the electrodes against carbon dioxide, some electrodes were calibrated with carbon dioxide mixed with the calibrating gas. Again, the electrodes proved highly selective to oxygen which is represented by a trace in figure 3.2.e.

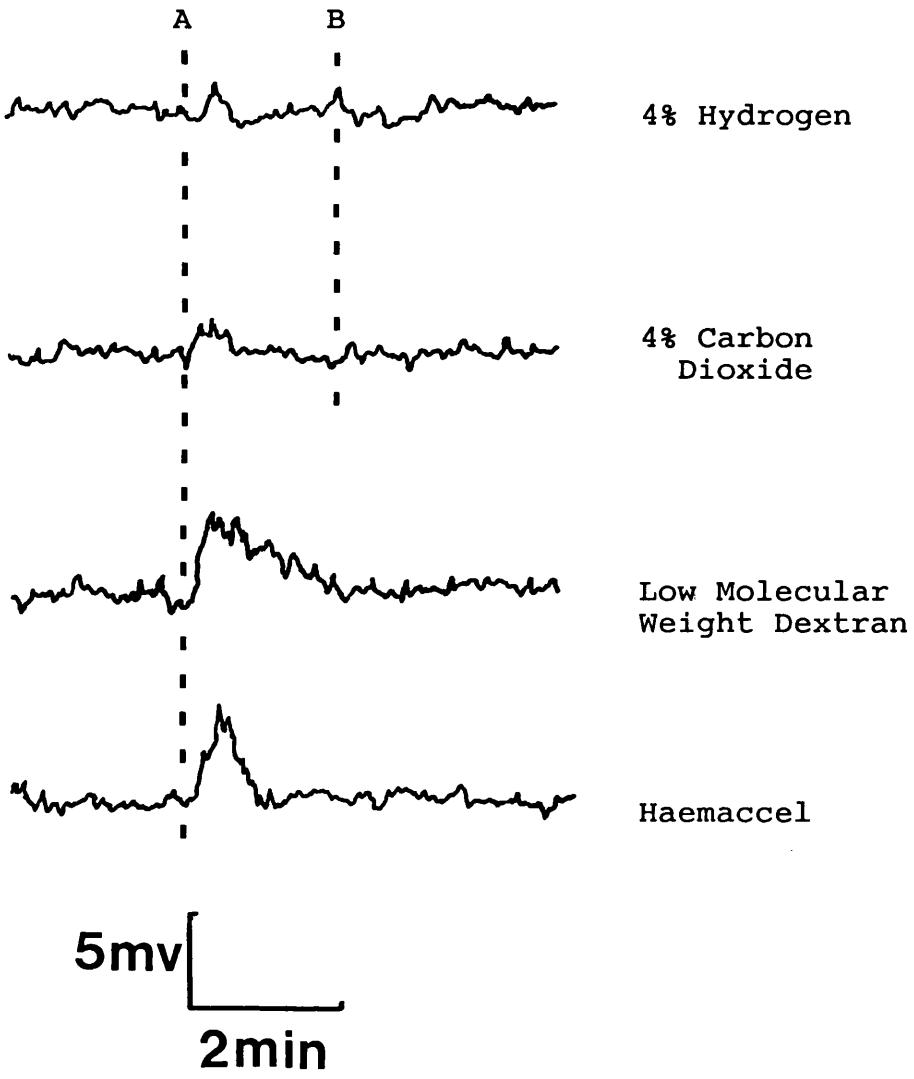


Figure 2.3.e
Output from a gold oxygen electrode when the above were added to the calibrating chamber containing a continuous throughput of 6% oxygen in nitrogen. Substances were added at A and stopped, where applicable, at B.

A major aim of this project was to evaluate the effect haemodilution might have on the oxygen availability to ischaemic regions of the brain. It was therefore necessary to test whether the haemodiluents used in the investigation, had any electroactive properties that might influence oxygen tension recording. Figure 3.2.e also shows an oxygen tension recording prior to and following an infusion of haemodiluent into the measuring chamber. A slight rise in electrode output was recorded, probably due to the 20% oxygen content of the infusate. The output returned to normal and no detectable difference in the electrode response was observed.

2.4 RHEOLOGY

Introduction

In the relatively new field of clinical blood rheology, it was found that the many techniques used for sampling, processing and measurement of the blood could influence the test result. To combat variation, an expert panel on blood rheology was established to make recommendations on the laboratory measurement of blood rheology (International Committee for Standardisation in Haematology (ICSH), 1984; 1986). All the techniques described below have been based on these recommendations.

Blood Sampling

Blood was collected without stasis into sterile tubes containing the potassium salt of edetic acid (EDTA) anticoagulant at a final concentration of 2mg/ml (Harkness, 1971). Two samples of 3ml were taken at each sample time. One sample was for blood viscosity and haematocrit measurement, the other for plasma viscosity measurement. After mixing both samples on a blood roller, the whole blood sample was stored in a refrigerator at 4°C and the sample for plasma viscosity evaluation was placed in a centrifuge at 2,000g for 5 mins to separate the plasma from the cells. If a centrifuge was not available immediately, the sample was stored upright at room temperature and was not remixed after red cell settling had occurred. Plasma samples separated from the settled cells were stored at room temperature and could be kept for up to one week without effect on viscosity (Harkness, 1971), but were normally measured within 24 hours.

Sample Measurement

Blood and plasma viscosity measurements were performed on a rotational viscometer (Contraves Low Shear 30, Metler-Toledo). The main advantage of this machine was its high accuracy even on a sample size of less than 1ml. The temperature of the sample was regulated at 37°C by a water jacket fed from a circulating water bath. The machine was frequently calibrated using Brookfield viscosity standards.

Whole blood that had been stored in a refrigerator was placed on a mixing roller to remix settled cells, and to bring the sample to room temperature. A 1ml sample was taken from the mixed blood using a disposable plastic pasteur pipette and placed into the clean, pre-warmed sample chamber. Care was taken to prevent bubbles being introduced into the sample as they would affect the recordings. The measuring head was lowered slowly into the sample chamber and high shear rotation begun. The time taken for the sample to reach 37°C could be reduced by rotating the sample at a high shear rate and by gently 'bobbing' the measuring head up and down in the sample. As the sample temperature equilibrated, the signal output steadily fell and stabilised after approximately 1 minute, when the full evaluation of viscosity was performed.

High shear viscosity was measured at a shear rate of $94.5s^{-1}$. When the sample measurement was stable at this shear rate, the rate of shear was reduced to $5.47s^{-1}$, and the output recorded when the signal had stabilised. The sample was then measured again at the high shear rate following repeated mixing through 'bobbing'. This procedure was repeated several times, until the same output was achieved by consecutive readings at each shear rate.

Plasma is a Newtonian fluid and its viscosity is constant at all shear rates, therefore it was measured only at a shear rate of $94.5\ s^{-1}$, in a similar fashion to whole blood. Care

was taken to mix the plasma sample in its container before removal for measurement as the evaporation of water could raise the viscosity value.

The haematocrit of the whole blood sample was evaluated using a Coulter analyser. This analysis required that the blood was well mixed and was advantageous in requiring only a small sample size (0.2ml).

Sources of Error

The temperature of a blood sample has a marked effect on its viscosity and therefore all measurements made during this study were done at a constant 37°C maintained by an accurate circulating water bath (Julabo). Even with an accuracy of 0.02°C it was recognised that the temperature of the water bath was not necessarily the same as that in the measuring chamber. This was therefore evaluated using a small thermometer inserted into the sample chamber. A small temperature difference was found, probably produced though radiation and conduction of heat from the circulating water. The error was eliminated by increasing the bath temperature to give an accurate sample temperature of 37°C.

The accuracy of our model of viscometer in measuring the viscosity of blood and plasma samples had been reported previously by numerous investigators (eg Black et al, 1986). One source of error which was found to be of relevance to this study was the loss of accuracy that can occur at low voltage outputs from the measuring head and when the machine was used at its most sensitive settings. To overcome these problems, the measurement of low shear blood viscosity was made at a shear rate of 5.45 s^{-1} which was found to encompass the best of the apparatus.

Red cell settling is a major problem in the measurement of whole blood in a rotational viscometer. The erythrocytes tend

to settle under the action of gravity and thereby reduce the effective haematocrit between the two shearing surfaces and lower the measured viscosity. To diminish this effect, the sample was mixed by repeated bobbing of the measuring head up and down in the sample cup (Matrai et al, 1984). As well as preventing settling, and disaggregating rouleaux formation, the shearing forces created by the movement of fluid in the cup helped to prevent the accumulation of minute bubbles released through the degassing effect of sample warming. The mixing also reduced the temperature equilibration time and prevented the formation of a semi-rigid surface film.

Calculation Formulas

A number of calculations and adjustments have been developed for the analysis of human blood rheology. Some of the calculated values were of interest to this investigation and so a small test of validity was carried out on a number of blood samples. Following plasma and blood viscosity measurement at the native haematocrit, the formula of Matrai et al (1987, and overleaf) was used to calculate blood viscosity at a standardised haematocrit of 0.35. The original blood samples were then manually adjusted by removal or addition of plasma to produce samples with an haematocrit of 0.35 ± 0.01. These samples were reanalysed and the blood viscosity measurements compared with the calculated values obtained from Matrai's formula. The plot of calculated viscosity against measured viscosity values is given in figure 2.4.a, and validates the use of human derived formulas for baboon blood analysis.

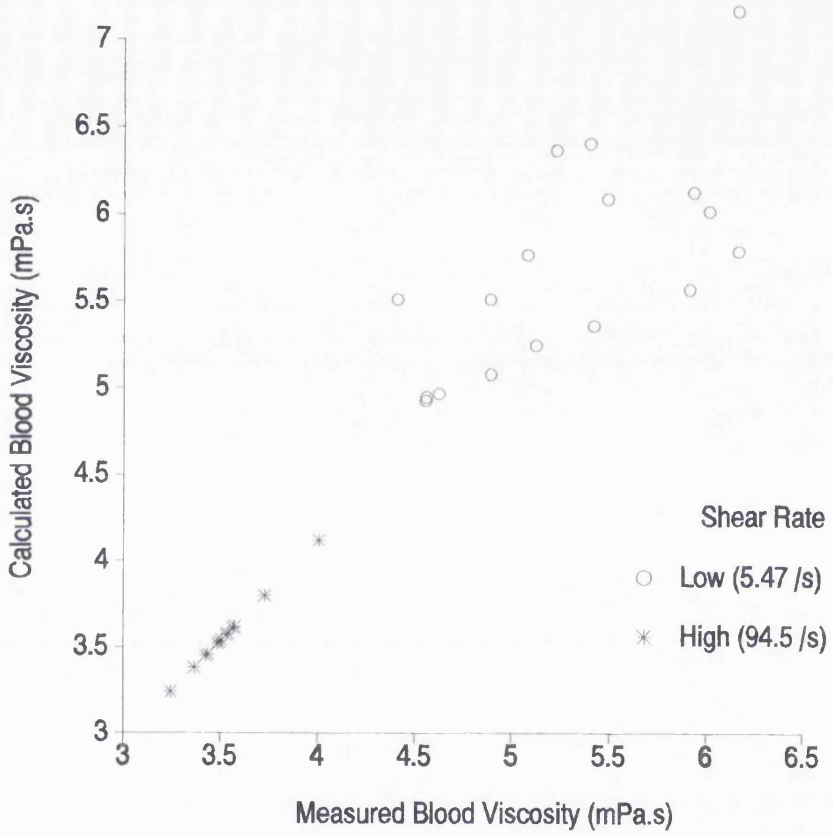


Figure 2.4.a

Correlation plot between measured blood viscosity following manual adjustment of haematocrit and a mathematically calculated blood viscosity using measurements from the control samples. Corr = 0.95, $P<0.001$, n=34.

Calculation formulas used in this thesis.

1) Adjusting blood viscosity for haematocrit:
(Matrai et al, 1987)

$$\frac{\text{Blood Visc (Hct 0.35)}}{\text{Plasma Viscosity}} = \left[\frac{\text{Blood Visc (Native Hct)}}{\text{Plasma Viscosity}} \right]^{0.35/\text{Hct}}$$

2) Relative Low Shear Blood Viscosity (ICSH, 1986)
Used as an estimate of erythrocyte aggregation

$$= \frac{\text{Low Shear Blood Viscosity}}{\text{Plasma Viscosity}}$$

3) Viscometric Aggregation Index (ICSH, 1986)
Used as an index of red cell aggregation

$$= \frac{\text{Low Shear Blood Viscosity}}{\text{High Shear Blood Viscosity}}$$

2.5 STATISTICAL ANALYSIS

Where mean values were calculated, checks were made that the data were normally distributed and the mean values were then expressed along with the calculated standard deviation. Where data were not normally distributed about the mean, attempts were made to transform the data into a normal distribution and in these instances, the error of mean values are given as 95% confidence ranges.

Within-group and between-group statistical analysis were performed using paired and unpaired student t-tests respectively. Statistical differences are expressed in the following format:

*** or +++	:	$P<0.001$
** or ++	:	$P<0.01$
* or +	:	$P<0.05$
n.s.	:	$P>0.05$ or Not Significant

Regression analysis of data was by the least squares method and multiple regression of a number of variables was by the backward stepwise method.

All calculations and statistical work was carried out using a microcomputer based statistical softwear package (Nanostat) following discussion of the methods used with the statistician at the Institute of Neurology and applied in accordance with the teachings of Altman (1991).

CHAPTER 3

RESULTS

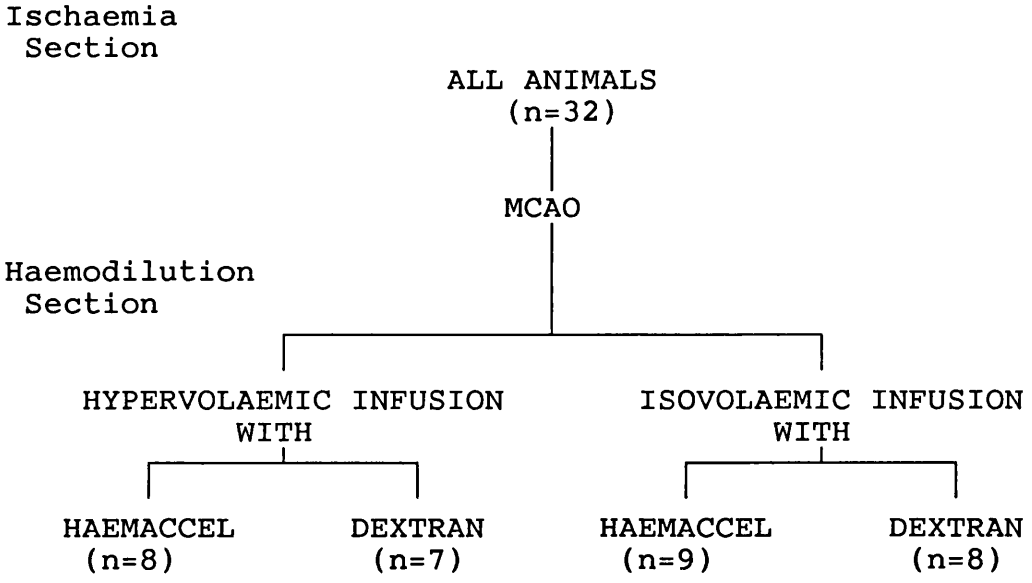
Introduction

As detailed in the Methods section, each experiment comprised of three separate recording stages:

1. The Control Period; prior to MCA occlusion.
2. The Ischaemic Period; after MCAO and prior to haemodilution.
3. The Dilution Period; during ischaemia but following haemodilution.

For clarity, the results have been presented in two distinct sections: Ischaemia and Haemodilution. The Ischaemia section presents the data obtained for all the animals in this investigation up to the point of haemodilution. The Haemodilution section presents data obtained from the sub-groups of animals diluted with one of the four infusion regimes.

A total of 32 animal experiments were successfully completed, sub-divided into the four infusion subgroups as outlined below.



3.1 ISCHAEMIA

3.1.1 Physiological Measurements

Table 3.1.a (below) contains data on the baseline physiological parameters measured throughout the control and post-MCAO phases of the experiments. The only significant changes were seen during induced hypercapnia when the raised arterial PCO_2 was accompanied by significant falls in pH (Pre-MCAO:-0.08 sd:0.04, $P<0.001$; Post-MCAO: -0.10 sd:0.03, $P<0.001$).

Table 3.1.a
The mean (SD) values of five physiological parameters recorded from all 32 animals during the control and post-MCAO periods.

		PARAMETER				
		MSBP (mmHg)	PaCO2 (mmHg)	pH	PaO2 (mmHg)	[GLUC] (µmol)
CONTROL	No CO2	111 (13)	38.7 (1.7)	7.35 (0.05)	504 (79)	5.3 (2.1)
	With CO2	113 (15)	51.1 (3.3)	7.27 (0.04)	490 (83)	--
ISCAEMIA	No CO2	112 (16)	40.0 (1.8)	7.34 (0.03)	504 (69)	5.9 (2.8)
	With CO2	117 (16)	52.7 (3.2)	7.24 (0.04)	501 (78)	--

MSBP : Mean Systemic Blood Pressure.
[GLUC] : Arterial Blood Glucose Concentration.

3.1.2 Haemorheology

Recorded data for a number of haemorheological parameters are presented in Table 3.1.b (below). The mean systemic haematocrit following MCAO was found to have fallen slightly from the mean level seen during the control phase, although this did not reach statistical significance ($P=0.06$, $n=32$). However, this fall was reflected in a small but statistically significant reduction in the mean whole blood viscosity at both high and low shear rates ($P<0.05$, $n=32$). Mathematical correction for haematocrit differences eliminated this difference in mean blood viscosity between pre and post-clip levels. No significant changes were seen in mean plasma viscosity or relative blood viscosity, but the mean viscometric aggregation index decreased significantly ($P<0.05$, $n=32$).

Table 3.1.b
Mean (SD) values for blood and plasma viscosity and haematocrit recorded from systemic blood samples from all 32 animals prior to and following MCAO. The table also includes calculations of: haematocrit corrected blood viscosity, relative blood viscosity, and the viscometric aggregation index (details in section 2.4)

	PRIOR TO MCAO		POST MCAO	
NATIVE HAEMATOCRIT	35.5 (3.4)		34.5 (3.3)	
PLASMA VISCOSITY (mPa.s)	1.18 (0.08)		1.17 (0.07)	
BLOOD VISCOSITY (mPa.s)	Low Shear	High Shear	Low Shear	High Shear
At Native HCT	6.55 (1.69)	3.77 (0.61)	5.92 (1.35)	3.61 (0.50)
At Adjusted HCT of 35%	6.40 (1.43)	3.75 (0.55)	6.03 (1.13)	3.67 (0.44)
RELATIVE LOW SHEAR BLOOD VISCOSITY	5.42 (1.11)		5.16 (0.87)	
VISCOMETRIC AGGREGATION INDEX	1.71 (0.19)		1.62 (0.17)	

3.1.3 Cerebral Blood Flow

Cortical cerebral blood flow was recorded at up to 16 individual electrode sites in each animal. The electrodes were distributed over the brain to record the varying depths of ischaemia that would appear following MCAO. Prior to MCA occlusion, the mean control blood flow was 57.8 ml/100g/min (SD 15.1, n=32). Vascular reactivity testing with raised $PaCO_2$ during the control period increased the mean blood flow by 30% to 74.8 ml/100g/min (SD 13.8). Combining this change in blood flow with the measured venous $PaCO_2$, the averaged vascular reactivity calculated out at a 2.6% increase in CBF per torr CO_2 (0.1-9.0, 95% confidence range).

Following MCAO, cerebral blood flow fell to various levels dependant on the remaining vascular perfusion. In order to analyse these changes, blood flow recordings in each animal were grouped depending on the post-MCAO value, and the presence or absence of vascular reactivity to raised $PaCO_2$.

The criteria for the four groups were:

Group 1: All electrodes placed in the cortex of the contralateral hemisphere to the ischaemic insult.

Group 2: All electrodes in the ischaemic hemisphere recording a blood flow of more than 30 ml/100g/min following MCAO and with preserved CO_2 reactivity.

Group 3: All electrodes in the ischaemic hemisphere recording a blood flow of less than 30 ml/100g/min following MCAO and with preserved CO_2 reactivity.

Group 4: All electrodes in the ischaemic hemisphere that showed no improvement in flow during hypercapnia following MCAO.

In each animal, the blood flow measurements obtained from all the electrodes in each group were averaged to provide a mean blood flow value for each of the 4 groups. The mean post-MCAO blood flows and vascular reactivities of the four electrode groups in all 32 animals are illustrated in figure 3.1.a.

The post MCAO blood flow data is also presented in numerical form in table 3.1.c (below), and includes a more detailed analysis of the residual CO_2 reactivity. All of the mean changes in blood flow, both to MCAO and hypercapnia, were statistically significant ($P<0.001$, $n=32$), except for the mean change in flow to MCAO recorded from the contralateral hemisphere (Group 1 electrodes : $P>0.05$, $n=32$).

Table 3.1.c
Mean cerebral blood flow following MCAO and its subsequent change during hypercapnic challenge.
(Variance expressed as SD or 95% Confidence ranges)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Post MCAO CBF (ml/100g/min)	57.5 (20.1)	48.4 (11.8)	20.6 (4.9)	23.7 (9.6)
Change in CBF to MCAO	+0.6 (10.9)	-13.4 (16.6)	-35.2 (17.4)	-35.4 (16.6)
Change in CBF to raised PaCO2	+17.7 (0.7-57.6)	+15.8 (0.6-51.5)	+2.9 (0.2-8.7	-3.7 (-0.1--13.3)
CBF reactivity to raised PaCO2 % increase/torr	+2.65 (0.16-8.19)	+2.75 (0.07-9.34)	+1.09 (0.03-4.02)	-1.22 (-0.01--4.37)

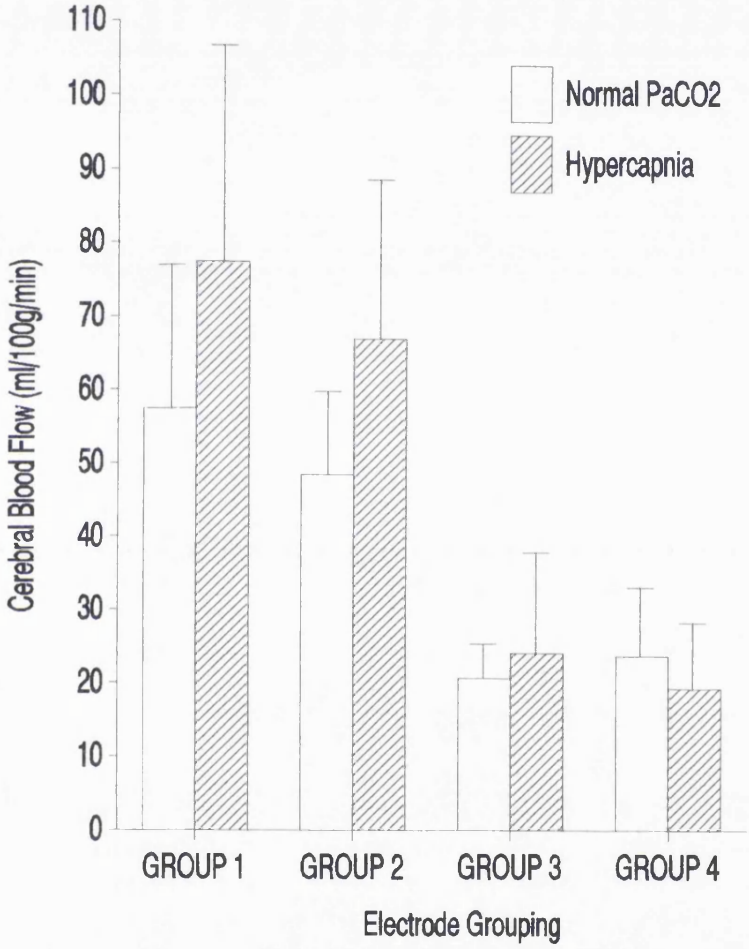


Figure 3.1.a

Mean basal cerebral blood flow in the four electrode groups following MCAO and corresponding mean blood flow levels during hypercapnic challenge. (n=32. Error bars indicate standard deviation. All changes significant at $P<0.001$).

3.1.4 Oxygen Transport

As outlined in the introduction, the calculation of regional oxygen transport to the brain has been used by a number of investigators as an index of benefit from haemodilution therapy. Using blood flow data and the measurements of systemic haematocrit collected during this investigation, regional oxygen transport was calculated from their product. As this calculation did not take account of influencing variables such as haemoglobin saturation, plasma oxygen content or the systemic to capillary haematocrit ratio, the resulting data has been used only as an index of change.

Prior to MCAO, the mean value for the whole brain oxygen transport index was 20.0 (sd: 5.9, n=32). This value is similar to that found in humans (20.4; Gottstein 1987), and other normal baboons (15.6; Tsuda et al 1987). Following MCAO, the graded fall in cerebral blood flow had a similar effect on the oxygen transport. The calculated oxygen transport values for each of the pre-defined blood flow groups are given in the table below.

Table 3.1.d
Changes in mean Oxygen Transport Index to MCAO in the four pre-defined recording areas.

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Post MCAO Oxygen Transport Index	20.3 (7.6)	17.0 (4.6)	7.0 (1.8)	8.1 (3.6)
Change in Oxygen Transport Index From Pre-Clip	+ 0.9 (4.0) P>0.05	- 4.8 (6.3) P<0.01	-11.9 (6.7) P<0.001	-11.8 (6.2) P<0.001

As demonstrated in section 3.1.2, the systemic haematocrit fell slightly with MCAO, but the effect of this on oxygen transport would be similar in all electrode groups. The changes in oxygen transport were therefore due mainly to the reduction in blood flow.

3.1.5 Tissue Oxygen Tension

Cortical oxygen tension was recorded at up to 8 individual sites in each animal. These electrodes were distributed over the brain in similar locations to the blood flow electrodes so that the relationship between blood flow and oxygen tension could be monitored. Mean brain tissue oxygen tension prior to MCA occlusion was 20.0mmHg (sd:7.1, n=32). During vascular reactivity testing through induced hypercapnia, the mean brain PtO_2 fell to 18.7mmHg (sd:6.5, n=32), but this fall was not statistically significant (P=0.2).

Following MCAO, brain tissue PO_2 fell by varying amounts depending upon electrode location. As tissue oxygen tension is a factor of blood flow, oxygen tension measurements were grouped according to the measured local cerebral blood flow. This produced groups similar to those used for the blood flow analysis above, the mean PtO_2 values for these are given in the table below. All electrode groups ipsilateral to the occlusion recorded statistically significant reductions in the mean PtO_2 ($P<0.01$, n=32). The change in mean PtO_2 in the contralateral hemisphere was not significant (P=0.3, n=32). During hypercapnic testing of vascular reactivity, the PtO_2 in all areas of the brain was not significantly affected.

Table 3.1.e
Mean tissue oxygen tension recorded from the four electrode groups following MCAO and their subsequent change during hypercapnic challenge.

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Post MCAO PtO2 (mmHg)	20.8 (9.1)	14.4 (6.3)	3.4 (2.8)	1.9 (2.6)
Change in PtO2 to MCAO (mmHg)	+1.2 (9.2)	-4.6 (6.5)	-17.3 (6.8)	-20.1 (8.8)
Change in PtO2 to raised PaCO2 (mmHg)	-0.5 (7.0)	-1.9 (4.4)	+1.0 (2.6)	+1.1 (1.9)

3.2 HAEMODILUTION

3.2.1 Physiological Measurements

The mean values obtained for measured physiological parameters following haemodilution are presented in table 3.2.a (Appendix B1). Haemodilution had no significant effect on basal systemic pH or $PaCO_2$. However, haemodilution was associated with changes in blood pressure, PaO_2 and blood glucose concentration, all of which are quantified below (Table 3.2.b).

Animals that were hypervolaemically haemodiluted tended to have raised blood pressure, but the mean rise was not statistically significant. However, comparing methods of infusion for a given haemodiluent revealed a significant difference between hypervolaemic and isovolaemic infusion and its effect on systemic blood pressure ($P>0.05$ unpaired t-test, for both Haemaccel and dextran)

Table 3.2.b
Change in mean (sd) blood pressure, PaO_2 and blood [Glucose] following the four infusion regimes.

	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
Blood Pressure (mmHg)	+6.5 (8.4)	-2.3 (6.9)	+5.4 (8.1)	-2.0 (5.8)
Signif.	P=0.06	P=0.34	P=0.12	P=0.36
Systemic PaO2 (mmHg)	-30.5 (61.5)	-35.2 (50.1)	-59.1 (52.3)	+19.5 (31.4)
Signif.	P=0.2	P=0.07	P<0.05	P=0.13
Systemic [Glucose] (µmol)	+1.7 (1.9)	+2.9 (2.6)	+0.6 (0.4)	+3.6 (3.3)
Signif.	P=0.07	P<0.05	P<0.05	P<0.05

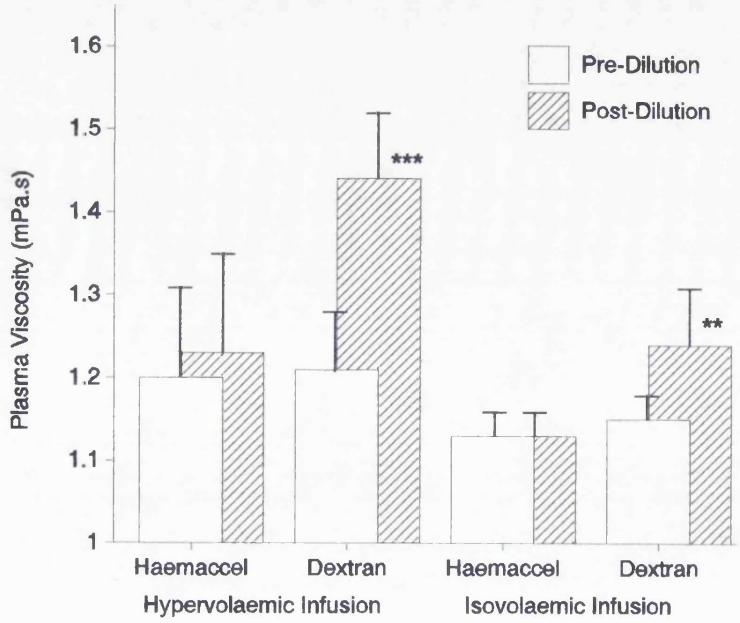
3.2.2 Haemorheology

The mean post-haemodilution values recorded for plasma viscosity, haematocrit and whole blood rheology are given in table 3.2.c (Appendix B2). For ease of reference, the plasma viscosity and haematocrit data are also presented in figure 3.2.a (overleaf).

Mean systemic haematocrit levels following haemodilution under any of the four infusion regimes were statistically similar. Mean systemic plasma viscosity values following haemodilution were varied with the lowest viscosity in animals infused isovolaemically with Haemaccel, and the highest in animals infused hypervolaemically with dextran. In order to assess the effect of the infusate type and the method of haemodilution on the blood rheology, the mean within animal changes of a number of rheological parameters are given in table 3.2.d.

The increase in plasma viscosity following haemodilution with dextran was statistically significant irrespective of the method of infusion. The degree of increase in plasma viscosity was significantly greater with hypervolaemic infusion of dextran than with isovolaemic infusion. There was no change in mean plasma viscosity following haemodilution with Haemaccel, irrespective of the method of infusion.

The reduction in haematocrit was statistically significant following haemodilution with any of the four infusion regimes. With dextran infusion, the fall in mean haematocrit was similar irrespective of the method of infusion. The fall in haematocrit following Haemaccel infusion was not as great as that seen with dextran, especially when infused isovolaemically. The slight difference in the changes in haematocrit were reflected in the changes in low shear blood viscosity but these trends were not statistically significant.



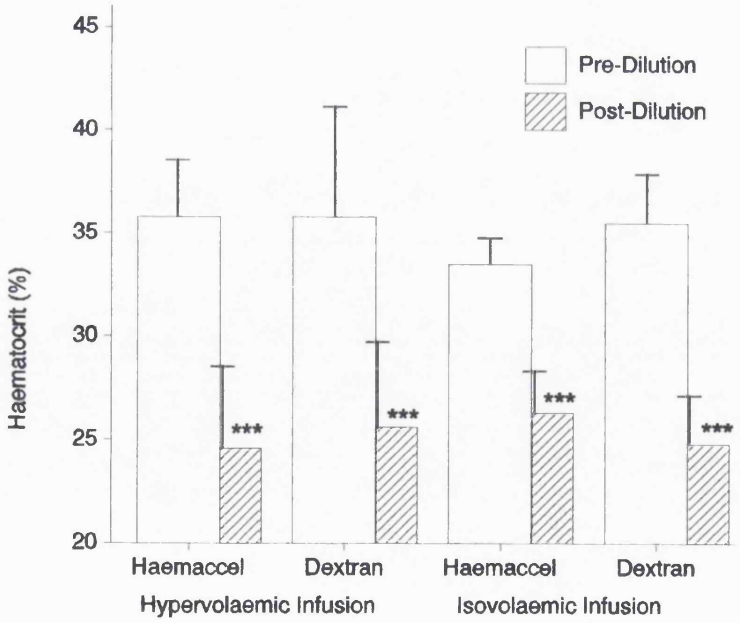


Figure 3.2.a
Mean systemic plasma viscosity and haematocrit prior to and following haemodilution (n=32, Error bars : sd).

Table 3.2.d

Changes in mean (sd) blood rheology changes following haemodilution under the four infusion regimes. All blood viscosity values are for the native haematocrit.

	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
Plasma Viscosity (mPa.s)	+0.03 (0.02) P=0.1	-0.01 (0.04) P=0.7	+0.23 (0.06) P<0.001	+0.09 (0.06) P<0.01
Haemato-crit (%)	-9.0 (4.3) P<0.001	-7.26 (1.58) P<0.001	-10.3 (2.5) P<0.001	-10.7 (2.5) P<0.001
Blood Viscosity Low Shear (mPa.s)	-2.22 (1.49) P<0.01	-1.92 (0.35) P<0.001	-2.74 (1.15) P<0.001	-2.29 (0.98) P<0.01
High Shear (mPa.s)	-0.69 (0.35)	-0.77 (0.16)	-0.79 (0.29)	-0.92 (0.27)
Signif.	P<0.001	P<0.001	P<0.01	P<0.001

In order to test the influence of haemodilution on the intrinsic rheology of the blood, it was necessary to use a number of calculation formulas described in the literature and examined in section 2.4. The changes in blood viscosity parameters derived using these formulas are given in table 3.2.e (overleaf). Using these calculated values, it appears that after adjusting blood viscosity for the fall in haematocrit that occurred on haemodilution, there was still a significant fall in low shear blood viscosity, but only in animals infused with Haemaccel. Calculated high shear blood viscosity at 35% haematocrit was not influenced by haemodilution except in animals hypervolaemically infused with dextran, where a statistically significant increase was recorded.

Table 3.2.e
Changes in calculated blood viscosity parameters following haemodilution, including blood viscosity and relative blood viscosity at a standardised haematocrit of 0.35, and the viscometric aggregation index.

	HAEMACCEL		DEXTRAN	
CHANGE IN:	HYPERVOL.	ISOVOL.	HYPERVOL.	ISOVOL.
VISCOSITY @ HCT of 35% Low Shear (mPa.s)	-0.56 (0.61) P<0.05	-0.72 (0.52) P<0.01	-0.29 (0.49) P=0.2	-0.28 (0.46) P=0.2
High Shear (mPa.s)	+0.32 (0.58) P=0.2	-0.08 (0.18) P=0.2	+0.45 (0.13) P<0.001	+0.17 (0.21) P=0.1
Relative Low Shear Blood Viscosity	-0.56 (0.37) P<0.01	-0.61 (0.39) P<0.01	-1.07 (0.41) P<0.001	-0.55 (0.47) P<0.05
Viscometric Aggregation Index	-0.26 (0.16) P<0.01	-0.26 (0.07) P<0.001	-0.40 (0.13) P<0.001	-0.29 (0.19) P<0.01

Relative viscosity, where the whole blood viscosity was corrected for differences in plasma viscosity, fell significantly following haemodilution with any of the infusion regimes. This fall in relative low shear viscosity indicated a reduction in red cell aggregation, which was confirmed by the significant decreases in the viscometric aggregation index.

3.2.3 Cerebral Blood Flow

The changes in mean cerebral blood flow that occurred following haemodilution with each of the infusion regimes are represented in figures 3.2.b and 3.2.c. The blood flow measurements remained grouped according to the basal post-MCAO flow values and the presence or absence of vascular reactivity to hypercapnia. The mean changes in blood flow and their statistical significance are given below.

Table 3.2.f
Changes in mean (sd) cerebral blood flow at predetermined electrode sites following haemodilution under four separate regimes.

	BLOOD FLOW (ml/100g/min)			
	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
GROUP 1 CONTROL HEMISPHERE	+17.5 (8.1) P<0.001	+ 8.1 (7.4) P<0.01	+15.0 (16.8) P<0.05	+14.8 (14.8) P<0.05
GROUP 2 ISCHAEMIA >30ml/100g /min.	+ 9.1 (6.5) P<0.01	+13.7 (7.0) P<0.001	+14.4 (12.1) P<0.05	+12.8 (9.2) P<0.01
GROUP 3 ISCHAEMIA <30ml/100g /min.	+ 9.3 (1.2) P<0.001	+ 6.8 (7.3) P<0.05	+ 9.6 (6.4) P<0.01	+ 9.5 (5.6) P<0.01
GROUP 4 ISCHAEMIA NON VASO-REACTIVE	+ 9.2 (6.6) P<0.05	+ 5.6 (7.1) P<0.05	+ 9.0 (6.2) P<0.05	+ 6.8 (3.5) P<0.001

Haemodilution with any of the four infusion regimes was associated with significant increases in mean cerebral blood flow irrespective of the depth of ischaemia or the degree of vascular reactivity present prior to dilution. The increases in blood flow recorded in each of the electrode groups were

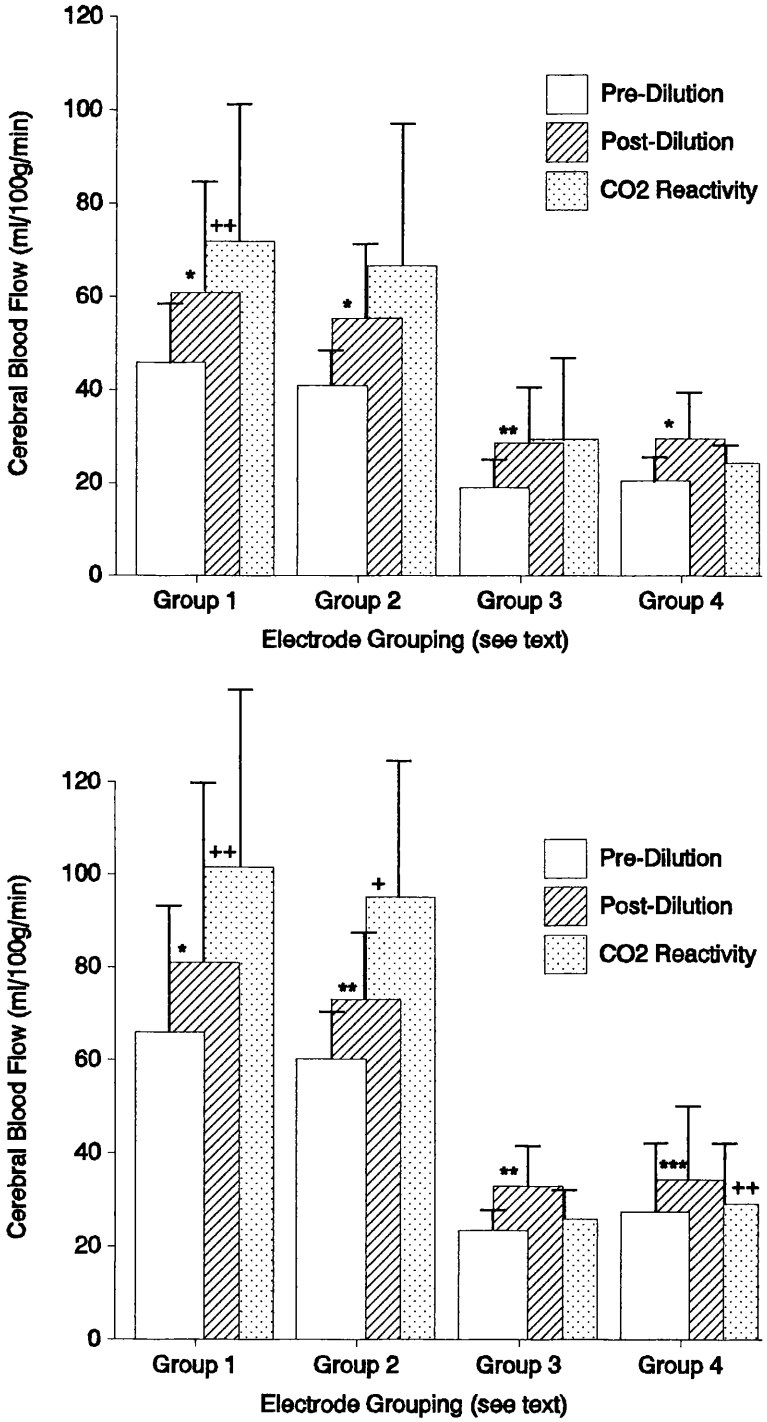


Figure 3.2.b

Mean cerebral blood flow in the four electrode groups prior to and following hypervolaemic (top) and isovolaemic (bottom) haemodilution with Dextran, and during post-dilution hypercapnic challenge.

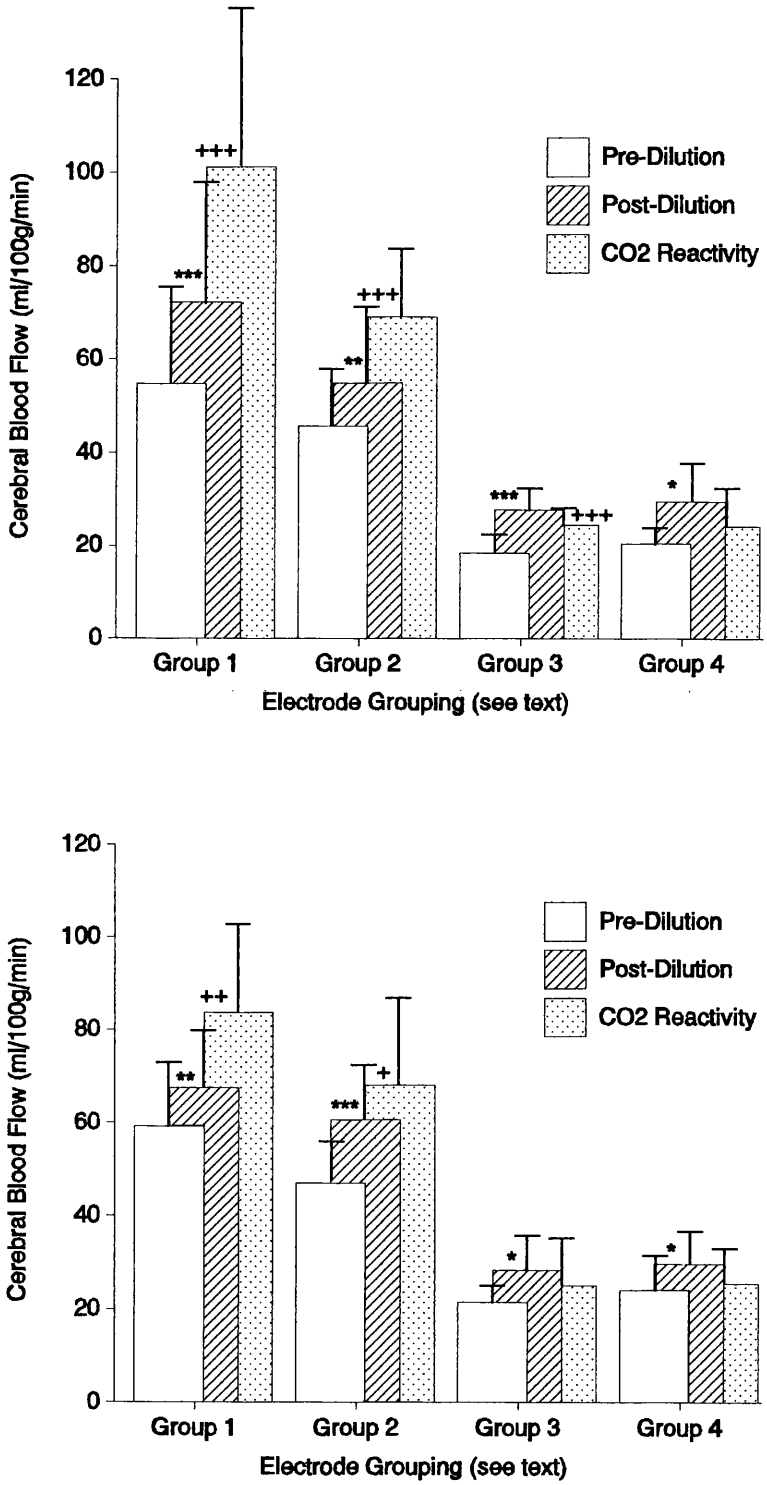


Figure 3.2.c

Mean cerebral blood flow in the four electrode groups prior to and following hypervolaemic (top) and isovolaemic (bottom) haemodilution with Haemaccel, and during post-dilution hypercapnic challenge.

statistically similar irrespective of the infusion regime used, except for the change in flow to the contralateral hemisphere following isovolaemic infusion of Haemaccel. In these animals, the significant rise in flow was lower than that recorded in animals hypervolaemically infused with Haemaccel.

Vascular Reactivity

The degree of residual vascular reactivity present in all areas of the brain following haemodilution has been included in figures 3.2.b and 3.2.c, and table 3.2.g (Appendix B3). Changes in mean cerebral blood flow during hypercapnic challenge have been presented as a percentage per CO_2 torr in the table below. These data have not been sub-divided into the four haemodilution groups because of large variance in the calculated data.

Table 3.2.h
Post haemodilution CO_2 reactivity (mean(sd), n=32) in the four flow groups, and the degree of change in reactivity from pre-dilution levels.

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Post Dilution CO2 Reactivity (% / torr)	2.0 (1.7)	1.8 (1.9)	-1.2 (2.1)	-1.4 (2.0)
Change in CO2 Reactivity with Dilution (% / torr)	-0.8 (2.3) P=0.06	-1.3 (4.4) P=0.12	-2.6 (2.4) P<0.001	-0.1 (1.5) P=0.9

The CO_2 reactivity within the contralateral hemisphere to the ischaemia fell slightly following haemodilution, a reduction that was almost statistically significant. Within the ischaemic hemisphere, a dramatic reduction in the vascular reactivity was seen in areas with a pre dilution blood flow of <30ml/100g/min. The positive increase in flow seen prior to haemodilution was lost completely.

3.2.4 Oxygen Transport

Values for calculated Oxygen Transport Index are presented in table 3.2.j (Appendix B4). The changes in Oxygen Transport following haemodilution with each of the four infusion regimes are presented below.

Table 3.2.k
Changes in the mean (sd) Oxygen Transport Index at predetermined electrode sites following haemodilution under four separate regimes.

	OXYGEN TRANSPORT INDEX			
	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
GROUP 1 CONTROL HEMISPHERE	+ 0.6 (2.4) P=0.49	- 2.0 (2.7) P=0.07	- 1.3 (3.6) P=0.38	- 2.8 (3.3) P=0.07
GROUP 2 ISCHAEMIA >30ml/100g /min.	- 0.9 (2.0) P=0.30	+ 0.4 (2.8) P=0.69	- 0.8 (2.4) P=0.38	- 3.3 (0.7) P<0.001
GROUP 3 ISCHAEMIA <30ml/100g /min.	+ 1.2 (1.0) P<0.05	+ 0.8 (1.2) P=0.09	+ 0.3 (0.5) P=0.15	- 0.4 (1.1) P=0.51
GROUP 4 ISCHAEMIA NON VASO-REACTIVE	+ 0.8 (1.5) P=0.28	+ 0.3 (1.3) P=0.59	0.0 (0.5) P=0.85	- 0.9 (1.5) P=0.19

Oxygen transport remained predominantly unaffected by haemodilution, but fell in a minority of areas under certain infusion regimes. Oxygen transport to the contralateral hemisphere fell considerably following isovolaemic infusion with either infusate, but this was not quite statistically significant. Isovolaemic infusion of dextran was associated with a significant fall in oxygen transport to high flow regions of the ipsilateral hemisphere. Oxygen transport

index was improved to ischaemic regions with some preserved vascular reactivity following hypervolaemic infusion of Haemaccel.

3.2.5 Tissue Oxygen Tension

Post haemodilution mean tissue oxygen tension values are given in table 3.2.1 (Appendix B4). The changes in mean tissue oxygen tension with the four haemodilution regimes are presented below.

Table 3.2.m
Changes in the mean (sd) tissue oxygen tension at predetermined electrode sites following haemodilution under four separate regimes.

	OXYGEN TENSION (mmHg)			
	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
GROUP 1 CONTROL HEMISPHERE	- 1.3 (2.0) P=0.13	- 5.4 (4.8) P<0.001	- 3.4 (3.2) P<0.05	- 5.4 (3.7) P<0.001
GROUP 2 ISCHAEMIA >30ml/100g /min.	- 1.3 (1.5) P=0.06	- 1.9 (1.0) P<0.001	- 3.6 (2.6) P<0.05	- 2.1 (1.4) P<0.01
GROUP 3 ISCHAEMIA <30ml/100g /min.	- 0.1 (0.7) P=0.86	- 0.8 (0.8) P<0.01	- 1.5 (1.2) P<0.05	- 0.2 (0.7) P=0.66
GROUP 4 ISCHAEMIA NON VASO-REACTIVE	+ 0.7 (2.6) P=0.6	- 1.1 (1.1) P<0.05	- 1.3 (0.9) P=0.13	- 0.8 (0.7) P=0.08

Haemodilution with any of the four infusion regimes had either no significant effect on, or was associated with significant reductions in, the mean brain PtO_2.

CHAPTER 4

DISCUSSION

Introduction

The following discussion has been presented in separate sections to help guide the reader towards the conclusions drawn at the end of this thesis. The effect that haemodilution had on the rheological parameters is discussed first, to give an insight into the different effects of the four infusion regimes and to discuss "the changes in plasma viscosity during haemodilution therapy of cerebral ischaemia". The changes in cerebral blood flow recorded during haemodilution are then discussed in relation to the different infusion regimes and the findings of others. The final sections of this discussion relate haemodilution induced changes in haematocrit, plasma and blood viscosity to the changes in: blood flow, vascular dilatation, oxygen delivery and tissue oxygen tension. These sections therefore aim to provide an insight into how these haemorheological changes may be beneficial or harmful in the use of haemodilution therapy in the treatment of cerebral ischaemia.

4.1 HAEMORHEOLOGY

Haematocrit

The haematocrit in normal baboons has been previously recorded as approximately 40 % (Harris et al, 1987; Tsuda et al, 1987a and b), which is slightly higher than found in this study. However, in both these previous publications, no indication was given of the method of haematocrit determination and so because of variations in values obtained by different methods of haematocrit determination (Dacie and Lewis, 1984) and the likelihood that haematocrit in baboons is age related, as in humans, it is difficult to make any direct comparison between the values obtained here and those of other studies. A similar argument can be used for comparison of baseline plasma viscosity data with other publications (Tsuda et al, 1987a and b), where the measurement temperature was not given.

Direct comparison of data obtained in one series of experiments with those of another research group can usually offer little more than an estimate of reliability and confidence in results. Invariably, the experimental conditions and measurement techniques in one investigation are very different to those in another and statistically, sample numbers are usually too small to make any reliable comparisons. The main objectives of this thesis were, however, to determine the within-animal changes in recorded parameters during focal ischaemia and more importantly with haemodilution. These observed changes could then be compared more reliably with changes seen in other studies.

One notable within-animal change with induced focal ischaemia was the small reduction in mean haematocrit and subsequent fall in whole blood viscosity and calculated red cell aggregation. The fall in the red cell count could have been produced by the removal of blood required for blood gas

analysis, haematocrit and rheology determination, blood glucose monitoring, and by the additional fluid infusions used during catheter flushing, and in the replacement of sample volumes. Even though sample volumes were kept to a minimum, it is likely that there were falls in erythrocyte count which, especially in small animals, would have produced this small reduction in the haematocrit value.

As was expected, all four haemodilution regimes induced significant reductions in the mean systemic haematocrit. However, even with the adjustments in the infusion volumes to take account of differences in oncotic pressure, dextran still induced a greater fall in haematocrit than Haemaccel. The greater effect of dextran on the extent of haemodilution was also reported by Tsuda et al (1987a and b) where baboon haematocrit was reduced to a greater degree than following similar infusions of hetastarch. It was possible that the different effect of dextran and Haemaccel on systemic haematocrit was due to differences in excretion rates of the two infusates. Although the mean excretion half-lives are similar for both infusates (3 hours dextran, 4-6 hours Haemaccel), Haemaccel comprises two different size molecules. 30% of the polygeline molecules are of a smaller molecular weight which have an elimination half time of 14-22 minutes. With a post-dilution recording period of just over an hour, a considerable amount of the smaller weight fraction will have been excreted along with associated electrolyte. Such an effect would inevitably reduce the volume of Haemaccel and therefore the degree of haemodilution.

The implications of these findings are small as any long term dilution in man would still be relatively stable due to the longer half life of the larger molecular weight fraction. However, during isovolaemic haemodilution with Haemaccel, it may be advisable to infuse slightly more fluid than the volume of blood withdrawn if slight hypovolaemia is to be avoided.

The induced haemodilution in these studies attained an average haematocrit of approximately 25%. This was lower than the 30% level reported by others (eg Tu et al, 1988a; Tsuda et al, 1987a and b; Sakaki et al, 1991), but the change in haematocrit was similar at approximately 10%.

Influence of haematocrit on blood viscosity

To establish the influence of the haemodilution induced reductions in haematocrit on blood viscosity, all the *in vitro* blood sample data were pooled to provide the correlation in figure 4.1.a; A and B. The regression between haematocrit and blood viscosity was significantly linear (not shown) but, as demonstrated by others (Matrai et al, 1987; Nicolaides et al, 1977), the relationship was better between the logarithm of viscosity and the haematocrit. The logarithmic relationship also fitted an extrapolation to zero haematocrit, giving a mean plasma viscosity of between 1 and 1.5mPa.s.

Plasma viscosity

Hypervolaemic infusion of dextran for haemodilution was associated with a significant rise in mean plasma viscosity. The 20% increase was smaller than the 34% found in patients after 10 days of hypervolaemic dextran infusion (Kroemer et al, 1987; Haass et al, 1986). In these patient studies, no plasma viscosity measurements were made immediately after the first infusion to allow accurate comparison with this study, but the differences could be explained by the lower blood dextran concentration seen on the first day (12mg/ml) compared to that recorded after ten days of infusion (18mg/ml). Kroemer et al (1987) suggested that the high plasma concentration after ten days was due to circulatory accumulation of the large molecular weight dextran molecules and so a lower plasma viscosity would be expected

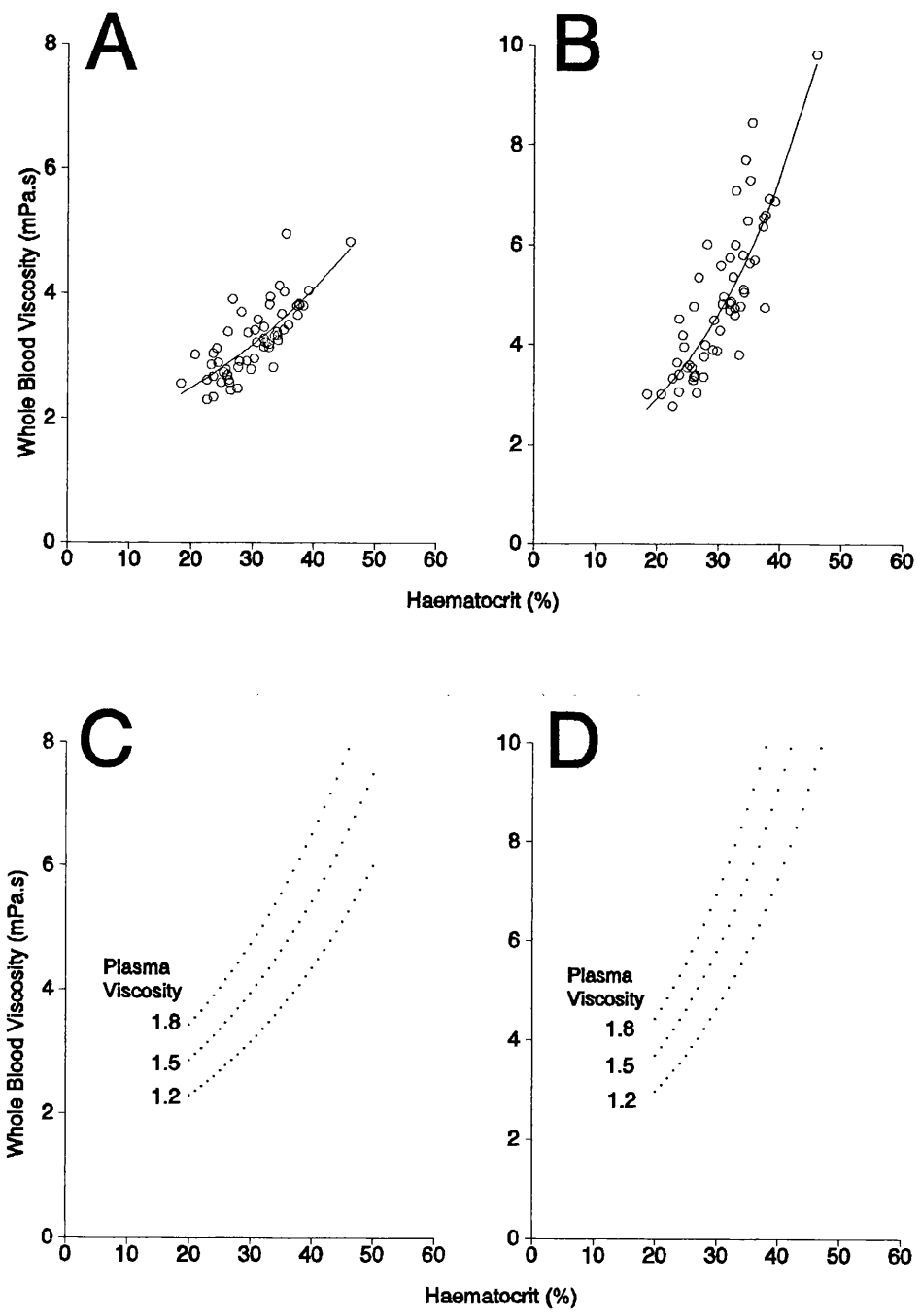


Figure 4.1.a.
A & B : Pre and post-dilutional systemic haematocrits and corresponding Blood Viscosity measurements from all animals (n=32) with superimposed calculated regression curves:

A) Log High Shear BV = 0.011 (HCT) + 0.178 Corr = -0.78

B) Log Low Shear BV = 0.020 (HCT) + 0.066 Corr = -0.84

C & D : Influence of plasma viscosity on the correlation between haematocrit and blood viscosity at high (C) and low (D) shear rates (see text).

immediately after a single infusion. This was demonstrated in similar studies where plasma viscosity in patients increased by 12%, five hours after a single 500ml hypervolaemic infusion of LMWD (Költringer et al, 1988), and by 15% immediately following a 120ml infusion over 60mins in baboons (Tsuda et al, 1987a and b). Our own studies (Farman et al, 1991b; Appendix A) found an 18% increase in plasma viscosity after three days of daily 500ml infusions of LMWD.

These data indicate that hypervolaemic infusion of dextran will induce a significant increase in plasma viscosity immediately following infusion. This increase can be sustained for up to three days following a single infusion (Költringer et al, 1988), and even exaggerated if further daily infusions are given (Farman et al, 1991b; Kroemer et al, 1987).

A smaller, but still significant, increase in plasma viscosity was seen with isovolaemic infusion of dextran. There have been no known studies on the changes in plasma viscosity following isovolaemic infusion of LMWD with which these data can be compared. In keeping with the findings of Kroemer et al (1987), the smaller rise in plasma viscosity could be explained by the fact that less dextran was infused during isovolaemic haemodilution. However, because infusion was accompanied by exsanguination, the calculated dextran to blood volume ratio in hyper and iso-volaemically infused animals was similar. This assumes that extravascular fluid was drawn into the vasculature to equilibrate the high intravascular oncotic pressure induced by dextran infusion. As more dextran was used in hypervolaemic haemodilution than in isovolaemic haemodilution, the extra tissue dehydration required, and presumably longer equilibration time, may have influenced the size and dynamics of the circulatory dextran pool, and could explain the greater plasma viscosity seen in animals hypervolaemically infused.

Hypervolaemic or isovolaemic infusions of Haemaccel were not associated with any significant changes in plasma viscosity. As a semi-synthetic plasma substitute, with a viscosity and oncotic pressure similar to plasma, no change in plasma viscosity was expected. No previous study on the use of Haemaccel for haemodilution has provided data on plasma viscosity, except for preliminary presentation of these results (Farman et al, 1990; 1991a and b). The use of Haemaccel as a haemodiluent, infused either hyper or isovolaemically, maintained a more normal plasma viscosity than infusions of dextran 40 under the same conditions. However, as discussed in the introduction, the effect that plasma viscosity has on the in vivo blood viscosity, subsequent blood flow and the possible tissue survival is of prime importance.

Influence of plasma viscosity on blood viscosity

This thesis has measured a number of rheological parameters in order to assess how the differences in plasma viscosity with the two infusates affect the haemorheology. Haematocrit being the single most determining factor of blood viscosity, it was inevitable that there would be significant reductions in blood viscosity following haemodilution with any infusate. With the recorded differences in plasma viscosity being small relative to the whole blood viscosity changes, it was therefore difficult to see any direct effect that changes in plasma viscosity might have on the viscosity of whole blood. However, multiple regression analysis of the pooled blood viscosity data, revealed both haematocrit and plasma viscosity as significant variables in determining whole blood viscosity ($P<0.001$).

As described by Matrai et al (1987), it was possible to derive logarithmic equations for the influence of plasma viscosity on the haematocrit dependant blood viscosity. In doing so, it was assumed that blood viscosity at zero

haematocrit was equal to the plasma viscosity.

Thus:
At low shear rates

$$\text{Log BV} = \text{Log PV} + 0.0195.(\text{HCT})$$

At High Shear Rates

$$\text{Log BV} = \text{Log PV} + 0.0141.(\text{HCT})$$

The haematocrit/plasma viscosity influence on blood viscosity is also presented graphically in figure 4.1.a; C and D. As reported previously (Lowe and Barbenel, 1988; Rand et al, 1970) at any given haematocrit and shear rate, the greater the plasma viscosity the higher the blood viscosity. The figures also demonstrate that plasma viscosity has an increasing effect on blood viscosity as haematocrit increases (Mayer, 1966).

Erythrocyte aggregation

The low shear viscosity of whole blood at standardised haematocrit was significantly reduced following Haemaccel haemodilution. This effect was also present following calculation of the relative low shear blood viscosity. The latter calculation eliminated the effect of plasma viscosity and therefore the reduced blood viscosity in Haemaccel infused animals must have been due to either reduced red cell aggregation or increases in red cell deformability.

A significant fall in red cell aggregation as measured by the viscometric aggregation index and relative low shear blood viscosity was also seen following haemodilution with dextran. The disaggregation properties of low molecular weight dextran have been well documented (Chein and Jan, 1973), and this investigation concurs with these findings. However, the probable disaggregation was not mirrored by an expected fall in standardised low shear blood viscosity

implying that the raised plasma viscosity seen following dextran infusion compromised any possible reduction in blood viscosity. This is in line with the observations of Gustafsson et al (1981) and Chen et al (1989) who showed that blood viscosity was more dependant upon plasma viscosity than on erythrocyte aggregation.

Early reports found that in vitro mixing of blood and Haemaccel increased red cell aggregation (Ehrly, 1969; Thorsen and Hint, 1950). However, later investigations using in vivo infusions of Haemaccel found no significant effect on aggregation rate (Harke et al, 1976). There have been no detailed investigations on the effect of Haemaccel on aggregation during haemodilution, but the results presented in this thesis suggest that red cell aggregation is reduced during both iso and hypervolaemic infusion of Haemaccel and that the magnitude of disaggregation is similar to that found with similar infusions of dextran.

In summary, these results demonstrate significant differences in the effect of the four infusion regimes on the in vitro haemorheology. Whether these differences are of importance is dependant on their effects in vivo. Of prime importance is the influence on local cerebral blood flow in the ischaemic penumbra.

4.2 CEREBRAL BLOOD FLOW

4.2.1 Prior to Haemodilution

The mean cerebral blood flow recorded in the baboon cortex (57.8ml/100g/min) was similar to that found by Harris et al (58ml/100g/min, 1987) using the same hydrogen clearance method. Prior to MCAO, the reactivity of the vascular bed to increases in tissue PCO_2 was found to be intact and of similar value to that found in another primate study (Jakubowski et al, 1982) and in cats (von Kummer, 1984).

Following MCAO, the reduction in blood flow in the ischaemic hemisphere was dependant on the degree of preserved collateral flow (Symon et al, 1974). As would be expected, the blood flow in the contralateral hemisphere remained similar to that prior to the occlusion due to an intact blood supply. The ischaemic hemisphere blood flow varied according to the degree of collateral supply from the anterior cerebral and posterior cerebral arteries. The topographical determination of blood flow in this model has been dealt with in earlier studies (Symon et al, 1974), although more recent studies have further outlined the heterogeneous nature of focal cerebral ischaemia (Obrenovitch et al, 1988) and the effect heterogeneity has on blood flow measurements using the hydrogen clearance method (von Kummer and Herold, 1986b). Therefore, in order to reduce the effects of ischaemic heterogeneity on blood flow measurements, recording electrodes were grouped and averaged according to their measurement values and not their location.

Cerebral reactivity to raised $PaCO_2$ was reduced at a number of recording sites in the ischaemic hemisphere, with the most ischaemic regions tending to have the poorest vascular reactivity. In some regions cerebral steal was recorded during hypercapnia, and in these regions mean cerebral blood

flow was less than approximately 40% of control levels. This figure concurred with more detailed studies on cerebral vascular reactivity (Symon et al, 1974). However, we also found that vascular reactivity could be preserved at such low blood flow levels and therefore the incidence of cerebral steal does not necessarily occur at a particular flow 'threshold'. Such a phenomenon is characteristic of the dynamically changing infarct seen early in the evolution of the ischaemic zone (Symon et al, 1975). These findings demonstrate the need to check for the presence or absence of vascular regulation as it can not be assumed that vasoreactivity has been lost at a particular level of blood flow.

Near normal vascular reactivity was observed in areas of the brain where blood flow was above 50% of the basal level. This confirms that the brain was not traumatised by flow down to such a low level otherwise metabolic (Kuschinsky, 1987), and possibly neurogenic disturbances (Edvinsson, 1987), would have induced significant changes in vessel diameter. Indirect evidence for this observation has been demonstrated by the maintenance of the SEP (Branston et al, 1984), K^+ clearance rates (Branston et al, 1977) and extracellular pH (Harris and Symon, 1984), at cerebral blood flows as low as 30-35 ml/100g/min.

4.2.2 Haemodilution

The increases in blood flow to all areas of the ischaemic brain following haemodilution were statistically significant irrespective of the infusion regime used.

Hypervolaemic haemodilution with dextran induced a 45-50% increase in blood flow to the most ischaemic areas. This was in agreement with studies by Wood (1983, 1984b) where hypervolaemic infusion of dextran reduced haematocrit by 14 points and increased blood flow by 42% in the MCAO territory

of focally ischaemic dogs. Coyer et al (1987a) found no significant increase in flow to the ischaemic areas of cat brain following hypervolaemic haemodilution with dextran. Data presentation was limited in the reporting of this latter study but, by measuring values from the graphs, haemodilution appears to have increased blood flow by 46% in the ischaemic hemisphere from 13.3 to 19.5 ml/100g/min. The lack of statistical significance in this change was possibly due to the authors method of data analysis demonstrated in a similar previous study (Coyer et al, 1986). Blood flow measurements below 5ml/100g/min were assigned a value of 0ml/100g/min which gave 5 out of the 10 animals in that study a post occlusion flow of 0ml/100g/min making any statistical analysis open to question. The measurement of such low flows with hydrogen clearance is also highly erroneous in itself (von Kummer and Herold, 1986b).

Wood et al (1982a) did not find an increase in flow with hypervolaemic dextran haemodilution of non-ischaemic dogs; and in normal baboon brain, Tsuda et al (1987a and b) failed to demonstrate any increase in blood flow following an 11 point reduction in haematocrit. In the normal brain regions of our animals, however, blood flow increased by over 30%, which is similar to the significant 27% rise in flow to the non-ischaemic hemisphere in another study (Coyer et al, 1987a). It could be argued therefore, that blood flow to normal brain regions will only increase following hypervolaemic haemodilution with dextran if ischaemia is present in another region of the brain. It is impossible to say how this might occur, but vasodilation in one region of the brain may induce a centralised mechanism for vasodilation further back in the vascular tree or at least prevent vasoconstriction in response to the reduced blood oxygen content. Such a mechanism could be a secondary neurogenic or metabolic response to the ischaemic insult. The variation in findings between studies in normal brain and true ischaemic stroke models emphasis the need for studies on the use of haemodilution therapy for treatment of

stroke to be performed on whole brain models of stroke and not by drawing conclusions from changes in normal brain or from studies on perfused isolated brain or hypotension induced models of focal ischaemia.

Isovolaemic haemodilution with dextran was also associated with increases in blood flow to both ischaemic and non-ischaemic tissue. The increases were of a similar size to those induced by hypervolaemic infusion of dextran, but as a percentage change the increases were less, due to slightly higher pre-dilution flows within each electrode group. Increases in blood flow through the ischaemic grey matter of dogs following isovolaemic infusion of dextran were also demonstrated by Tu et al (1988b), although the 22% increase in flow was not statistically significant and the experimental ischaemia was very mild: mean pre-dilution blood flows through the grey matter of the MCA area were at least 30ml/100g/min and at over 65% of the control level. Isovolaemic haemodilution of focally ischaemic cats using dextran also produced an improvement in cerebral blood flow when compared to a non haemodiluted group (Yamashita et al, 1989), but the within animal changes in blood flow in this latter study were not analysed.

In normal cats, a 10 point fall in haematocrit was associated with a 14ml/100g/min rise in mean cerebral blood flow (von Kummer et al, 1988; Scharf et al, 1989), which was similar to the 15ml/100g/min increase in flow to the non-ischaemic hemisphere for an 11 point fall in haematocrit reported here. However, vonKummer et al (1988) could not demonstrate an increase in flow to regions of the brain devoid of compensatory vasodilatation induced by hypotension. However, no measure of vascular reactivity to CO_2 change was performed during this study and it is therefore difficult to say whether their induced hypotension mimicked the ischaemic brain especially when the hypotensive mean blood pressure was at least 90mmHg in some animals and blood flow was no lower than at normotension. The authors

concluded that "haemodilution cannot improve CBF in regions of severe ischaemia". This thesis has however demonstrated significant increases in blood flow to regions of the brain with no vascular reactivity to hypercapnia and therefore reiterates the necessity for determining effectively the presence or absence of vascular reactivity during haemodilution studies.

There have been no known previous studies on the effect of either hypervolaemic or isovolaemic haemodilution with Haemaccel on cerebral blood flow. One of the aims of this thesis was to determine whether Haemaccel could be used as a haemodiluent instead of dextran. The data shows that hypervolaemic infusion of either Haemaccel or dextran induced statistically similar increases in cerebral blood flow to all areas of ischaemic and non-ischaemic brain. However, isovolaemic infusion of Haemaccel produced less of an increase in blood flow compared with isovolaemic infusions of dextran, especially in the contralateral hemisphere. This could be due to the smaller reduction in haematocrit produced by isovolaemic infusion of Haemaccel the reasons for which are discussed fully at the beginning of this chapter. It appears therefore, that following some further adjustment for the differences in oncotic pressure, hyper and isovolaemic infusion of Haemaccel could offer a similar effect on cerebral blood flow to infusions of dextran.

Isovolaemic or hypervolaemic haemodilution with either Haemaccel or dextran improves cerebral blood flow to both ischaemic and non-ischaemic regions of the focally ischaemic brain. Although the increase in flow was possibly smaller in animals isovolaemically infused, the difference was not statistically significant. The similarity in the effect of iso and hypervolaemic infusion indicates that the significant difference in mean blood pressure recorded between these groups of animals had little effect on the changes in blood flow. The difference in MABP was small at

approximately 7mmHg and was probably due to the induced difference in blood volume and therefore the probable higher cardiac output in hypervolaemically infused animals. Much debate has centred on the effect of volume on the blood flow to ischaemic regions during haemodilution therapy. A number of studies have demonstrated a rise in cardiac output following hypervolaemic haemodilution, and it seems likely that this is caused by volume induced increases in right ventricular pressure (Grotta et al, 1985; Tu et al, 1987; Wood et al, 1984a) rather than the reduction in blood viscosity (Fan et al, 1980). Although raised cardiac output may assist in haemodilution induced increases in cerebral blood flow and be of benefit to vasospasm induced ischaemia, the increased demand on the cardiovascular system and the possibility of increased intracranial pressure may be contraindicated in a number of stroke patients. However, this thesis demonstrates that isovolaemic haemodilution is as effective as hypervolaemic infusion at increasing cerebral blood flow to ischaemic regions. In focally ischaemic dogs, Tu et al (1987) also found that "increases in CBF can be achieved with isovolaemic haemodilution without increasing CO" and without exaggerating any ischaemia induced increases in intracranial pressure. Although recent clinical trials using isovolaemic haemodilution have not been successful in improving clinical outcome (SSSG, 1987), these findings suggest that factors other than blood volume are involved in increasing cerebral blood flow and that isovolaemic infusion is still a viable method of haemodilution therapy.

4.2.3 Haemodilution and Vasoreactivity

As detailed above, haemodilution increased blood flow to all areas of the ischaemic brain irrespective of the degree of vasoreactivity present. Despite these increases in flow, areas of brain devoid of any vasodilatory capacity prior to dilution remained maximally vasodilated and a period of

hypercapnia induced a certain degree of intracerebral steal. However, this phenomenon was also observed at the group 3 electrodes. Prior to haemodilution these areas of the brain had basal flows of less than 30ml/100g/min, but possessed at least some preserved vasodilatory capacity. The loss of the vasodilatory reserve following haemodilution could either be due to vessel dilatation caused by haemodilution induced reductions in oxygen availability, or an increase in the degree of intracerebral steal during the post dilution hypercapnic challenge.

The former theory would be in line with Brown et al (1985b) who showed that the blood flow in normal vasoreactive brain is dependant upon the oxygen content of the blood. The reduction in oxygen content through haemodilution may therefore have induced vasodilatation in areas of the brain with preserved vasoreactivity. In group 3 areas of the brain where vascular reactivity was already significantly compromised by the ischaemic insult, cerebral vessels may have become maximally dilated. No further dilatation could have occurred during hypercapnia and therefore blood flow would not have increased. During hypercapnia, the increase in blood flow through the control hemisphere and the normal regions of the ischaemic hemisphere would inevitably induce some cerebral steal (Symon et al, 1974) and therefore reduce blood flow in the areas of the brain with no vasodilatory reserve (Group 3 and 4).

The alternative to this argument is that no vasodilation occurred following haemodilution and that the reduction in viscosity induced the increases in blood flow in all areas of the brain as seen in the group 4 electrodes. During hypercapnia, vessel dilatation would have been of the same degree as during hypercapnia prior to dilution. The increase in flow in the control hemisphere was therefore greater than prior to dilution due to the reduced viscosity even though the %flow increase per torr CO_2 was the same. This overall higher level of flow could have induced a greater

intracerebral shunt of blood to the contralateral hemisphere and therefore deprive more areas in the ischaemic hemisphere, hence the enhanced steal observed in group 3 electrodes.

It is impossible to determine from these data whether one, both or neither of these explanations is correct at present, but it is clear that increases in $PaCO_2$ during haemodilution therapy of cerebral ischaemia should be strongly avoided.

4.3 CEREBRAL BLOOD FLOW / HAEMATOCRIT RELATIONSHIP

Negative correlations between systemic haematocrit and cerebral blood flow have been demonstrated in non-ischaemic brain (von Kummer et al, 1988; Maruyama et al, 1985) ischaemic brain (Wood et al, 1982b; Cavestri et al, 1986; Kusunoki et al, 1981) and patients with high basal haematocrits (Thomas et al, 1977a; Humphrey et al, 1979). A similar correlation study was carried out on our data, pooling the pre and post haemodilution haematocrits of all 32 animals and plotting them against the mean cerebral blood flow from each of the four electrode groupings (Figure 4.3.a). There were significant negative correlations between haematocrit and blood flow in ischaemic and non-ischaemic tissue, even in areas without vascular reactivity to hypercapnia. Regression analysis of the relationships indicated that lower haematocrits were significantly associated with higher blood flows, and the slope of the regression line was shallower in most ischaemic regions. In normal cats von Kummer et al (1988) demonstrated a steeper relationship between haematocrit and cerebral blood flow :

$$CBF = -1.16 \cdot HCT + 86.0$$

(Transposed from : $HCT = -85.8 \cdot CBF + 73.8$)

than found in the focally ischaemic brains of 12 separate cats (Yamashita et al, 1989):

$$CBF = -0.88 \cdot HCT + 39.1$$

These data suggest that in ischaemic brain, a reduction in haematocrit has less of an effect on blood flow than in normal brain. This may be so, but as blood flow in ischaemic brain is already at a very low level, even these small changes in flow can be a significant percentage of the residual flow.

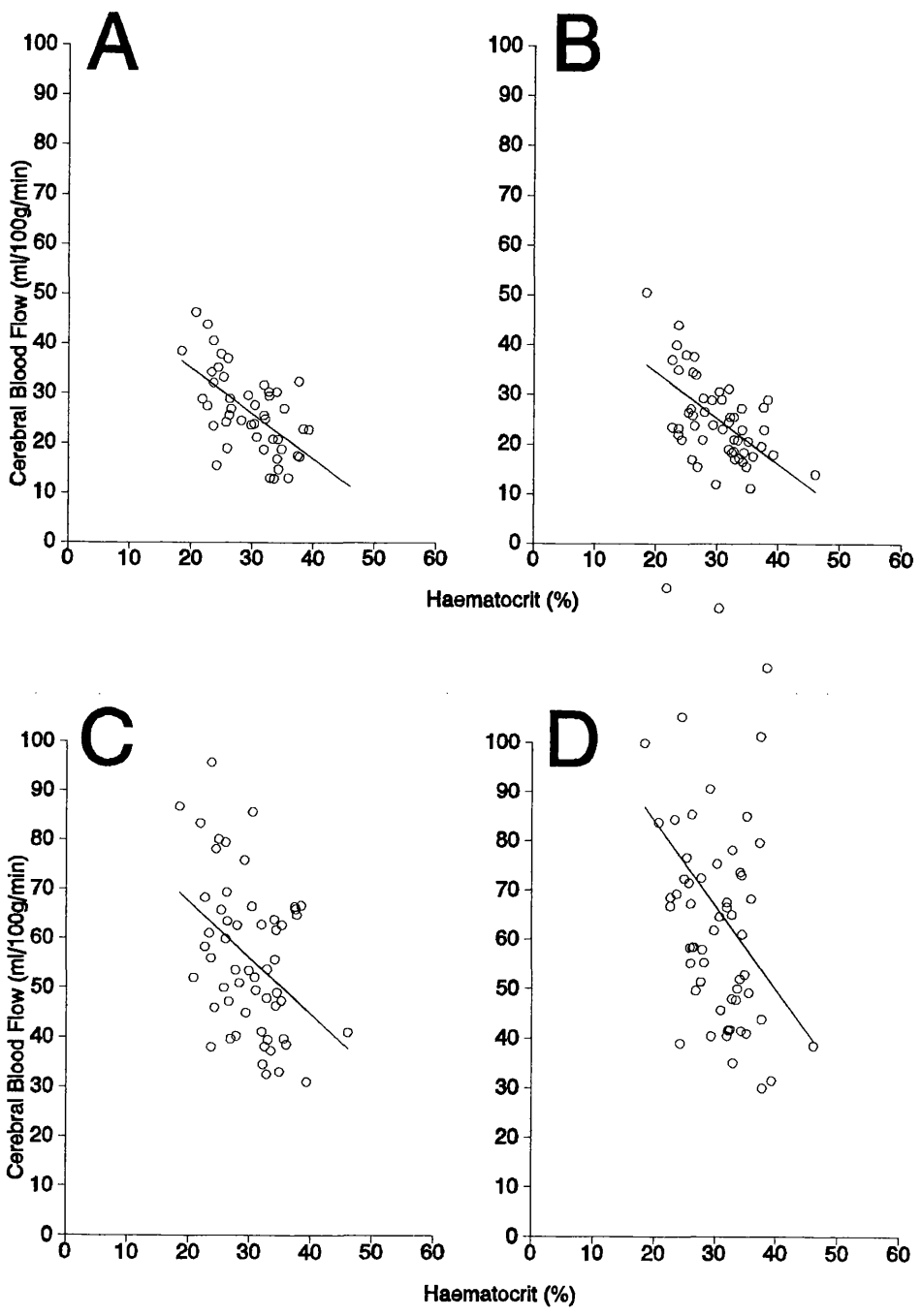


Figure 4.3.a
Pre and post-dilutional systemic haematocrit and corresponding cerebral blood flow in all animals (n=32) where pre dilutional blood flow was :

A. Non-vasoreactive
CBF = -0.92 . HCT + 53.4 Corr = -0.60 P<0.001
B. Vasoreactive but <30ml/100g/min
CBF = -0.93 . HCT + 53.0 Corr = -0.60 P<0.001
C. Vasoreactive but >30ml/100g/min
CBF = -1.15 . HCT + 90.4 Corr = -0.41 P<0.01
D. In the Contralateral Hemisphere
CBF = -1.72 . HCT + 118 Corr = -0.37 P<0.01

The increases in cerebral blood flow following haemodilution with all four infusion methods, and the significant correlations between haematocrit and blood flow add weight to the argument that reducing haematocrit can improve cerebral blood flow. Assuming Poiseuille's equation applies, the increase in blood flow following haemodilution could have been due to vasodilatation in response to reduced oxygen carrying capacity (Brown and Marshall, 1985b) or through the reduction in whole blood viscosity (Thomas et al, 1977b). This thesis has presented data demonstrating increases in blood flow to ischaemic brain where there was no vascular reactivity to hypercapnia (Group 4 electrodes). In these regions, the capillary bed was maximally dilated and therefore the improvement in blood flow could not be attributed to local vasodilatation in response to the reduction in oxygen carrying capacity, but was more likely due to the reduced blood viscosity.

As blood viscosity is a logarithmic function of haematocrit (Section 4.1) and according to Poiseuille, flow is an inverse linear function of viscosity, one could expect the in vivo blood flow to be linearly related to blood viscosity or a logarithmic function of haematocrit. Few investigations into haemodilution have addressed this concept except that Wood et al (1984b, 1982b) demonstrated a linear viscosity-CBF relationship in ischaemic dogs and Shimojo et al (1979, Maruyama et al, 1985) demonstrated a log.Haematocrit relationship with blood flow in normal dogs. Brown et al (1985b) and Humphrey et al (1979, 1980) both demonstrated significant whole blood viscosity-CBF relationships in patients without cerebrovascular disease and exactly the same relationship was demonstrated in patients with focal cerebral ischaemia (Korosue et al, 1988). Our data confirmed a strong inverse correlation between cerebral blood flow and the systemic blood viscosity at both high or low shear rates (Figures 4.3.b and 4.3.c) in ischaemic and non-ischaemic brain. The correlations were better in the most ischaemic areas, perhaps emphasising the greater role of viscosity in

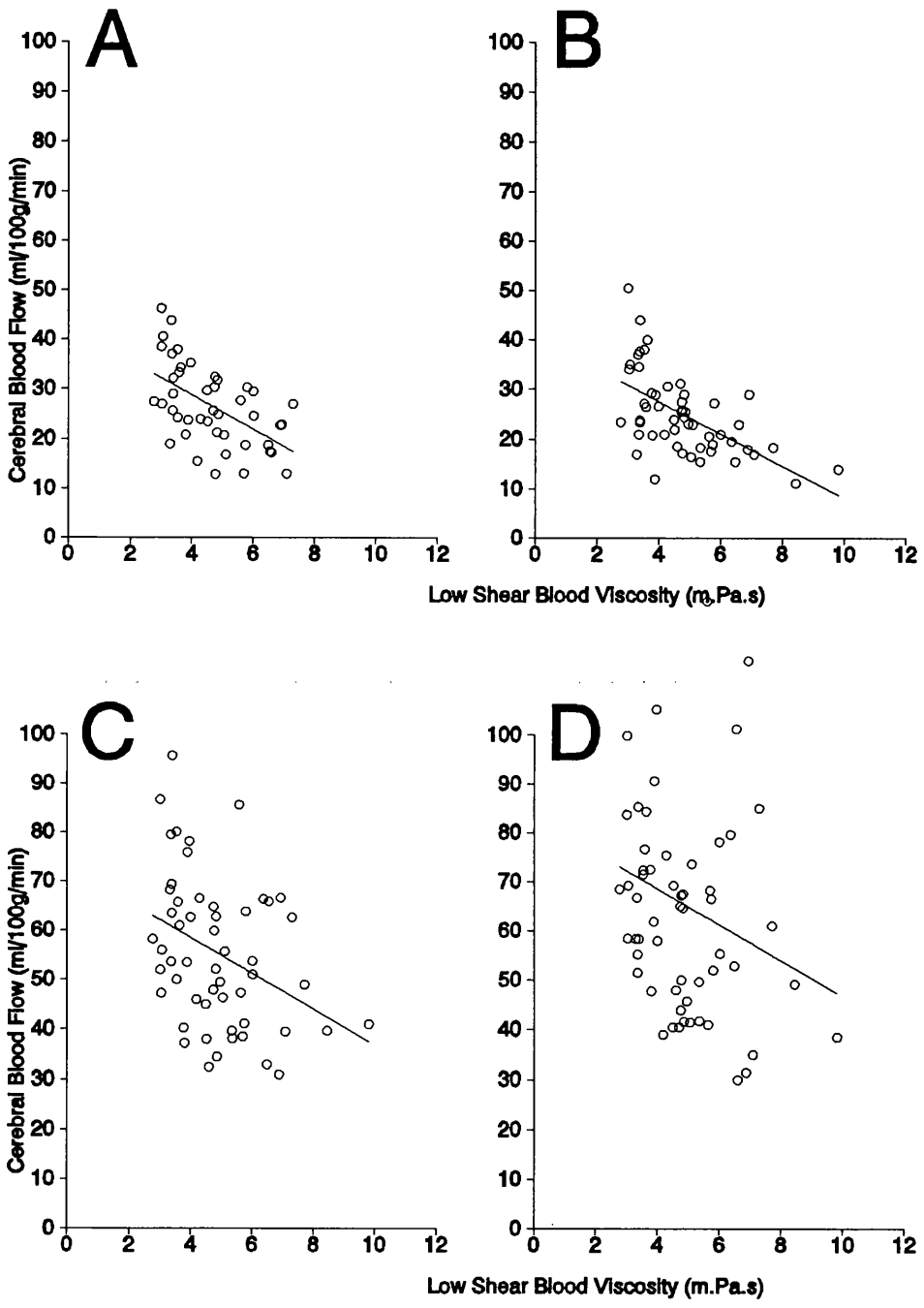


Figure 4.3.b
Pre and post-dilutional low shear whole blood viscosity (WBV) and corresponding cerebral blood flow in all animals (n=32) where pre dilutional blood flow was :

A. Non-vasoreactive
CBF = -3.43 . WBV + 42.5 Corr = -0.55 P<0.001
B. Vasoreactive but <30ml/100g/min
CBF = -3.23 . WBV + 40.5 Corr = -0.59 P<0.001
C. Vasoreactive but >30ml/100g/min
CBF = -3.62 . WBV + 73.0 Corr = -0.36 P<0.01
D. In the Contralateral Hemisphere
CBF = -3.64 . WBV + 83.0 Corr = -0.22 P>0.05

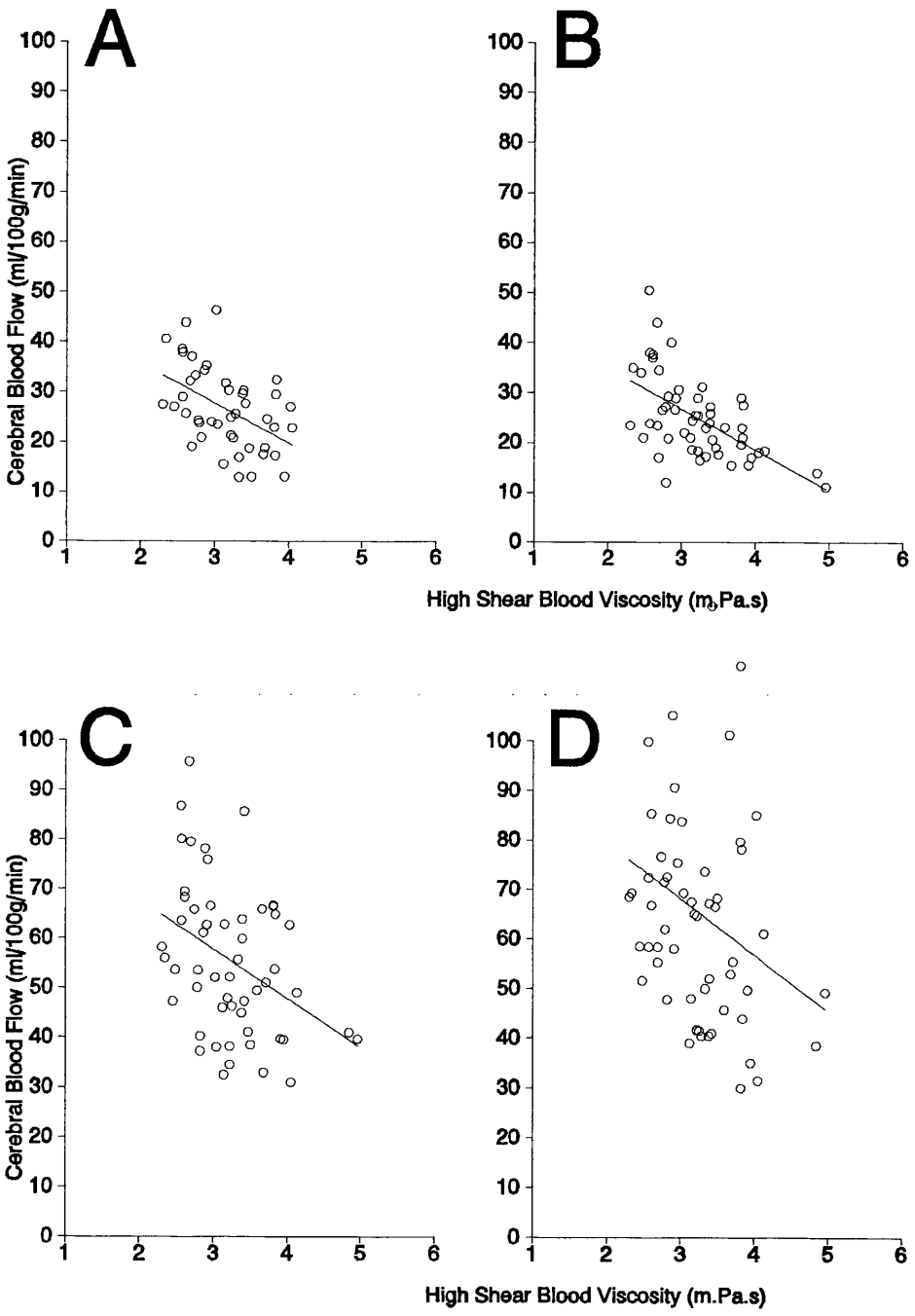


Figure 4.3.c
Pre and post-dilutional high shear whole blood viscosity (WBV) and corresponding cerebral blood flow in all animals (n=32) where pre dilutional blood flow was :

A. Non-vasoreactive
CBF = -8.00 . WBV + 51.7 Corr = -0.48 P<0.001
B. Vasoreactive but <30ml/100g/min
CBF = -8.19 . WBV + 51.3 Corr = -0.58 P<0.001
C. Vasoreactive but >30ml/100g/min
CBF = -9.92 . WBV + 87.5 Corr = -0.38 P<0.01
D. In the Contralateral Hemisphere
CBF = -11.4 . WBV + 102 Corr = -0.27 P<0.05

determining blood flow in these areas. Therefore, although "the viscosity effect on increasing blood flow is of a lower order" than the reduction in oxygen-carrying capacity (Thomas, 1992), where vasoreactivity has been lost, blood viscosity appears to become the major determinant of blood flow.

It was possible, using the viscosity/haematocrit equations from figure 4.1.a (Section 4.1) and the viscosity/CBF equations (figures 4.3.b and 4.3.c), to calculate the theoretical haematocrit for optimal oxygen delivery where the latter was expressed as haematocrit x CBF. This was done using the regression formula obtained from both high and low shear rate blood viscosity measurements and using flow analysis equations from each of the electrode groupings. The resolved formula are given in Appendix C1 and the mathematical functions have also been plotted in figure 4.3.d along with the actual oxygen delivery measurements from all the animals.

Considering the significant effect of shear rate on blood viscosity <u>in vitro</u> it was interesting that the high and low shear rate curves were so similar. The similarity of the curves was a good test of the calculated equations as the amalgamation of the two sets of formulae effectively eliminated the blood viscosity measurements. Measured blood flow is a composite measurement of all factors included in Poiseuille's equation and therefore encompasses changes in shear stress induced by pressure changes, vessel diameter and haematocrit induced variations in viscosity (see section 1.3.2). The rate of shear is therefore significant in <u>in vitro</u> measurement of blood viscosity but is probably superfluous in the link between haematocrit and blood flow; as blood flow itself is a representation of the changes in viscosity and shear.

These arguments are emphasised by the observation that calculated maximum oxygen delivery rates occur at similar

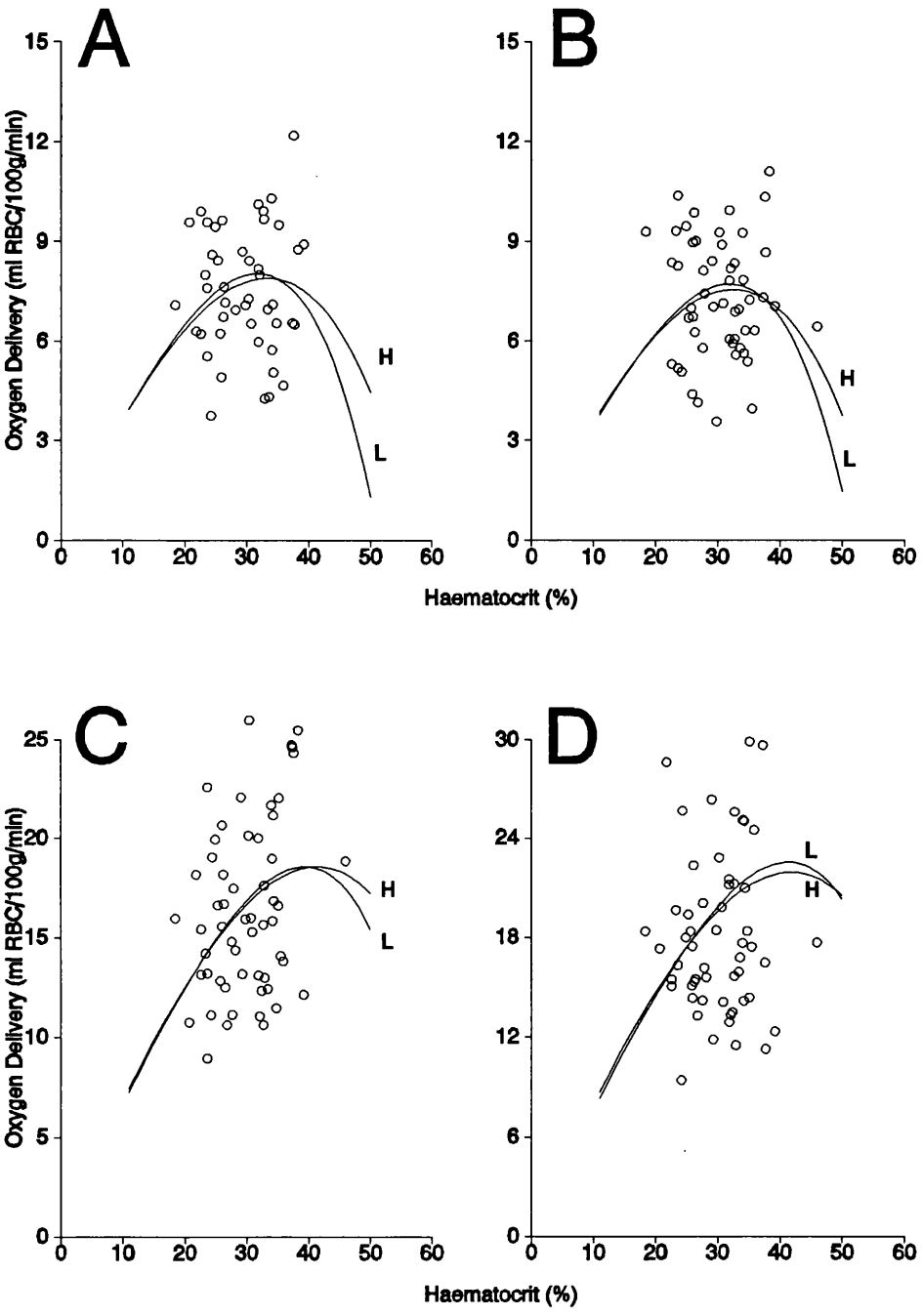


Figure 4.3.d

Pre and post dilution mean oxygen transport data plotted against systemic haematocrit. Superimposed curves are estimates of oxygen delivery as calculated from the derived functions for haematocrit/blood viscosity and blood viscosity/cerebral blood flow (Appendix C1), where H=High and L=Low shear blood viscosity.

A. Group 4 Electrodes B. Group 3 Electrodes
C. Group 2 Electrodes D. Group 1 Electrodes

values irrespective of shear rate, but are dependent upon the predilution level of blood flow:

Maximum oxygen delivery (ml RBC/100g/min)

	Low Shear	High Shear
Non reactive areas (Group 4) :	32	34
Reactive areas with CBF : <30ml/100g/min	32	33
Reactive areas with CBF : >30ml/100g/min	39	41
Contralateral Hemisphere :	41	42

These data agree with the observations of Dormandy (1983), who stated that "As the shear stress decreases, and this is analogous to the pathological poor flow situation, so the optimal haemoglobin concentration for oxygen delivery also decreases. Thus, the optimal haematocrit for oxygen delivery will also depend on blood flow".

In normal regions of the brain, maximum oxygen delivery was calculated to be around 40%, but in ischaemic vasodilated tissue, the optimal haematocrit for delivery was much lower at 32%. These findings contrast with those of Kusunoki et al (1981) who found an optimal haematocrit of 40-45% in stroke patients where "vasomotor adjustment to haematocrit variations [was] impaired". However, mean cerebral blood flow in these patients was 40ml/100g/min which according to our data and others (Symon et al, 1974) was high enough for vasoreactivity to be preserved. The level of blood flow corresponds well with values from group 2 electrodes in this series and where the optimal haematocrit values are similar.

The optimal haematocrit of 42% calculated from amalgamated data from studies in normal patients (Gaehtgens and Marx, 1987) also agrees with the value obtained for maximum oxygen delivery to the contralateral hemisphere in this study. These curves also explain the lack of increase in oxygen

transport expected by Hino et al (1992) following hypervolaemic haemodilution of normal patients to reduce haematocrit from 43% to 37%. Using the calculation formula obtained for the contralateral hemisphere, this reduction in haematocrit would produce a small reduction in oxygen transport.

The oxygen delivery/haematocrit curves presented here give an indication of why previous investigations had failed to prove the existence of an optimum haematocrit for maximum oxygen delivery to the brain that is below the normal physiological range. Previous studies have not studied these changes in truly ischaemic tissue where compensatory vasodilation had been lost and where haemodilution was most likely to improve oxygen delivery. By reducing systemic haematocrit to around 30%, calculated oxygen delivery to the penumbral regions (group 4) reaches a maximum while oxygen delivery to other areas of the brain may fall. This was confirmed by the oxygen delivery data in table 3.2.k, where the largest and most significant reductions in oxygen delivery occurred in the control hemisphere and high flow regions of the ischaemic hemisphere. As detailed in the introduction however, reduction in oxygen delivery to reasonably normal brain is not too significant as the oxygen delivery far outweighs the brain's oxygen requirement.

One of the major criticisms of the recent multi-centre haemodilution trials was the small size of the reductions in haematocrit (Heros and Korosue, 1989). Both the Scandinavian (SSSG, 1987, 1988) and the Italian trials (IASSG, 1988) reduced haematocrit from around 43% to 37% over periods of more than 48 hours. Applying these changes to the curves generated in this thesis, oxygen delivery would have remained fairly constant in the normal perfused regions of the brain but would have increased by over 30% in the ischaemic brain regions. Although a further 7% increase in oxygen delivery could have been achieved had the haematocrit been reduced to around 32%, it seems unlikely that this

additional reduction would have had a significant effect on the outcome of the studies. Unless increased blood flow without increases in oxygen delivery are significantly beneficial through clearance of harmful metabolites, the depth of haemodilution induced by these trials appears scientifically sound. Failure of these trials to demonstrate any positive benefit of haemodilution in recovery from ischaemic stroke was therefore more likely due to other factors such as the timing of haemodilution (Grotta, 1987b; Heros and Korusue, 1989; Harrison, 1989). These observations seem warranted if the penumbral regions remain viable for only a few hours (Frackowiak, 1985; Strong et al, 1983).

The generated haematocrit/oxygen delivery curves agree well with the clinical guidelines described by Kee and Wood (1987) where stroke patients with haematocrit levels above 40% should be treated with haemodilution to reduce haematocrit to around 33%. They also concur well with the recommended treatment of non-stroke patients with polycythemia by the reduction of systemic haematocrit to below 45% (Pearson, 1988).

Within this primate study, the generated haematocrit/oxygen delivery curves also offer an explanation for the lack of improvement in oxygen delivery to the ischaemic regions of the brain following haemodilution with either of the infusion regimes. The mean fall in haematocrit from 34.5% to 25.8% occurs around the calculated maximum oxygen delivery point of 33% haematocrit. The calculated change in oxygen delivery to the ischaemic regions for this change in haematocrit was therefore very small at around a 4% fall.

It was disappointing to find such a large degree of scatter of the measured oxygen delivery data when plotted next to the calculated delivery curves (figure 4.3.d). However, the significant changes in plasma viscosity found with the different infusion regimes may have produced some of this variation.

4.4 INFLUENCE OF PLASMA VISCOSITY ON THE HAEMATOCRIT / CEREBRAL BLOOD FLOW RELATIONSHIP

Section 4.1 revealed that plasma viscosity, as well as haematocrit, was a significant variable in determining *in vitro* blood viscosity. Section 4.3 demonstrated that blood flow correlated well with changes in blood viscosity (figures 4.3.b and c). Theoretically therefore, it could be expected that the significant influence of plasma viscosity on blood viscosity may also affect blood flow. This can be demonstrated by amalgamation of the two sets of regression equations. The amalgamated functions are given in Appendix C2 and are plotted in figures 4.4.a and 4.4.b.

The figures demonstrate that increases in plasma viscosity at any given haematocrit could be associated with reduced oxygen delivery to all regions of the ischaemic brain. The effect of systemic plasma viscosity on oxygen delivery appears greatest at the higher haematocrits. Figures 4.4.a and 4.4.b (A and B) demonstrate that, at a plasma viscosity of 1.8mPa.s, maximum oxygen delivery to areas of the brain where vasoreactivity has been lost or reduced can be obtained at a haematocrit of around 25%. If plasma viscosity was lowered to 1.2mPa.s however, a similar haematocrit would increase oxygen delivery by over 28%. Even more striking is the opposite effect of increasing plasma viscosity from 1.2 to 1.8mPa.s at a haematocrit of 32%, where oxygen transport could fall by over 55%. The marked influence of plasma viscosity on blood flow and oxygen delivery was also demonstrated by Humphrey et al (1980) who reported that a 0.6mPa.s increase in plasma viscosity in non-ischaemic patients was associated with a 20ml/100g/min lower mean cerebral blood flow. Using the equations generated above, calculations using the figures published in Humphreys' study, confirm a 20ml/100g/min fall in cerebral blood flow for a 0.6mPa.s reduction in plasma viscosity. Tsuda et al (1987a and b), who demonstrated a lack of increase in blood

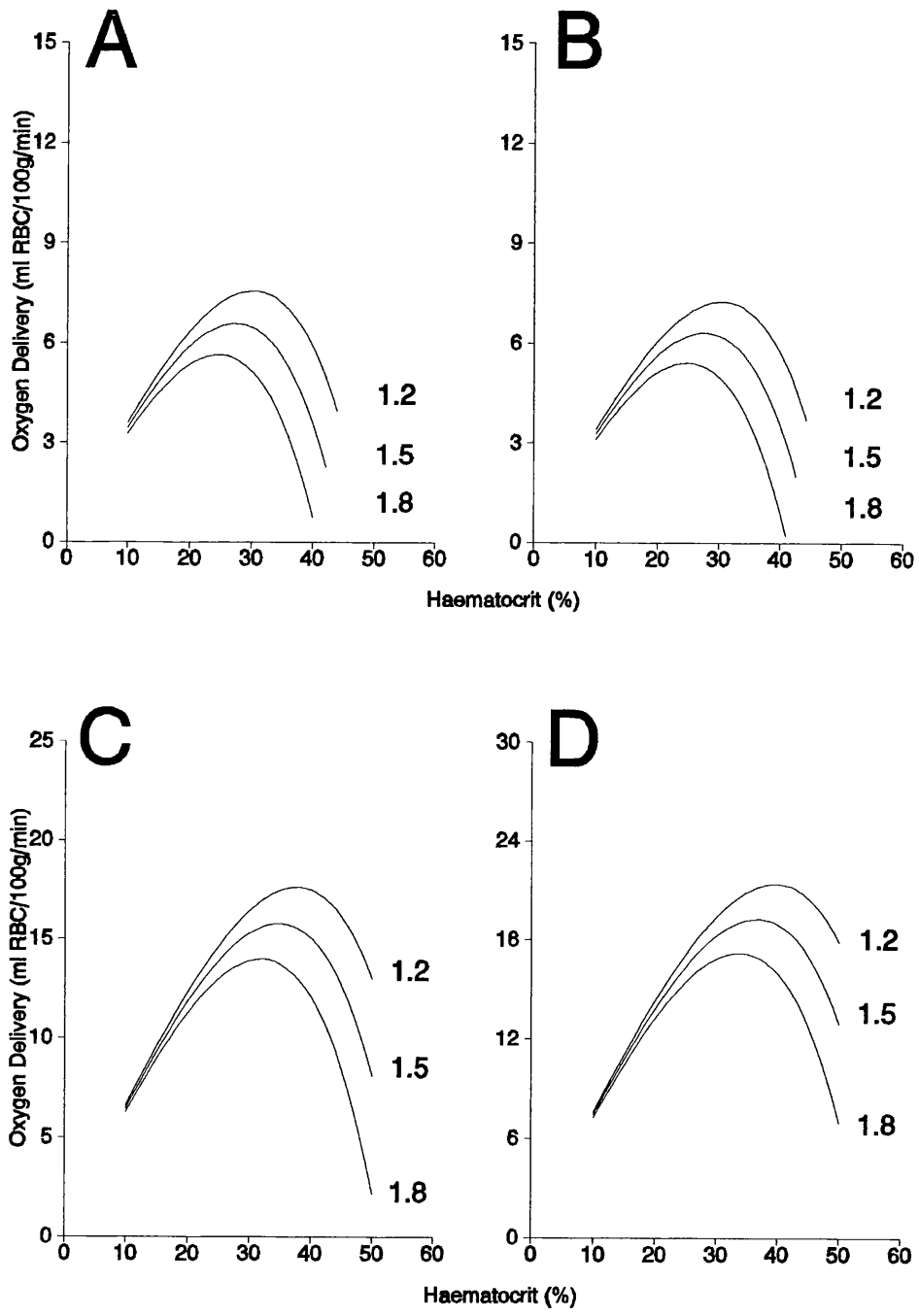


Figure 4.4.a
Plots of the derived functions for oxygen delivery to the brain demonstrating the influence of both systemic haematocrit and plasma viscosity (calculated from low shear blood viscosity data: see Appendix C2).
Where:
A: Group 4 electrodes B: Group 3 electrodes
C: Group 2 electrodes D: Group 1 electrodes

1.2, 1.5, 1.8 represent plasma viscosities in mPa.s

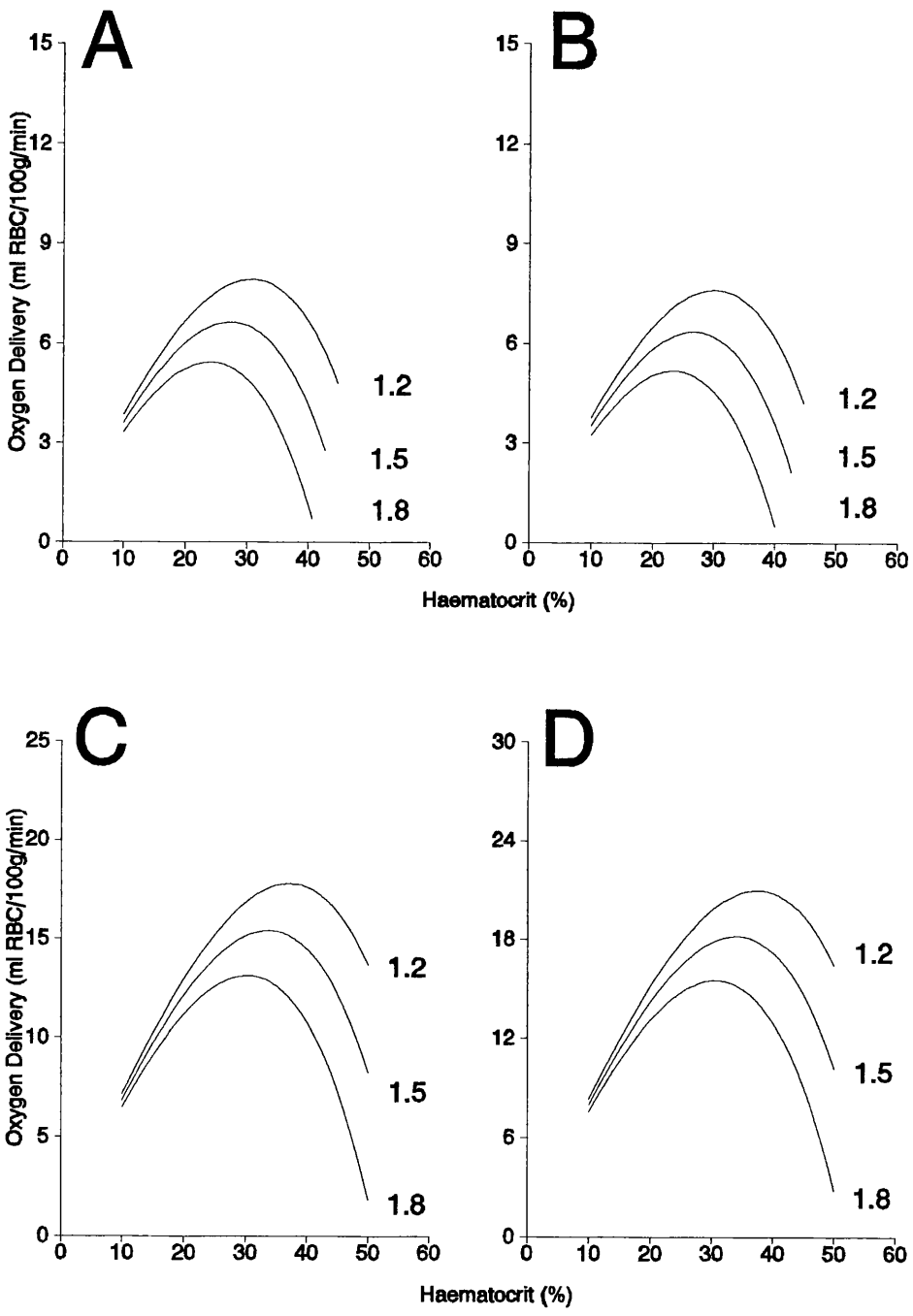


Figure 4.4.b
Plots of the derived functions for oxygen delivery to the brain demonstrating the influence of both systemic haematocrit and plasma viscosity (calculated from high shear blood viscosity data: see Appendix C2).
Where:
A: Group 4 electrodes B: Group 3 electrodes
C: Group 2 electrodes D: Group 1 electrodes

1.2, 1.5, 1.8 represent plasma viscosities in mPa.s

flow and oxygen delivery to normal baboon brain following haemodilution with dextran, also demonstrated a significant correlation between the fall in haematocrit and an increase in plasma viscosity and concluded that the lack of change in blood flow was attributed in part to the increase in plasma viscosity.

The data presented in this thesis indicate that the increase in plasma viscosity produced by haemodilution with dextran is likely to be detrimental to blood flow and oxygen delivery to the ischaemic regions of the brain. However, attempts to correlate measured cerebral blood flow with the systemic haematocrit and plasma viscosity values failed to demonstrate any statistically significant influence of plasma viscosity. Having calculated the oxygen delivery curves from the in vitro blood viscosity formulae (Fig 4.1.a) and in vivo blood flow formulae (Fig 4.3.b and c), we would expect blood flow, haematocrit and plasma viscosity to be related:

$$\text{Log } (f \text{ CBF}) = \text{Log } (\text{PV}) + f \text{ HCT} + C$$

Multiple regression analysis between Log CBF, Log PV and HCT indicated haematocrit to be a significant variable in determining cerebral blood flow to all areas of the focally ischaemic brain, but failed to indicate any in vivo role for plasma viscosity in the determination of cerebral blood flow and oxygen delivery.

The failure to demonstrate any significant effect of plasma viscosity on blood flow concurs with the findings of Brückner and Messmer (1990). In normal dogs, haemodilution from a haematocrit of 30% to 19% using dextran 70 increased cerebral blood flow, even though plasma viscosity was raised from 1 to 3mPa.s. The authors demonstrated that the haematocrit / oxygen transport curve remained unaltered from that previously reported (Messmer et al, 1972) and concluded therefore that "..blood flow is independent of plasma

viscosity..". However, it could be argued that the similarity between the curves in their recent study and those previously reported could be due to two other possibilities: that plasma viscosity was elevated in both studies, as haemodilution in the earlier study was also with a high viscosity infusate, dextran-60; or because the oxygen transport values quoted were the calculated product of cardiac output and oxygenated haemoglobin concentration ie, for the plasma viscosity to have influenced oxygen transport, it would have had to change cardiac output.

This thesis has obtained data that adds weight to the theory that increased plasma viscosity is detrimental to blood flow and oxygen delivery to the focally ischaemic brain. However, the data fail to prove this theory conclusively. Whilst there is still doubt as to the effects of dextran induced increases in plasma viscosity, it would seem prudent to infuse dextran isovolaemically, as less dextran is required to produce a similar degree of dilution. Moreover, Haemaccel infusion can induce a similar degree of haemodilution to dextran without increasing plasma viscosity and could therefore be of more benefit to the haemodiluted.

4.5 CEREBRAL OXYGEN TENSION

A mean pre-MCAO oxygen tension of 20.0mmHg (sd: 7.1) in brain cortex was similar to values recorded in another baboon study (23.8 ± 14mmHg; Crockard et al 1976a) and in a recently reported study in rats (17.9 ± 10.2mmHg; Raffin et al 1991). As discussed by Lubbers (1973) and Silver (1965), the measured PtO_2 in the brain is dependant upon the geometrical location of the capillaries and the recording electrode and increases with increasing proximity to a blood vessel. The use of a relatively large electrode with a large surface area was intended to reduce the influence of the intercapillary oxygen gradients and to provide a mean measurement of tissue PO_2 within a comparatively large volume of tissue. The mean tissue PO_2 therefore gave a measure of the oxygen gradient between the blood PO_2 and that in the cellular mitochondria (Guyton 1991) and was determined by both the oxygen delivery and the cellular utilisation of oxygen.

The period of hypercapnia prior to the ischaemic insult, that induced significant increases in cerebral blood flow, was not associated with increases in brain tissue PO_2. This agreed with the findings of Crockard et al (1976b), who "emphasised the danger of equating increases in blood flow with improvement in supply of oxygen to the tissue". Critical to this statement is the fact that oxygen delivery is the product of blood flow and blood oxygen content and therefore an increase in one parameter does not necessarily produce an increase in oxygen delivery as the other parameter may decrease. This was the case in the above scenario, where hypercapnia increased cerebral blood flow, but the marked effect of raised $PaCO_2$ and pH on the oxygen dissociation curve of haemoglobin (Bohr effect) reduced the oxygen content of the blood. The combined effect was that oxygen availability to the brain was not significantly affected.

Following MCAO, the greatest reductions in tissue oxygen tension were recorded in areas of the brain where the largest reductions in cerebral blood flow occurred. Figure 4.5.a, demonstrates a linear relationship between mean cerebral blood flow and PtO_2 in the ischaemic hemisphere following MCAO. It is interesting that the slope of the curve indicates that a zero tissue oxygen tension would occur at a blood flow of 10ml/100g/min, or possibly even higher if we look at the scattering of data points around the baseline at blood flows around 20ml/100g/min. This suggests that the tissue oxygen tension is not linearly related to blood flow at these low flow levels and that other factors are causing oxygen tension to fall. As outlined above, these factors could include a fall in blood oxygen content or an increase in tissue oxygen metabolism. The former is most likely, due to the reduced blood flow allowing increased extraction of oxygen by the tissues and thereby leaving the blood to carry less oxygen further down the vascular network. This would explain the apparent fall off in oxygen tension as blood flow was reduced to around 20ml/100g/min. The further reduction in oxygen tension to zero at between 10 and 20ml/100g/min ties in with the thresholds for the loss of cellular ion homeostasis (Harris et al, 1987) and cellular function (Branston et al 1974) which are secondary to hypoxia induced disruption of cellular metabolism (Siesjo 1981).

Haemodilution with either of the four infusion regimes was not accompanied by any increases in normal or ischaemic brain tissue PO_2 despite the significant increases in cerebral blood flow. This was in line with results of Chan et al (1983), where the oxygen tension histogram for normal and ischaemic brain of cats remained constant during extreme isovolaemic haemodilution with dextran to reduce haematocrit from 40% to 20%.

The mean oxygen tension and local cerebral blood flow values recorded in the ischaemic hemisphere following haemodilution

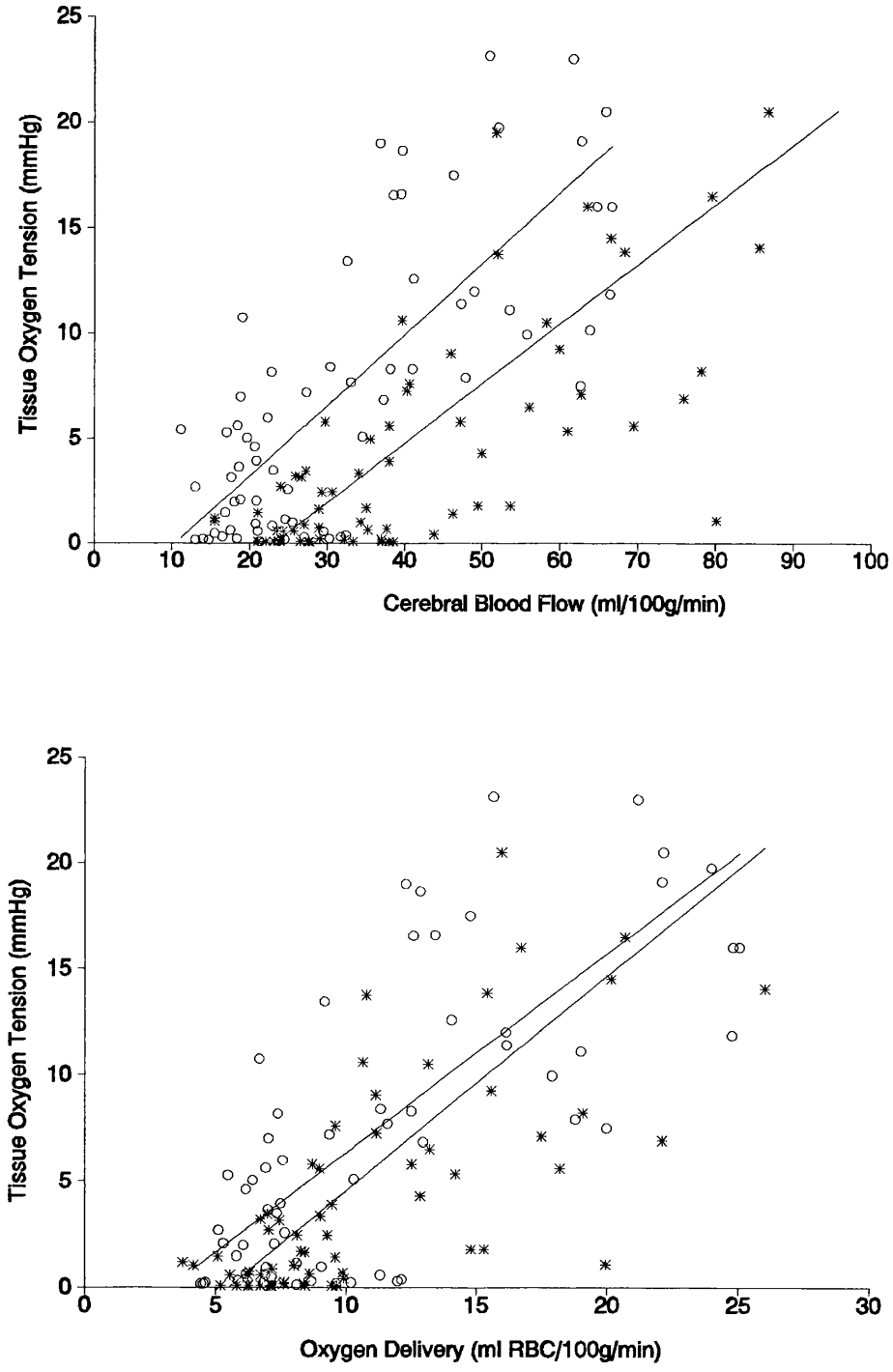


Figure 4.5.a

Relationship between tissue oxygen tension and cerebral blood flow (top) or calculated oxygen delivery (bottom) in the ischaemic hemisphere prior to (circles) and following (stars) haemodilution. All linear regression lines represent significant ($P<0.001$) correlations between the sets of data.

have been included in figure 4.5.a.

Pre-Haemodilution

$PtO_2 = 0.33 \cdot CBF - 3.48$

Post-Haemodilution

$PtO_2 = 0.28 \cdot CBF - 6.54$

The regression analysis demonstrates a similar relationship between flow and oxygen tension both before and after haemodilution, but the slope of the correlation has been shifted to the right ie more flow for the same tissue oxygen availability. The same level of blood flow before and after haemodilution was associated with a 5mmHg lower tissue oxygen tension.

Figure 4.5.a also includes the haematocrit values in the relationship by relating oxygen tissue tension to calculated oxygen transport. The regression lines were very similar for pre and post haemodilution comparisons:

Pre-Haemodilution

$PtO_2 = 0.010 \cdot (CBF \cdot HCT) - 5.5$

Post-Haemodilution

$PtO_2 = 0.009 \cdot (CBF \cdot HCT) - 3.0$

The same oxygen delivery levels prior to and following haemodilution were associated with tissue oxygen tensions that differed by less than 2mmHg. These data therefore demonstrate that cerebral oxygen tension was linearly dependant upon the cerebral oxygen delivery. The lack of increase in oxygen tension following haemodilution with any of the four haemodilution regimes was probably due to the lack of increase in oxygen delivery. As discussed in section 4.3, the haematocrit was reduced to below the calculated optimum (fig 4.3.d) for increased oxygen delivery and therefore neither oxygen delivery to, nor oxygen availability in the brain were improved. These findings were in line with the observations of Ehrly (1986), who

emphasised that "improvement of haemorheological parameters in patients does not necessarily mean an improvement in tissue supply and clinical symptoms ...[and] simultaneous measurements of haemorheological data as well as direct *in vivo* measurements of tissue supply can help us to establish and quantify the beneficial effect of hemorheological therapy". Although blood viscosity was reduced and blood flow increased to all areas of the ischaemic brain following haemodilution, the reduction in haematocrit to below the optimum values (section 4.3) resulted in no beneficial effect on oxygen delivery and tissue oxygen availability.

4.6 SUMMARY AND CONCLUSIONS

Hypervolaemic haemodilution with dextran-40 induced significant increases in plasma viscosity. Increases in plasma viscosity were also produced by isovolaemic infusion of dextran-40, although the increases were not as great as with hypervolaemic infusion.

Hypervolaemic or isovolaemic infusions of Haemaccel were not associated with significant increases in plasma viscosity.

Isovolaemic and hypervolaemic infusions of Haemaccel and dextran induced significant reductions in systemic haematocrit, although isovolaemic infusion of Haemaccel was least successful in this respect.

Red cell aggregation, as measured by viscometric means, was reduced by both Haemaccel and dextran infusion. Whilst the Haemaccel induced reduction in aggregation produced a fall in relative blood viscosity, the dextran induced increase in plasma viscosity compromised the benefit of reduced red cell aggregation.

Isovolaemic or hypervolaemic haemodilutions with either Haemaccel or dextran were associated with significant increases in cerebral blood flow to the focally ischaemic primate brain. Increases in flow occurred in both normal brain regions and in areas where ischaemic flow was reduced sufficiently to induce a state of maximal vascular dilatation. Increases in flow to the latter areas could not therefore be due to compensatory vasodilation in response to reduced blood oxygen content.

Isovolaemic infusion induces significant increases in cerebral blood flow to ischaemic regions of the brain and may therefore be a beneficial method of haemodilution in stroke patients with additional cardiac problems or at risk of intracerebral haemorrhage.

The increases in blood flow following either of the four infusion regimes were not associated with significant increases in cerebral tissue oxygen tension, and merely shifted the linear oxygen tension / blood flow relationship to the right.

Cerebral vessel dilatation to hypercapnia was compromised in low flow regions following haemodilution probably due to haemodilution enhanced intra cerebral steal.

The cerebral blood flows recorded before and after haemodilution were significantly inversely correlated with both the *in vitro* blood viscosity and a logarithmic function of haematocrit.

Using the cerebral blood flow / blood viscosity relationship, calculation of oxygen delivery revealed haematocrit values at which maximum oxygen delivery to ischaemic and normal regions of the focally ischaemic brain occurred. The theoretical systemic haematocrit for maximum oxygen delivery to ischaemic regions was significantly lower (32%) than required in normal brain (41%).

The shift in the tissue oxygen tension / cerebral blood flow relationship was alleviated by correcting for the change in haematocrit. The brain tissue oxygen tension was therefore better related to changes in blood oxygen transport than to changes in cerebral blood flow. The linear nature of the relationship also demonstrated that maximum oxygen availability in the brain would occur at a theoretical optimum haematocrit.

The changes in plasma viscosity induced by haemodilution with dextran-40 had a significant effect on the *in vitro* whole blood viscosity.

Incorporating the *in vitro* influence of plasma viscosity on whole blood viscosity into the haematocrit / cerebral blood

flow relationship demonstrated the real possibility of remarkable reductions in oxygen delivery to both ischaemic and normal regions of the brain when plasma viscosity is increased. However, this investigation failed to demonstrate any direct evidence to this effect.

4.7 RECOMMENDATIONS

The differences of opinion in the literature as to the effect of haemodilution on cerebral blood flow is remarkable and seems extremely dependant on the experimental model used and the presence or absence of any degree of ischaemia. It seems logical to suggest, but is still being overlooked, that studies into haemodilution therapy of stroke should be performed on as close an experimental model to the true changes seen during stroke in man. With ischaemia and haemodilution affecting so many variables, anything but a true representation of the real problems may fail to include a significant influencing factor, either known or unrecognised.

Fundamental to the theory of haemodilution therapy is that reduction in blood viscosity improves flow through maximally dilated tissue. It is therefore vital that any studies on changes in cerebral blood flow to ischaemic brain should confirm the degree of vascular reactivity present at the recording site, so that changes in flow can be directly attributed to changes in either viscosity or vessel diameter. This thesis has shown that the depth of ischaemia is not an accurate enough indicator of vessel reactivity.

The results of this thesis suggest that Haemaccel would be a suitable haemodiluent substitute for dextran-40, due to its similarities to plasma. The increases in plasma viscosity with dextran-40 may not be desirable, but the hypertonic properties may be of benefit in preventing post-

ischaemic oedema formation and increases in intracranial pressure. Further studies would therefore be beneficial to ensure that the maintenance of a normal plasma viscosity is not compromised by increases in brain water content.

Although not as effective as hypervolaemic infusion at reducing haematocrit, isovolaemic haemodilution with either Haemaccel or Dextran produced a significant increase in cerebral blood flow to ischaemic regions. Without the associated rise in blood volume and cardiac output, this method of haemodilution may be advantageous in certain groups of people and therefore clinical trials may be warranted.

Although data have been presented to support the theory that plasma viscosity is a significant determinant of blood flow and oxygen delivery during haemodilution therapy of cerebral ischaemia, more detailed studies are required to conclusively demonstrate this relationship. However, there are major difficulties in determining the influence of plasma viscosity when so many other variables are changing during haemodilution. This might be overcome if plasma viscosity became a routine clinical blood measurement such as haematocrit. The data generated in this fashion would make the clinician more aware of the influence of infusions such as LMWD and may identify plasma viscosity induced trends in clinical outcome.

APPENDICES

CLINICAL INVESTIGATION

Introduction

This section contains data and information presented at the 7th European Conference on Clinical Haemorheology 1991 and the associated abstract publication (Farman et al, 1991b). The study was performed on patients undergoing surgery for sub-arachnoid haemorrhage or aneurysm clipping at the National Hospital, London. Normal post-operative treatment of these patients included daily prophylactic infusions of fluid to reduce haematocrit and increase cerebral perfusion pressure. Such treatment is common and is aimed at reducing the probability of, and increasing blood flow through, any post operative vasospasm. The patients of two neurosurgical consultants were studied. One of the consultants used Haemaccel as the post operative infusate the other preferring dextran.

Materials and Methods

Immediately following surgery, patients were given a 500ml infusion of either dextran 40 (Rheomacrodex, Pharmacia) or polygeline (Haemaccel, Hoechst). Infusion was over 4-5 hours and was repeated each day post operatively for a total of 5 days. No restrictions were placed on the normal patient treatment. Two venous blood samples (3ml in EDTA) were taken both before and after each infusion. One sample was used for evaluation of plasma viscosity the other for haematocrit and whole blood viscosity. A control blood sample was also taken immediately prior to surgery where possible. All blood sampling was by qualified medical staff under the direction of the consultant or senior registrar and the rheological studies were carried out as detailed in section 2.4.

Results

A total of 19 patients were included in the study. Seven received haemodilution therapy using Rheomacrodex, while twelve received Haemaccel.

The mean systemic plasma viscosities and haematocrits of the two infusion groups are presented in figure A.i. The figure gives the mean values over a period of four days with the origin of the time scale from when a blood sample was taken immediately following the operation. The horizontal bars near to the x-axis represent the periods of each infusion. The statistical significance of the differences between the two groups are presented in table A.ii.

The increase in mean plasma viscosity following dextran infusions was from 1.47mPa.s to 1.73mPa.s (17.7%) over the four day period. Following Haemaccel infusions over the same period, the change was from 1.44mPa.s to 1.48mPa.s (2.8%).

Mean haematocrit fell from 43% to 37% during dextran infusion and from 40% to 35% with Haemaccel infusion.

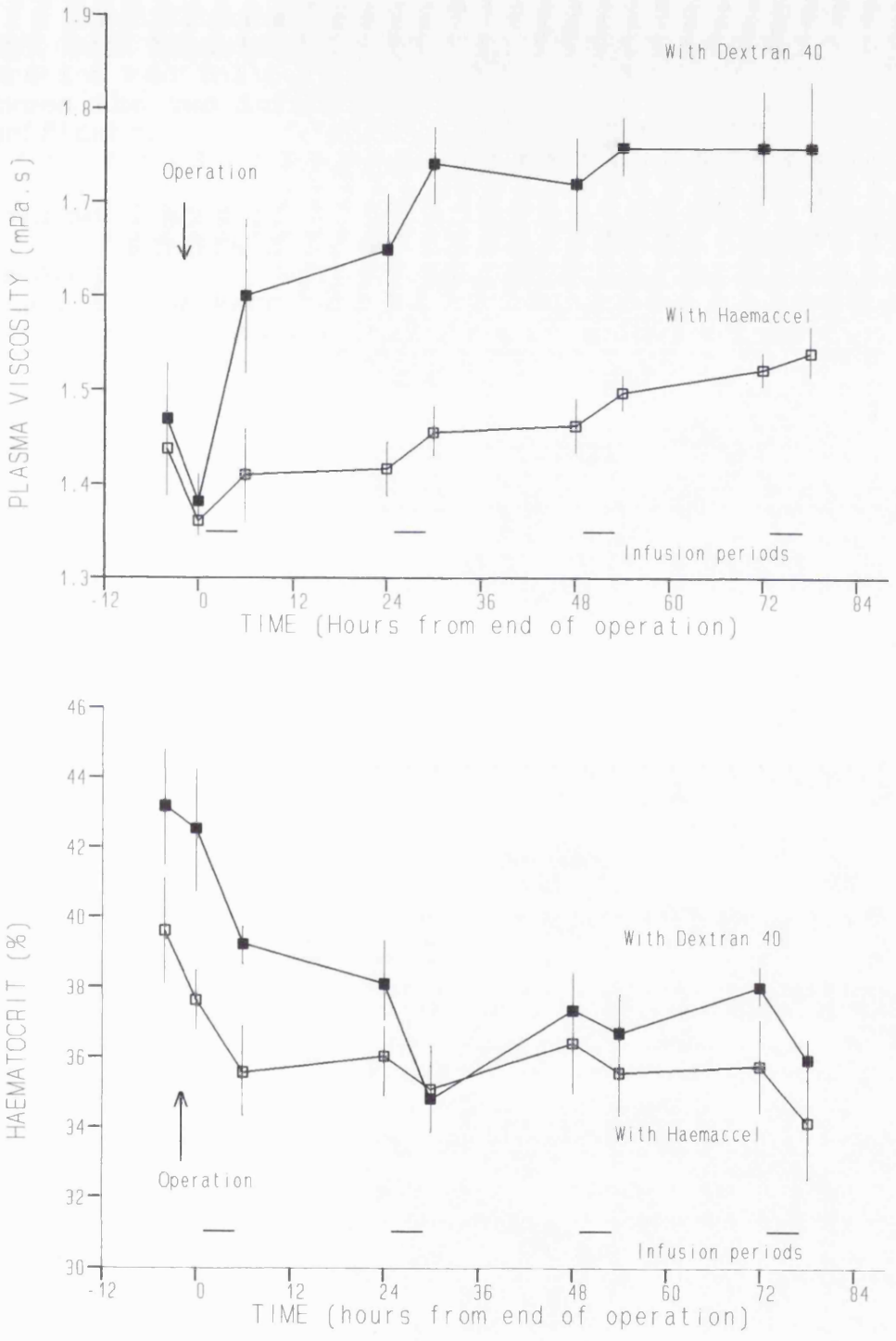


Figure A.i

Changes in mean (±SEM) plasma viscosity (top) and haematocrit (bottom) during a series of daily infusions of dextran or Haemaccel. Samples were taken prior to and following each infusion. n=12 for Haemaccel, n=7 for Dextran.

Table A.ii

Significance of differences in mean plasma viscosity (top) and haematocrit (bottom) between levels prior to operation and following the infusions (Paired t-tests), and between the two infusion types (unpaired t-test). NS : Not Significant.

A. PLASMA VISCOSITY

Sample (Time,h)	BETWEEN SAMPLE AND PRE-OP LEVEL		BETWEEN THE TWO INFUSIONS
	HAEMACCEL	DEXTRAN 40	
POST OP (0h)	NS	NS	NS
POST 1st (6h)	NS	P<0.01	P<0.05
PRE 2nd (24h)	NS	P<0.01	P<0.01
POST 2nd (30h)	NS	P<0.001	P<0.001
PRE 3rd (48h)	NS	P<0.001	P<0.001
POST 3rd (54h)	NS	P<0.01	P<0.001
PRE 4th (72h)	NS	P<0.01	P<0.001
POST 4th (78h)	NS	P<0.01	P<0.001

B. HAEMATOCRIT

Sample (Time,h)	BETWEEN SAMPLE AND PRE-OP LEVEL		BETWEEN THE TWO INFUSIONS
	HAEMACCEL	DEXTRAN 40	
POST OP (0h)	P<0.01	NS	NS
POST 1st (6h)	P<0.001	NS	P<0.05
PRE 2nd (24h)	P<0.01	P<0.05	NS
POST 2nd (30h)	P<0.01	P<0.01	NS
PRE 3rd (48h)	P<0.05	P<0.01	NS
POST 3rd (54h)	P<0.01	P<0.05	NS
PRE 4th (72h)	P<0.01	P<0.05	NS
POST 4th (78h)	P<0.01	P<0.01	NS

Discussion

A significant increase in plasma viscosity was seen in patients infused with Dextran-40 over the four days post-operatively. This has been demonstrated in other studies on patients (Schievink, 1988; Kroemer et al, 1987) and animal studies (Tsuda et al, 1987a and b). The increase in plasma viscosity was probably due to the systemic accumulation of high molecular weight dextran molecules (Haass et al, 1987).

This investigation demonstrates that infusion of Haemaccel instead of Dextran-40 can prevent such a large increase in plasma viscosity. However, the reduction in haematocrit following Haemaccel infusion was not as large as that induced by dextran. This was probably due to the hypertonic nature of Dextran, whereby extravascular fluid is drawn in to the blood stream to maintain the osmotic balance. Increasing plasma volume in this way would therefore reduce haematocrit comparatively more than with Haemaccel. This effect could probably be alleviated by additional infusion volumes of Haemaccel.

In conclusion, the use of Haemaccel for post-operative prophylaxis of vasospasm may therefore be advantageous over Dextran-40 by preventing significant long term increases in plasma viscosity which may have a detrimental effect on blood viscosity, red cell aggregation and ultimately blood flow (Haass et al, 1987).

Table 3.2.a

Mean (SD) values of five physiological parameters recorded from animals following hypervolaemic (A) or isovolaemic (B) haemodilution with Haemaccel or Dextran.

(A) HYPER-VOLAEMIC		PARAMETER				
		MSBP (mmHg)	PaCO2 (mmHg)	pH	PaO2 (mmHg)	[GLUC] (µmol)
HAEMACCEL	No CO2	119 (24)	39.1 (2.1)	7.32 (0.04)	487 (122)	7.1 (2.0)
	With CO2	120 (24)	50.8 (2.7)	7.23 (0.04)	516 (106)	--
DEXTRAN	No CO2	119 (15)	40.1 (1.3)	7.35 (0.03)	411 (132)	6.9 (2.5)
	With CO2	113 (6)	49.9 (4.0)	7.28 (0.04)	439 (119)	--

(B) ISO-VOLAEMIC		PARAMETER				
		MSBP (mmHg)	PaCO2 (mmHg)	pH	PaO2 (mmHg)	[GLUC] (µmol)
HAEMACCEL	No CO2	112 (9)	41.9 (1.3)	7.33 (0.03)	511 (58)	11.0 (4.5)
	With CO2	114 (11)	55.2 (1.9)	7.24 (0.03)	504 (51)	--
DEXTRAN	No CO2	106 (16)	39.8 (2.4)	7.32 (0.02)	497 (34)	10.9 (2.5)
	With CO2	111 (9)	50.9 (3.1)	7.23 (0.01)	517 (29)	--

Table 3.2.c

Mean (SD) values for blood and plasma viscosity and haematocrit recorded from systemic blood samples taken following hypervolaemic (A) and isovolaemic (B) haemodilution. The table also includes calculations of haematocrit corrected blood viscosity, relative blood viscosity and the viscometric aggregation index (details in section 2.4)

(A) Hypervolaemic Infusion With :

	HAEMACCEL		DEXTRAN	
NATIVE HAEMATOCRIT	24.6 (3.9)		25.6 (4.1)	
PLASMA VISCOSITY (mPa.s)	1.23 (0.12)		1.44 (0.08)	
BLOOD VISCOSITY (mPa.s)	Low Shear	High Shear	Low Shear	High Shear
At Native HCT	3.93 (0.98)	2.91 (0.38)	4.35 (0.81)	3.26 (0.45)
At Adjusted HCT of 35%	5.71 (1.43)	3.95 (0.83)	6.47 (0.88)	4.40 (0.44)
RELATIVE LOW SHEAR BLOOD VISCOSITY	4.66 (1.16)		4.52 (0.64)	
VISCOMETRIC AGGREGATION INDEX	1.34 (0.19)		1.34 (0.08)	

(B) Isovolaemic Infusion With :

	HAEMACCEL		DEXTRAN	
NATIVE HAEMATOCRIT	26.3 (2.0)		24.8 (2.5)	
PLASMA VISCOSITY (mPa.s)	1.13 (0.03)		1.24 (0.07)	
BLOOD VISCOSITY (mPa.s)	Low Shear	High Shear	Low Shear	High Shear
At Native HCT	3.52 (0.35)	2.63 (0.19)	3.54 (0.30)	2.75 (0.13)
At Adjusted HCT of 35%	5.13 (0.37)	3.56 (0.25)	5.34 (0.71)	3.74 (0.27)
RELATIVE LOW SHEAR BLOOD VISCOSITY	4.55 (0.27)		4.34 (0.58)	
VISCOMETRIC AGGREGATION INDEX	1.34 (0.02)		1.29 (0.05)	

Table 3.2.g
Changes in mean cerebral blood flow during hypercapnia following haemodilution with each of the four infusion regimes.

	BLOOD FLOW (ml/100g/min)			
	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
GROUP 1 CONTROL HEMISPHERE	+28.9 (11.2) P<0.001	+ 8.5 (6.8) P<0.001	+10.9 (7.4) P<0.01	+20.7 (13.0) P<0.01
GROUP 2 ISCHAEMIA >30ml/100g /min.	+14.2 (5.5) P<0.001	+ 7.4 (11.1) P>0.05	+11.1 (16.3) P>0.05	+22.1 (18.6) P<0.05
GROUP 3 ISCHAEMIA <30ml/100g /min.	- 3.3 (1.1) P<0.001	- 4.7 (9.2) P>0.05	+ 0.9 (12.5) P>0.05	- 6.9 (8.8) P>0.05
GROUP 4 ISCHAEMIA NON VASO-REACTIVE	- 5.4 (6.6) P>0.05	- 4.2 (7.1) P>0.05	- 5.2 (8.2) P>0.05	+ 5.0 (4.5) P<0.05

Table 3.2.j

Mean Oxygen Transport values following haemodilution with each of the four infusion regimes.

	OXYGEN TRANSPORT INDEX			
	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
GROUP 1 CONTROL HEMISPHERE	19.1 (7.1)	18.4 (3.1)	14.9 (3.7)	23.4 (9.0)
GROUP 2 ISCHAEMIA >30ml...	14.3 (5.8)	16.6 (2.8)	13.7 (2.1)	19.7 (2.7)
GROUP 3 ISCHAEMIA <30ml...	7.5 (1.9)	7.8 (1.6)	7.0 (1.9)	8.1 (2.2)
GROUP 4 NON VASO-REACTIVE	7.5 (1.5)	7.9 (1.3)	6.9 (2.2)	9.5 (4.7)

Table 3.2.l

Mean Oxygen Tension values following haemodilution with each of the four infusion regimes.

	OXYGEN TENSION (mmHg)			
	HAEMACCEL		DEXTRAN	
	HYPERVOL. (n=8)	ISOVOL. (n=9)	HYPERVOL. (n=7)	ISOVOL. (n=8)
GROUP 1 CONTROL HEMISPHERE	15.0 (9.8)	14.9 (10.0)	15.3 (7.0)	20.3 (9.5)
GROUP 2 ISCHAEMIA >30ml...	9.3 (5.0)	10.9 (8.5)	8.3 (3.5)	13.1 (3.9)
GROUP 3 ISCHAEMIA <30ml...	4.0 (2.3)	3.6 (3.8)	2.7 (0.9)	5.7 (2.9)
GROUP 4 NON VASO-REACTIVE	2.4 (2.9)	1.7 (2.3)	5.0 (3.9)	2.0 (1.3)

Caclulated oxygen delivery (HCT x CBF) using amalgamated equations derived from data in figures 4.1.a, 4.3.b and 4.3.c.

Non reactive areas (Group 4)

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (42.5 - 3.98 \cdot 10^{0.02\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (51.7 - 12.1 \cdot 10^{0.011\ HCT})$$

Reactive areas (Group 3):

with Pre-dilutional blood flow <30ml/100g/min

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (40.5 - 3.76 \cdot 10^{0.02\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (51.3 - 12.4 \cdot 10^{0.011\ HCT})$$

Reactive areas (Group 2):

with Pre-dilutional blood flow >30ml/100g/min

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (73.0 - 4.21 \cdot 10^{0.02\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (87.5 - 15.0 \cdot 10^{0.011\ HCT})$$

Contralateral Hemisphere (Group 1)

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (83.0 - 4.24 \cdot 10^{0.02\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (102 - 17.2 \cdot 10^{0.011\ HCT})$$

Caclulated oxygen delivery (HCT x CBF) using amalgamated equations derived from data in figures 4.1.a, 4.3.b and 4.3.c and including variations in plasma viscosity.

Non reactive areas (Group 4)

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (42.5 - 3.43 \cdot PV \cdot 10^{0.0195\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (51.7 - 8.00 \cdot PV \cdot 10^{0.0141\ HCT})$$

Reactive areas (Group 3):

with Pre-dilutional blood flow <30ml/100g/min

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (40.5 - 3.23 \cdot PV \cdot 10^{0.0195\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (51.3 - 8.19 \cdot PV \cdot 10^{0.0141\ HCT})$$

Reactive areas (Group 2):

with Pre-dilutional blood flow >30ml/100g/min

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (73.0 - 3.62 \cdot PV \cdot 10^{0.0195\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (87.5 - 9.92 \cdot PV \cdot 10^{0.0141\ HCT})$$

Contralateral Hemisphere (Group 1)

@ Low shear Viscosity

$$O2\ Delivery = HCT \cdot (83.0 - 3.64 \cdot PV \cdot 10^{0.0195\ HCT})$$

@ High shear Viscosity

$$O2\ Delivery = HCT \cdot (102 - 11.4 \cdot PV \cdot 10^{0.0141\ HCT})$$

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 4. 245:0/0.10     $aPlasma viscosity changes during haemodilution therapy of ce
                   rebral ischaemia
 5. 300:0/0.00     $a177 leaves$bill.
 6. 502:0/0.00     $aThesis: (PhD) University of London 1994
 7. 505:0/0.00     $aLeaves 153-163 are appendices
 8. 960:0/0.01  *  $aPhysiology (Board of Studies)
 9. 970:0/0.01  *  $aPhD (Sci) 1994 IN
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