EXPERIMENTAL MIDDLE CEREBRAL ARTERY OCCLUSION

A study of the effects of controlled hypotension on infarct size, cerebral oedema, and cerebral blood flow in a rat model of focal cerebral ischaemia, and a preliminary evaluation of the effects of immunosuppression by irradiation on the development of cerebral oedema.

By

Roger D. Strachan FRCSE

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DECLARATION

The work presented in this thesis has been planned, performed and interpreted by myself. The author has personally designed, written and typed the entire presentation. Any reference to, or adaptation of, other published work is clearly acknowledged in the text. Where others have contributed in both thought and technical support, due credit is given in the acknowledgments.

signed:

(adopted as holograph)

March, 1994.

PREFACE

The mortality and morbidity related to occlusive cerebrovascular disease, head injury and subarachnoid haemorrhage remains so enormous that a great deal of time and effort has been spent on research into the pathogenesis and pathophysiology of cerebral ischaemia. A better understanding of the mechanisms leading to neuronal injury has brought improvements in the treatment of head injury and subarachnoid haemorrhage. Sadly, however, the hope that specific medical therapies might reduce the disabling impact of occlusive cerebrovascular disease has not been realised.

Neuronal tissue dies four to eight minutes after its blood supply ceases (Kabat et al 1941). Nothing can be done to prevent the irreversible consequences of arresting the circulation to an area of the brain. If changes in medical therapy are to be beneficial after the ischaemic insult, they must prevent either systemic complications such as hypotension or hypoxia, or secondary local complications such as cerebral oedema and raised intracranial pressure. These complications increase morbidity and mortality because they cause further cell damage and death, especially in areas where the circulation is already compromised (but not halted) by the primary event.

This thesis addresses further aspects of these secondary complications. In a rat model of focal cerebral ischaemia, it looks at the effect that systemic hypotension has on the extent of cerebral infarction, the development of cerebral oedema, and the regional cerebral blood flow. It demonstrates that following middle cerebral artery occlusion, there are changes in autoregulatory phenomena that are not isolated to the ipsilateral hemisphere. Staining and histological techniques have shown differences in anatomical and physiological areas of ischaemic damage that indicate disparities in perfusion deficit and anatomical infarction. New techniques for precise experimental blood pressure control are introduced, with comments on the variability of infarct size despite accurate blood pressure control, yet clear evidence that even a modest reduction in blood pressure after an ischaemic insult causes significant increases in infarct size and cortical oedema formation. It explains why the maintenance of blood pressure and cerebral perfusion is so important if the extent of the ischaemic damage is to be minimised. Finally, it looks at the possible role of peripheral blood components in the generation of cerebral oedema. A better understanding of the immune mechanisms involved following an ischaemic insult to the brain may have major therapeutic implications.

ACKNOWLEDGMENTS

This project would never have been possible without the initial encouragement of my consultants at Middlesbrough General Hospital, Mr. Fred Nath and Mr. Sid Marks. They insisted I should apply for a Northern Regional Research Fellowship, and after I successfully gained the fellowship in May 1989, they allowed me to take 1 year off from my substantive post at Middlesbrough to carry out this work.

I am indebted to Mr. A. D. Mendelow, Reader in Neurosurgery at Newcastle General Hospital, for providing laboratory facilities and materials with which to conduct the research, for laying the foundations on which the project was built, for teaching me the necessary surgical techniques, and for providing encouragement and advice when it was required.

Thanks to Phil Kane, a co-researcher in the laboratory, with whom I could share ideas, conversation, humour, and sometimes despair when experiments went terribly wrong.

Thanks to Iain Chambers, Senior Medical Physicist at Newcastle General Hospital, a fellow Scot who invested a great deal of time and technical expertise into the construction and computer management of the equipment essential to experimental success.

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When it seemed that the neuropathological studies might never be satisfactorily completed, Professor David Graham of the Institute of Neurological Sciences, Glasgow,

offered his assistance, and acted as an independent assessor in mapping out the areas of infarction on independently prepared slides. As a leading authority in the field of cerebral ischaemia, I am indebted to him for his invaluable help and advice.

Thanks to Mr. Ian Whittle, Department of Clinical Neurosciences at Edinburgh University, who as nominated supervisor diligently proof-read, criticised and commented upon this dissertation. His constructive advice encouraged me to redesign the lay-out of this thesis, improving its clarity of thought and presentation.

Finally, I need to thank my wife, Jennifer, who never complained when I returned home late *after* the children were in bed, or when I insisted on working on Bank holidays. She spent many hours painstakingly plotting over 15,000 points on logarithmic graph paper, to enable me to calculate by hand over 1000 cerebral blood flows. This thesis is dedicated to her.

March 1994

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ABBREVIATIONS

Alphabetical list of abbreviations used in the text:

ABG	*Arterial Blood Gas*
BBB	*Blood Brain Barrier*
CBF	*Cerebral Blood Flow*
$CMRO_2$	*Cerebral Metabolic Rate for Oxygen*
CPP	*Cerebral Perfusion Pressure*
CSEP	*Cortical Somatosensory Potentials*
CVA	*Cerebrovascular Accident*
CVR	*Cerebrovascular Resistance*
ICC	*Ischaemic Cell Change*
ICP	*Intracranial Pressure*
MABP	*Mean Arterial Blood Pressure*
MCA(O)	*Middle Cerebral Artery (Occlusion)*
PAP	*Pial Artery Pressure*
PBBF	*Percentage Baseline Blood Flow*
PMNL	*Polymorphonuclear Leucocyte*
SAH	*Subarachnoid Haemorrhage*
SG	*Specific Gravity*
TTC	*2,3,5 - Triphenyltetrazolium Chloride*

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FIGURE INDEX

TABLE INDEX

The data tables are grouped together in their own section at the end of the text (after chapter 5) from pages 111 to 129. All data is listed as means ± standard errors.

CONTENTS

Page no.

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Chapter 1

INTRODUCTION

The epidemiology and treatment of acute stroke, literature review, overview of experimental background, and aims of thesis.

1.1 EPIDEMIOLOGY & THERAPY OF ACUTE STROKE

1.1.1. Stroke is defined as rapidly developing clinical signs of focal or global disturbance of cerebral function, lasting more than 24 hours or leading to death, the cause of which is vascular in origin (WHO definition). The overall incidence is 150 - 200 per 100,000 of the general population per annum (Robinson & Toole 1989). In the United States there are 500,000 to 600,000 strokes each year (Robinson and Toole 1989). These cause death in 10-20%, hemiplegia in 60-70%, and dysphasia in 5-10% of cases (Oxfordshire Community Stroke Project 1983). About 70-80% of all strokes are due to thromboembolism, the rest occurring as a result of intracerebral haemorrhage (4%), subarachnoid haemorrhage (10%), or other rarer causes (Robinson & Toole 1989). In the United States the cost of stroke-related health care in 1988 was estimated at 12.9 billion dollars (Stroke Statistics, American Heart Association 1988).

1.1.2. Research into stroke-related problems remains a high priority because the morbidity is high, the cost to the community is great, and because satisfactory medical treatment remains elusive. Anticoagulants, antiplatelet drugs, corticosteroids, barbiturates, fluid restriction, plasma expanders, inotropic drugs, and cerebral vasodilators have all been tried with variable success in the treatment of thromboembolic disease. The current failure of medical treatment has lead to increasing interest in therapeutic strategies designed to limit the extent of infarction i.e. to salvage enough of the "ischaemic penumbra" to alter significantly the patient's functional outcome. This is based on the premise that some of the presenting neurological deficit is due to regions where cessation of neuronal electrical activity has occurred without an inevitable or irreversible cascade to cell death (the ischaemic penumbra). Methods of limiting brain injury include improving perfusion by surgical or medical means, treating ischaemic cerebral oedema, and using drugs to inhibit or arrest the destructive metabolic mechanisms that contribute to tissue damage (Harrison & Russell 1983; Allen et al 1988).

1.1.3. Although prophylactic carotid endarterectomy is of proven benefit in patients with transient cerebral and retinal ischaemia where the stenosis is greater than 70%, surgical

treatment in *acute* thromboembolic stroke remains limited. In established cerebrovascular occlusion, the results of carotid endarterectomy and vascular by-pass procedures have been disappointing (Blaisdell et al 1969; Sundt & Dyken 1983; Allen et al 1988). Revascularisation is only indicated in patients whose stroke is caused by occlusion of a major vessel, and involves identifying the cause by urgent angiography and proceeding to immediate surgery (Allen et al 1988). Increased mortality occurs from haemorrhage into the infarct after revascularisation (Wylie et al 1964; Blaisdell et al 1969). Current clinical trials suggest that emergency cerebral revascularisation is dangerous after an ischaemic deficit is established. Experimentally, Ito et al (1979) showed that restoration of CBF less than 1 hour after left hemisphere ischaemia in a gerbil model significantly reduced brain oedema, but if blood flow was restored more than 3 hours after the ischaemic insult, brain oedema was made much worse.

1.1.4. The use of thrombolytic agents and anticoagulants in acute cerebral infarction may seem rational given the mechanism of the vascular occlusion, but there remains an unacceptable risk of haemorrhage into the infarct (Matthews 1978; Allen et al 1988). It is accepted that anticoagulation has no place in the treatment of completed infarction, but it may have a role to play in patients whose deficit is evolving because of *progressive* thrombotic occlusion of a major vessel (Harrison & Russell 1983). Perhaps the development of thrombolytic agents with more specific clot-dissolution activity will be of some benefit.

1.1.5. In circumstances of reduced cerebral perfusion, cerebral vasodilation also seems a rational therapeutic strategy. However, lactic acidosis within the ischaemic penumbra already leads to maximum vasodilation, so that vasodilators are more likely to have a greater effect on blood vessels within *non*-ischaemic brain. Here, CBF may increase at the expense of the vasoparalysed penumbra (the intracerebral steal phenomenon) (Allen et al 1988). Therapeutic vasodilatation may exacerbate cerebral oedema in ischaemic areas and increase cerebral blood volume in non-ischaemic areas. These changes increase ICP and local tissue pressures making ischaemia worse rather than better (Heiss et al 1976). The recent introduction of the specific cerebral vasodilator Nimodipine, a calcium-channel antagonist, has been beneficial in treating delayed ischaemia after subarachnoid haemorrhage, if treatment can be initiated

before established infarction (Allen et al 1988; Koos et al 1985). Experimentally, *pretreatment* with nimodipine has been shown to reduce infarct size in rats after middle cerebral artery occlusion by 20-60%, with a proportional reduction in cortical oedema (Mohamed et al 1985; Meyer et al 1986; Jacewicz et al 1990). However, its clinical efficacy *after* acute infarction has not been established, although a few studies have reported some benefit (eg. Gelmers et al 1986, 1988).

1.1.6. Alterations in the $paCO_2$ alter cerebral blood volume. Hypercapnia produces cerebral vasodilatation and hypocapnia cerebral vasoconstriction in normal brain by dilatation or constriction of responsive arteries (Harper 1965). The former will increase the size of the vascular compartment and may lead to increased extracellular (interstitial) oedema if the blood-brain barrier is damaged. However, deliberately using hypocapnia to cause vasoconstriction and reduce intracranial pressure might help where brain oedema after infarction causes raised ICP. If it is assumed that autoregulation of cerebral vessel calibre is impaired within the ischaemic area, a "reverse" intracerebral steal phenomenon might be encouraged by vasoconstriction of *normal* cerebral blood vessels, thus "stealing" blood into the ischaemic area (Harrison & Russell 1983; Allen et al 1988). However, trials of vasoconstriction after infarction using a reduction of $paCO_2$ by hyperventilation have shown no benefit (Christensen et al 1973; Harrison & Russell 1983), although it may be of short-term benefit in preventing uncal herniation with large infarcts (O'Brien 1979).

1.1.7. The benefits of corticosteroid treatment for peritumoral cerebral oedema are well recognised, but their mechanism of action is not clearly understood (Maxwell et al 1972; Miller 1987). The results of treatment with corticosteroids after acute stroke remain disappointing and has largely been abandoned. It may be that they are ineffective when the BBB is severely damaged. Enthusiastic reports emerged in the early 1950's, but many later studies never confirmed any significant beneficial clinical effect of corticosteroids on ischaemic oedema after cerebral infarction (Dyken & White 1956; Bauer & Tellez 1973; Wright 1974; Candelise et al 1975; Gilsanz et al 1975). In experimental MCA occlusion, steroids have also been unsuccessful in reducing ischaemic oedema, although they may alter the distribution of large molecules (Hoppe et al 1974). Perhaps the anecdotal improvements

reported with steroid treatment in patients after cerebral infarction are due to more subtle effects of steroids on neuronal function rather than any direct effect on the generation of ischaemic oedema (Harrison & Russell 1983).

1.1.8. Oxygen carriage to the tissue is maximal when the haematocrit is about 33% (Wade 1983; Allen et al 1988; Harrison 1989), and several trials have been carried out using infusions of albumin or low-molecular weight dextran combined with venesection to reduce blood viscosity and theoretically improve local blood flow (Wood et al 1983; Strand et al 1984; Dietis et al 1986; Scandinavian Stroke Study Group 1987; Italian Acute Stroke Study Group 1988). These trials suggest that haemodilution needs to be achieved quickly after ischaemic stroke which may be logistically difficult. Overall, results have been conflicting, some studies suggesting a beneficial effect (eg. Gilroy et al 1969; Wood et al 1983) and others proving disappointing (eg. Matthews 1976; Strand et al 1984; Scandinavian Stroke Study Group 1987; Italian Stroke Study Group 1988). Outcome at 6 months has not been altered (Matthews 1976). Perhaps in some patients, *early* treatment is helpful in reducing neurological deficit (Grotta 1987).

1.1.9. Massive infarction with associated brain swelling causes severe disability or death by uncal herniation (Ng & Nimmannitya 1970). In smaller infarcts, although survival is commonplace, cerebral oedema may compound the disability by increasing intracranial pressure and contribute to reduced blood flow in areas where perfusion is already compromised. The preservation of CBF and the reduction of brain swelling after focal cerebral ischaemia are important factors in determining outcome. It is for this reason that these aspects of cerebral ischaemia have been intensively studied for half a century. This project is designed to develop these studies further, evaluating the importance of maintaining blood pressure, reducing brain swelling, and maintaining cerebral blood flow after cerebrovascular occlusion.

1.2 CEREBRAL ISCHAEMIA & BRAIN OEDEMA

1.2.1. Cerebral ischaemia develops when the delivery of oxygen to the brain is impaired so that cellular energy production by aerobic glycolysis is insufficient to meet cellular demand. Systemic events such as cardiac arrest or respiratory obstruction lead to global ischaemia by a reduction in adequate arterial oxygenation. Other causes of hypoxia or hypotension will contribute to cerebral ischaemia depending on the nature, extent and duration of the insult. Local cerebrovascular causes such as occlusion, haemorrhage or vasospasm will lead to focal ischaemia.

1.2.2. Complete brain ischaemia causes cessation of neuronal electrical activity, failure of the sodium/potassium pump, depletion of glucose, and increase in lactate (O'Brien 1979). This leads to irreversible cell death. Following occlusion of a cerebral vessel, ischaemia is rarely if ever total. Experimentally, after MCA occlusion in the rat, it is estimated that the ischaemic core (no blood flow) is confined to a small portion of the olfactory cortex and the medial aspect of the caudate (Shiraishi & Simon 1989). Cerebral blood flow (CBF) beyond the site of occlusion of the middle cerebral artery (MCA) may still be 25% of normal (Symon et al 1974b). Residual perfusion persists in the ischaemic area and is dependent on the collateral circulation and local perfusion pressures. Measures that maintain this residual perfusion are important in determining the final outcome after stroke. Failure of synaptic transmission, Na^+/K^+ pumping and energy metabolism will occur if perfusion falls below a certain level. This "critical threshold" probably occurs when CBF falls below 9-10 ml/100g/min (Symon et al 1979; Bell et al 1985). Below this threshold, depletion of high-energy phosphates, intracellular potassium loss, efflux of sodium and water, and membrane disruption lead to irreversible cell death.

1.2.3. Even in situations where a degree of energy failure exists, residual perfusion can supply sufficient oxygen to maintain normal concentrations of ATP. It is therefore important to encourage perfusion of the "ischaemic penumbra", as reversal of moderate energy imbalance can lead to neuronal recovery. Histologically, infarction appears to correlate well with areas where CBF has fallen below 10-12 ml/100g/min (Symon & Brierley 1976).

Maintaining cerebral perfusion above the critical threshold where energy failure occurs can prevent infarction (Astrup et al 1981). Early ischaemic dysfunction of the membrane Na^+/K^+ pump with efflux of sodium and water is one of the factors leading to the generation of cytotoxic brain oedema. Where ischaemia exists, oedema is sure to follow, unless perfusion is re-established within 30 minutes (Bell et al 1985).

1.2.4. Cerebral oedema is the abnormal accumulation of water associated with an increase in brain tissue volume (Klatzo 1967; O'Brien et al 1979; Ito et al 1979). After ischaemic damage to the brain, it is one of the most significant clinical complications, and remains the most important cause of death. The formation of oedema is divided into cytotoxic and vasogenic types (Klatzo 1967). Following cerebral ischaemia these are not mutually exclusive. Cytotoxic oedema is caused by any factor which damages cell metabolism, especially the membrane sodium-potassium pump, resulting in an increase in intracellular water. Vasogenic oedema results from the extravasation of fluid from intravascular to extravascular (interstitial) compartments. Cytotoxic oedema appears to be important in situations of permanent ischaemia without reflow (Ito et al 1979).

1.2.5. Vasogenic oedema is caused by increased blood-brain barrier (BBB) permeability, but the passage of water across the endothelium can occur even if the endothelial tight junctions are preserved (intact BBB) (Hossmann & Olsson 1971). It is important in situations of incomplete ischaemia or where blood flow has been re-established after complete ischaemia. The restoration of blood flow to areas where BBB damage has occurred permits the extravasation of fluid from the intravascular compartment. Katzman et al (1977) commented that significant increases in brain tissue volume and fluid occurred *before* extravasation of protein or other indicators of BBB breakdown. Recent studies confirm that the increase in brain water content seen within 4 hours of focal cerebral ischaemia is not related at that time to BBB breakdown (Hossmann & Olsson 1971; O'Brien et al 1973, 1974; Bell et al 1985; Hatashita & Hoff 1986b, 1988, 1990). Therefore, the evidence suggests that brain oedema due to focal ischaemia is initially cytotoxic in type, later followed by vasogenic oedema.

1.2.6. The effect of a modest short-term fall in mean arterial blood pressure on the extent of early oedema formation has not been clearly defined. The importance of systemic blood pressure in the generation of cerebral oedema is emphasised by O'Brien (1979). He comments that any evaluation of ischaemic cerebral oedema must "take cognizance of the rate of development and the duration and depth of ischemia *as well as the effect of variations in blood pressure*" (my italics). A significant *elevation* in blood pressure can fuel hydrostatic pressures, especially in any situation where dysautoregulation has lead to maximal arteriolar dilatation (eg. experimentally by raising MABP in association with hypercapnia) or where BBB breakdown has occurred eg. hypertensive encephalopathy. This leads to a rise in the ECF volume, and the rapid spread of oedema, particularly through white matter, by flow along hydrostatic pressure gradients. Matakas et al (1972) demonstrated experimentally in monkeys that following cerebral compression by epidural balloon inflation, elevation of the blood pressure not only increased CBF and CPP, but increased oedema formation and ICP. A significant *reduction* in systemic blood pressure, despite reducing the hydrostatic pressure, can lead to a critical reduction in blood flow especially where autoregulation has failed (para 1.3.10). In one particular study, brain water and electrolyte content remained unchanged after MCA occlusion in dogs. However, significant oedema and infarction developed when the ischaemic insult was made greater by a reduction in mean blood pressure (Schibata et al 1974).

1.2.7. The fluid that accumulates within the brain following ischaemia must be from the vascular compartment. Despite considerable metabolic defects, oedema does not develop in areas of *complete* circulatory arrest (Hatashita & Hoff 1986a, 1986b). It develops in areas where the circulation is compromised, and is proportional to depth and duration of ischaemia. When CBF is reduced below 20 ml/100g/min, significant oedema occurs in parallel with early synaptic transmission failure, but before membrane ionic pump failure (CBF $<$ 11 ml/100g/min) (Symon et al 1979). Moreover, in the MCA occlusion model, oedema is greater in the core of the infarct where the depth of ischaemia is greater, rather than in the periphery (Hatashita & Hoff 1986a). In this study, although significant oedema developed within the core of the MCA territory at 1 hour post-occlusion, a significant decrease in specific gravity of the periphery was not demonstrated before 6 hours.

1.2.8. The factors responsible for the development of oedema include hydrostatic and osmotic pressure gradients, tissue compliance and resistance, and the ability of the capillary endothelium to convey water (hydraulic conductivity) (Hatashita & Hoff 1990). Early experimental studies confirmed that oedema formation begins before blood-brain barrier (BBB) breakdown is demonstrable. Kapuściński (1974) induced massive but reversible cerebral oedema without impairment of the BBB using Levine's model (bilateral carotid ligation + hypoxia in rats; Levine 1960). Other studies confirm that the permeability of the BBB to albumin or Evans' blue dye is unchanged in the early stages of ischaemia (O'Brien et al 1974b; Hatashita et al 1988). Hydraulic conductivity is initially more important; osmotic pressure gradients develop later.

1.2.9. Most studies confirm that oedema is detectable as early as 1 hour after the onset of deep ischaemia, when CBF is below the threshold for membrane failure, representing alterations in intracellular/interstitial concentrations of sodium and potassium (Bell et al 1985; Hatashita & Hoff 1986a). However, at the periphery of an infarct where the CBF is normally greater than 15-20 ml/100g/min, the situation is different. Significant intracellular oedema will develop by 3 hours, which appears to parallel an increase the sodium concentration and a decrease in the potassium concentration, but the correlation between brain water and sodium contents only becomes significant after 6 hours (Gotoh et al 1985). Early ischaemic oedema is therefore related to (1) increased hydraulic conductivity caused by changes in BBB permeability to water; (2) alterations in hydrostatic pressure gradients between blood and brain parenchyma; and (3) energy failure and dysfunction of the membrane Na^+/K^+ pump (Hatashita & Hoff 1986a). After some hours, the hydrostatic pressure gradient will fall as tissue pressure rises, but loss of autoregulation will allow arterial pressure to be directly transmitted to the brain parenchyma.

1.2.10. In parallel with the haemodynamic mechanisms postulated to explain the generation of cerebral oedema, the activity of the cellular components within the blood is also important. In situations of cerebral ischaemia and endothelial damage, a complex interaction between blood components and endothelial collagen occurs with platelet aggregation and liberation of many factors eg. plasminogen, kallikrein, and prostaglandins. Cell-surface

membrane changes take place with increased endothelial permeability and vesicular transendothelial transfer of proteins, electrolytes and water (Hallenbeck et al 1982). This heralds the onset of BBB breakdown and associated vasogenic oedema. It develops more rapidly in situations where the circulation is re-established after a period of ischaemia with faster and greater increases in brain water (Ito et al 1979; Shigeno et al 1984).

1.2.11. The precise timing of the onset and peaking of cerebral oedema varies slightly from one study to another. In this thesis, experiments have been designed to assess oedema formation at 24 and 72 hours after proximal MCA occlusion, to ascertain if there was any significant progression of oedema over that time period. This also allowed the selection of a time-course for experiments designed to assess the effects of immunosuppression on brain oedema. The onset of experimentally measurable oedema is between 1 hour (Hatashita & Hoff 1986a, 1990) and 3 hours (Gotoh et al 1985). In non-survivor experiments, it was hoped that any difference in oedema formation between experimental groups would be detected by measuring specific gravity at 4 hours post-occlusion. Many studies have confirmed that MCA occlusion in the rat is a useful model for studying the pathophysiology of cerebral oedema. However, very few studies have specifically studied the effect of modest hypotension on the extent of oedema formation in this particular model.

1.3 CEREBRAL BLOOD FLOW & AUTOREGULATION

1.3.1. The brain represents approximately 2% of the total body weight, but it requires 15% of the resting cardiac output and at rest uses 25% of the total inspired oxygen (McHenry 1983). The utilisation of oxygen by the brain can be calculated if arterial and venous (jugular bulb) oxygen concentrations and the CBF are known ($CMRO_2 = A_0 - V_0 / CBF$). Cerebral blood flow (CBF) in blood volume per unit tissue mass per unit time will depend on the cerebral perfusion pressure (CPP) and the cerebral vascular resistance (CVR) i.e. $CBF = CPP / CVR$. At rest, the pressure in the jugular bulb is essentially zero, so the CPP will equal the mean arterial blood pressure (MABP) minus the intracranial pressure (ICP). Under physiological conditions, the ICP is low (0 - 10 mmHg) so cerebral vascular

resistance will generally vary directly with the mean arterial blood pressure, and inversely with the cerebral blood flow.

1.3.2. The vascular resistance (R) depends on three factors: the length of the vessel (l), blood viscosity (η), and the vessel calibre (r). Pouseuille's Law relates these as $R = l \times \eta \times 8/r^4$. The vascular resistance is greatest in the small arteries and arterioles: about 30% of the vascular resistance comes from arteries over 100 μm and 40% from arterioles between 40 and 100 μm in diameter (Hacke et al 1991). Blood viscosity is determined by serum protein concentration and blood cell content (especially red cell mass). Where blood flow is rapid as in large vessels, viscosity is low, but it increases in small vessels because of erythrocyte aggregation. This is important in situations where perfusion pressure is reduced and may contribute to the "no-reflow" phenomenon, potentially made worse in areas where a reduction in MABP has augmented the fall in CPP.

1.3.3. Control of blood flow through the brain differs from that of other organs because of its unique requirements and demands. In an excellent review of the physiology of cerebral blood flow, Lassen & Christensen (1976) divided the control of CBF into four components: metabolic, autoregulatory, chemical and neurogenic. The final common pathway will involve a complex interaction between all these components; they are not mutually exclusive. Under normal conditions, although the average hemispheric blood flow in man is about 50 ml per 100 gram tissue per minute, regional differences in blood flow are apparent depending on local neuronal function and metabolic activity. At a *regional* level, increased blood flow is required to support increased oxidation of glucose to provide more energy for ion-pumping and transmitter synthesis and uptake. In its extreme form, this relationship is demonstrated during seizure activity. The exact mechanisms that couple metabolism to local alterations in blood flow in the normal physiological state are still not clearly understood, but local acidosis and changes in extracellular potassium concentration may be involved (Lassen & Christensen 1976).

1.3.4. *Autoregulation* is the term attributed to the inherent ability of the brain to maintain a relatively constant blood flow despite variations in systemic blood pressure. As soon as

it was possible to make direct measurements of cerebral blood flow, the concept that CBF responded passively to systemic blood pressure was abandoned. In terms of biological survival, it is not surprising that the brain should demonstrate a remarkable capacity to autoregulate its own blood supply in view of its unique physiological demands. CBF is maintained at a relatively constant level within a systemic arterial pressure range of 50 - 150 mmHg (Hacke et al 1991) and above and below this range CBF tends to become passively dependent on systemic blood pressure. These limits will differ depending on the age of the individual, and the co-existence of any chronic disease state eg. in chronic hypertension, the curve is shifted to the right (so hypotension will be less well tolerated). Experimentally, the limits differ depending on the particular animal model under investigation, but range from 50-60 mmHg to 150-180 mmHg.

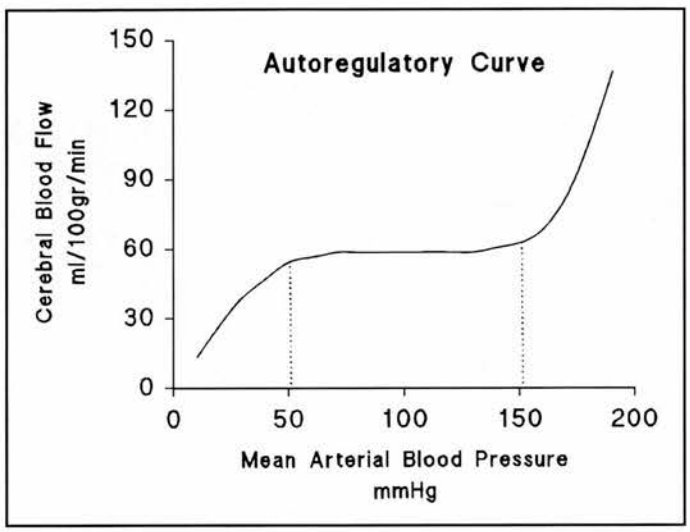

Figure 1
Schematic diagram of typical autoregulatory curve.

1.3.5. The cerebrovascular resistance is controlled by constriction or dilatation of the cerebral arterioles. In the intact animal or man where the circulation is physiologically normal, as the blood pressure is increased, there is vasoconstriction of the cerebral arterioles increasing vascular resistance maintaining a constant blood flow. Similarly, vasodilatation occurs in response to a reduction in blood pressure. The intrinsic regulation of cerebrovascular tone was first suggested by Fog (1938) who observed that changes in the calibre of pial vessels were inversely related the systemic blood pressure. Since then, the phenomenon of autoregulation has been intensely investigated, as the loss of normal control of CBF is crucial to the degree of cerebral ischaemia and extent of neuronal damage that occurs after a vascular event. The physiological mechanisms that generate the capacity to autoregulate are still incompletely understood, but three factors are implicated: the intrinsic ability of smooth muscle to contract in response to stretching (the so-called *Bayliss*† effect), a response to local metabolites, and a neurogenic reflex.

† *Bayliss (physiologist), described effect in 1902.*

1.3.6. Aaslid et al (1989) studied the flow velocity in the middle cerebral artery in 10 normal awake human subjects, and noted that the flow velocities returned to normal within 4 seconds after a fall in blood pressure. They suggested that such a response was mediated through a metabolic mechanism, but it seems more likely that such a rapid response is myogenic or neurogenic in origin. In contrast to this, dilatation of pial vessels is noted to occur after a relatively long delay of 2 to 3 minutes (Mchedlishvili et al 1973), suggesting that a causal relationship between intravascular pressure changes and vascular responses is unlikely. Harper (1966) also pointed out that a *sudden* fall in blood pressure causes a marked reduction in CBF, which lasts for at least 2 minutes, and concludes that such a reaction time is too slow if a myogenic mechanism is responsible. In studies of the cerebral collateral circulation involving the direct microscopic observation of pial vessels, Meyer & Denny-Brown (1957) noted that rapid alterations in vascular calibre occurred in anastomotic vessels (diameter 50 to 250 mμ) within 30 seconds of MCA occlusion in monkeys. They concluded that the Bayliss effect was the most important *immediate* factor in response to changes in intraluminal pressure.

1.3.7. The *chemical* control of cerebral blood flow is intimately related to arterial pCO_2 and pO_2. Variations in pCO_2 have enormous impact on CBF; hypercapnia causes marked cerebral vasodilation and hypocapnia marked vasoconstriction. Within normal pCO_2 values, CBF changes about 4% for each mmHg change in arterial pCO_2 (Reivich 1964). CO_2 reactivity is also dependent on blood pressure: when blood pressure is low, the vessels are maximally dilated, and CO_2 regulation is lost. This effect of CO_2 on vascular calibre is related to local variations in pH and tissue acidosis. There are good physiological reasons to increase local CBF when tissue CO_2 concentration and pH falls so that the accumulating CO_2 is washed out and pH is restored. Local tissue acidosis should not be confused with changes in *arterial* H^+ concentration which does not alter cerebral blood flow providing the pCO_2 is kept constant (Harper 1966). Alterations in extracellular K^+ concentration and vascular reactivity to potassium have also been implicated in the control of CBF (Somjen 1979), and adenosine is known to cause experimental vasodilatation of cortical vessels when applied topically (Winn et al 1981). The pO_2 also has a regulatory effect on CBF, but this is only significant in situations of relative hypoxia ($pO_2 < 50$ mmHg) when cerebral

perfusion rises rapidly (Hacke et al 1991). This effect may also be mediated via tissue acidosis due to hypoxia, since significant rises in brain lactate occur at pO_2 levels below 50 mmHg (Siesjö & Nielson 1971).

1.3.8. The *neurogenic* control of CBF still remains controversial. The existence of nerve fibres innervating cerebral vessels has been known for over 200 years, yet the importance of neurogenic factors controlling cerebral blood flow still remains unclear. Mchedlishvili (1980) reviewed the experimental evidence for neurogenic mechanisms controlling CBF, and describes several studies that demonstrate a positive relationship. Arteries and arterioles over the cortical surface and within the brain are supplied by a network of both sympathetic and parasympathetic nerve fibres, yet maximal stimulation of these nerves reduces (sympathetic) or increases (parasympathetic) cerebral blood flow by only 5-10% i.e. equivalent to changes in arterial pCO_2 of only 1-2 mmHg (Lassen & Christensen 1976).

1.3.9. Hypovolaemic hypotension is less well tolerated than drug-induced hypotension, because the former causes increased sympathetic activity, including sympathetic vasoconstriction fibres to the cerebral vessels. This shifts the autoregulatory curve to the right, increasing the lower limit of autoregulation so that brain ischaemia develops at a higher mean arterial blood pressure (Fitch et al 1975; Lassen & Christensen 1976). Following partial sympathetic block in baboons, autoregulation in response to haemorrhagic hypotension was present down to a pressure of 40 mmHg compared to 60-70 mmHg in intact animals (Harper 1975). While this effect in situations of hypovolaemia is not of obvious physiological benefit, increased sympathetic activity by enhancing arteriolar tone and preventing arterial over-stretching will protect the brain in situations of acute *hyper*tension eg. arousal, fear or anger (Fitch et al 1975). Neurogenic mechanisms therefore have some physiological role to play in the control of CBF, but there is no substantial evidence linking local changes in blood flow (eg. from increased metabolic activity) to neuronal reflexes (McHenry 1983). This aspect of the autonomic control of cerebral blood flow was eloquently presented by Harper at the 9th conference on Cerebral Vascular Diseases (Harper 1975). The author supports Harper's hypothesis that the control of autoregulation involves extraparenchymal and intraparenchymal vascular resistances working

in series so that the control of large vessels differs from that of the smaller pial arterioles and capillaries. Thus, the maintenance of normal CBF will involve an integration of neurogenic, myogenic and metabolic influences. However, the "drive" for normalising CBF must be occurring at a tissue level, since it is cellular demands for homeostasis and supply of metabolic substrates that is of paramount importance to the physiological well-being of the brain.

1.3.10. If the ability to maintain a constant blood flow is dependent on alterations in cerebrovascular resistance, then it will be lost if blood vessels are already maximally dilated or constricted. Kindt et al (1967) confirmed in their model (carotid occlusion in goats) that blood flow varied directly with blood pressure in areas where the vessels were maximally dilated because of a marked fall in intravascular pressure and rise in pCO_2. Harper (1966) has confirmed that cerebral ischaemia impairs autoregulation, where vessels are already maximally dilated because of a low pO_2 and/or a high pCO_2. Similarly, significant hypotension (MABP $\leq$ 50 mmHg) causes cerebrovascular dilatation because of a low tissue pO_2; under these circumstances, CBF does not alter with changes in arterial pCO_2, because the maintenance of normal cerebral perfusion takes precedence, and the vasoconstrictive effect of hypocapnia is abolished (Harper 1965). Moreover, autoregulation is abolished by hypercapnia, or profound hypocapnia (eg. hyperventilation), because the vessels are already fully dilated or constricted (Harper 1975). In situations of poor cerebral perfusion and associated cerebral ischaemia (impaired autoregulation), the "therapeutic" benefits of hyperventilation may be questionable.

Other mechanisms may be contributing to the failure of autoregulation in situations of reduced perfusion. Sundt & Michenfelder (1972) noted falls in ATP and 10-fold increases in lactate concentrations 2 to 4 hours after MCA occlusion in monkeys, and postulated that the loss of autoregulation and "luxury perfusion" seen in cerebral ischaemia is due to a localised metabolic acidosis from the accumulation of lactic acid. In addition, the ischaemic insult may impair the vascular responses to alterations in flow by directly damaging the vessels (Waltz & Sundt 1967).

1.3.11. The importance of autoregulation, and the circumstances under which it fails, are

central to the aims of this thesis. Previous studies have shown that where the mean blood pressure is reduced to the lower limits of autoregulation (or beyond), then there are significant deleterious effects on the extent of ischaemic injury (eg. Osborne et al 1987). However, Symon et al (1976) have shown that there is a proportional relationship between autoregulatory impairment and depth of ischaemia i.e. the autoregulatory capacity is *less* impaired in areas that are *less* ischaemic. Their results suggested that autoregulation was maintained to some extent where regional CBF was greater than 40% of control flow values (although the autoregulatory curve was still shifted to the right), with vasoparalysis occurring below this level. Similarly, Dirnagl & Pulsinelli (1990) have shown that in ischaemic neocortex, although autoregulation is attenuated, it is not lost altogether until CBF falls below 30% of normal. At a mean blood pressure reduction of only about 30%, it might be expected that autoregulation would still be effective in lessening the effects of reduced perfusion. The author has therefore ascertained the autoregulatory characteristics of the particular experimental model used in this thesis.

1.4 THE IMPORTANCE OF BLOOD PRESSURE

1.4.1. Maintaining mean systemic blood pressure and normal cerebral perfusion minimises neurological deficits and optimises recovery in situations of brain injury and ischaemia. In some patients, increasing mean arterial blood pressure (MABP) after occlusive stroke can reverse ischaemic neurological deficits and improve long-term outlook (Denny-Brown 1951; Wise et al 1972). Farhat & Schneider (1967) cited cases where significant neurological deficits in four patients, arising as a result of a fall in blood pressure in two of them, were reversed by pharmacologically raising the blood pressure above normal. Experimentally, Waltz & Sundt (1967) observed changes in the cortical microcirculation of squirrel monkeys after middle cerebral artery occlusion. These changes (vasoconstriction, cessation of blood flow, aggregation of erythrocytes and formation of platelet thrombi) developed more rapidly and were more widespread in animals where the blood pressure fell by more than 20 mmHg. Crowell & Olsen (1972) demonstrated impairment of filling in small vascular channels after permanent or temporary MCA occlusion in monkeys. Non-filling of the microvasculature

was far more extensive where hypotension was combined with focal ischaemia, involving almost the entire MCA territory. Other studies have confirmed that haemodynamic changes in cortical vessels supplied by the middle cerebral artery (eg. reduced CBF and intraluminal aggregation of blood elements) are much worse if hypotension is combined with MCA occlusion (Thomson & Smith 1951; Waltz & Sundt 1968; Waltz et al 1974; Hayakawa et al 1975).

1.4.2. Intracranial collateral circulation prevents significant brain injury after occlusion of an extracranial artery unless an additional stress such as hypotension is added (Graham 1988). In a rat model of cerebral ischaemia, whereas hypotension or carotid occlusion alone does not lead to ischaemic brain damage, unilateral carotid ligation in the presence of hypotension (40 - 50 mmHg) causes significant infarction (Mendelow et al 1984). In this latter study, the most vulnerable areas appeared to be the lateral neocortex, caudate nucleus, hippocampus and thalamus. Acute hypotension is also implicated in the production of boundary zone infarcts. Autoregulation is unable to maintain satisfactory blood flow in areas remote from parent arteries if blood pressure is reduced rapidly (Adams 1974). Brierley et al (1969) produced typical boundary-zone infarcts in primates by rapidly reducing the blood pressure, providing cerebral perfusion pressure fell below 25 mmHg for at least 15 minutes. Clinically, hypotensive stresses during cardiopulmonary bypass procedures are known to cause significant post-operative cerebral dysfunction, including irreversible coma and death. Neuropathological studies in patients have shown cortical necrosis worse in arterial boundary zones (Stockard et al 1974).

1.4.3. Many pathophysiological mechanisms act to increase and decrease ischaemia during periods of hypotension. The extent to which each contributes to further neuronal injury is still not clear. Morphological changes take place in the cortical microvasculature after MCA occlusion which include changes in vessel diameter, increases in capillary length and changes in capillary surface to volume ratio, representing adaptations to the reduced blood volume and acting to improve blood flow (Huniziker et al 1974). This adaptation is greater in situations of reduced blood pressure leading to improved cortical microcirculation and higher O_2 diffusion capacity. The relationship between hypotension, reduced blood flow and

availability of oxygen to the brain is of particular importance. Thomson and Smith (1951) suggested that the greater histological evidence of brain injury in hypotensive animals occurred because of decreased hydrostatic pressure and *anaemic anoxia*. Gygax et al (1974) demonstrated that although normal EEG activity was maintained in normoxic animals even in the face of a failure of autoregulation and a 50% reduction in cortical microflow, EEG activity in oligaemic animals was extraordinarily sensitive to arterial pO_2. This suggested that the arterial oxygen supply was already being fully utilised. The importance of oxygen availability rather than blood flow *per se* has been demonstrated by Astrup et al (1981). They concluded that if hypotension is combined with hypoxia, the critical level of blood pressure at which neuro-electrical and ion pump failure occurs is almost doubled i.e. much higher than in normoxic animals. Hypotension is known to abolish the normal rise in cerebrovascular resistance and fall in CBF seen in response to hypocapnia (Harper & Glass 1965; Artru & Colley 1984), and Harper (1965) postulated that tissue oxygen levels in situations where O_2 delivery is confounded further by hypotension will override the vasoconstrictive effects of a low pCO_2.

1.4.4. With respect to infarct size, Thomson & Smith (1951) showed that in groups of dogs and monkeys rendered hypotensive for 1 hour after MCA occlusion, neurological deficits were greater, refilling of cortical surface vessels was more sluggish, and post-mortem histological signs of ischaemic damage were more extensive. Ralston et al (1954) examined the effect of oligaemic and pharmacologically induced hypotension on the histological extent of infarction after MCA occlusion in monkeys. They noted that infarcts were topographically more consistent and much more extensive in the group rendered hypotensive by exsanguination (but less so in the chemically-induced hypotensive group). In more recent experimental studies, hypotension after either unilateral carotid occlusion or MCA occlusion is known to increase infarct size (Mendelow et al 1984; Osborne et al 1987). Cerebral autoregulation is effective in maintaining blood flow at levels of MABP levels above 50 mmHg. Most experimental studies looking at the effects of hypotension on cerebral ischaemia have maintained MABP around this level, or even lower. However, there is a paucity of information relating the effect of a modest reduction in mean arterial blood pressure to infarct size, oedema formation, and cortical CBF.

1.5 NEUROPATHOLOGY OF FOCAL CEREBRAL ISCHAEMIA

1.5.1. Brierley and colleagues (1973) define ischaemic cell change (ICC) as a process that "transforms a normal neuron into a more or less naked and very shrunken nucleus which will sooner or later be engulfed by phagocytes". It arises as a result of neuronal hypoxia, irrespective of the mechanism that gives rise to the hypoxic insult. Clinically, ICC is seen in the brains of patients dying 6 hours after cardiac surgery (Brierley 1963), and Brierley and co-workers confirm that ICC is evident when the interval between the hypoxic episode and death is only 5 to 6 hours (Brierley et al 1973). However, experimentally histological changes are seen as early as 15 minutes after the insult. Initially, small "microvacuoles" are seen in the cytoplasm, axon and dendrites, and electron microscopy suggests that these are expanded mitochondria with intact membranes but disordered cristae. This progresses to the typical appearances of ICC between 30 minutes and 4 hours (depending on the size of the neuron), where the cell cytoplasm becomes darker, shrunken and more electron dense, and the nucleus becomes smaller, darker, loses microscopic detail, and typically becomes triangular in appearance (Garcia et al 1971; Brierley et al 1973; Ginsberg et al 1978).

1.5.2. When these features are apparent, the neuron is already irreversible damaged. The cells then develop incrustations on the their surface, continue to shrink, and become progressively darker in appearance. Interestingly, ultrastructural studies of the vascular endothelium during this period have demonstrated that it remains *intact* (Garcia et al 1971), in keeping with physiological studies confirming blood-brain barrier integrity. The number of affected neurons is proportional to the severity of the ischaemic insult, but in each affected neuron the time course of the ischaemic process is not altered by the depth of the insult. These microscopic changes are easily confused with "dark cell" and "hydropic cell" changes - cytological artefacts that occur if fixation techniques are inappropriate or inadequate (ie. *immersion* or poor perfusion fixation; surface injury to the brain; excessive preliminary saline washout) (Cammermeyer 1961; Brierley et al 1973). The "dark cell" is a hyperchromatic neuron that is irregular, shrunken and stains darkly with all stains; the "hydropic cell" is swollen and paler on staining than normal.

1.5.3. Precise histological studies demand well-established perfusion and fixation techniques to obtain specimens that are free of cytological artifacts. Iizuka and co-workers (1989) have recently commented that "uniform and reproducible perfusion fixation is difficult to carry out in brains that have suffered *focal ischaemia due to permanent arterial occlusion*" (my italics). Because of the problems of inter-observer reliability and histological accuracy, histological grading systems are currently being devised and evaluated (Eke et al 1990), but problems still remain in defining early cytological evidence of ischaemia that is comparable between studies. Finally, although many studies have described histological changes as a result of *severe* ischaemia, there is a paucity of information on acute histological changes arising in areas of presumed reversible ischaemic damage i.e. in the ischaemic penumbra (Iizuka et al 1989). It is for these reasons that the author has avoided detailed histological studies that may be controversial and open to criticism. In this thesis, the specific histological or ultrastructural changes seen as a result of cerebral ischaemia (with and without hypotension) have not been studied, as they have been extensively described in many other studies (eg. Brown & Brierley 1968; Brierley et al 1969; Garcia et al 1971; Ginsberg et al 1978; Tyson et al 1981; Osborne et al 1987).

1.6 IMMUNOLOGICAL ASPECTS OF BRAIN OEDEMA

1.6.1. The concept that white blood cells are implicated in the pathogenesis of cerebral ischaemia and stroke is not a new one, but their precise role remains unclear. Leucocytes (polymorphonuclear leucocytes [PMNL], monocytes and macrophages) have been primarily implicated (Kochanek & Hallenbeck 1992), but lymphocytes and platelets may be involved. Earlier experimental work has demonstrated the accumulation of white cells in cerebral infarcts, and more recent studies have implicated leucocytes in the development of early ischaemic injury and neuronal dysfunction (Garcia & Kamijyo 1974; Pozzilli et al 1985; Dukta et al 1989; Grøgaard et al 1989). Garcia & Kamijyo (1974) demonstrated the accumulation of PMNL's in the penumbra around the infarct core after stroke in humans, which was maximal at 48-72 hours. Hallenbeck et al (1986) demonstrated the accumulation of indium^{111} - labelled autologous PMNL's during the first 4 hours of reperfusion after

cerebral ischaemia in dogs induced by air embolism. They noted that the accumulation of PMNL's correlated with the extent and depth of ischaemia, assessed by the reduction of amplitude of cortical somatosensory evoked potentials (CSEP). Several recent studies have implicated circulating neutrophils as contributing to the generation of ischaemic brain injury and cerebral oedema (Dutka et al 1989; Grøgaard et al 1989; Vasthare et al 1989; Schoëttle et al 1990). Lymphocytes have also been shown to accumulate within a cerebral infarct (Pozzilli et al 1985) and platelets have been shown to accumulate in regions of low blood flow, although their role in contributing to the extent of oedema has not been established (Obrenovitch & Hallenbeck 1985).

1.6.2. The effect of PMNL depletion in experimentally induced ischaemia using a wide variety of models is under intense study, but the evidence still remains conflicting. Romson et al (1983) established that neutrophil depletion in the dog reduced the extent of ischaemic myocardial injury, with a 43% reduction in the size of myocardial infarction. In *global* ischaemia models, PMNL depletion has attenuated post-ischaemic hypoperfusion and improved CBF and CSEP amplitude (Grøgaard et al 1989; Vasthare et al 1990). In other *focal* ischaemic models, PMNL depletion appears to have had beneficial effects on CBF and CSEP amplitude (Dutka et al 1989) and on CBF, infarct size and ICP (Bednar et al 1991). However, other studies have failed to demonstrate either a reduction in infarct volume after experimental MCA occlusion (Takeshima et al 1991) or a reduction in post-traumatic oedema (Schürer et al 1990; Uhl et al 1990) with neutropenia induced by monoclonal antibodies or antineutrophil serum. No studies have been designed that have assessed the effect of selectively depleting lymphocytes or platelets rather than PMNL's on the extent of ischaemic injury or generation of cerebral oedema.

1.6.3. Recent studies have demonstrated that using a form of immunosuppression† by whole body irradiation reduces brain oedema following experimental intracerebral haemorrhage in the rat (Kane et al 1992). These studies confirmed that whole body irradiation was effective in depleting the animals of circulating white blood cells and platelets. This method of global immunosuppression was used in this thesis to establish if an analogous depletion of circulating leucocytes and platelets would produce similar effects in a different experimental model of ischaemic cerebral oedema, i.e. after right middle cerebral artery occlusion. Other

† *for definition, see page 61, paragraph 3.8.2.*

studies designed to assess the time-course of cerebral oedema after proximal MCA occlusion suggested that cerebral oedema was well established at 24 hours, so the author chose to study the effects of white cell and platelet depletion on cerebral oedema at 24 hours post-occlusion.

1.7 AIMS OF THESIS

1.7.1. Although cerebral autoregulation is impaired in situations of cerebral ischaemia, it is not lost altogether until cerebral blood flow falls below 30-40% of normal values (Symon et al 1976; Dirnagl & Pulsinelli 1990). Thus, a modest reduction in systemic blood pressure in the presence of focal cerebral ischaemia might be better tolerated than expected. The author believes that this is not the case. The hypothesis to be tested is that even a small reduction in mean arterial blood pressure (and therefore cerebral perfusion pressure) will significantly affect the extent of ischaemic injury after focal cerebral ischaemia. The author also believes that the area most vulnerable to a fall in cerebral perfusion after arterial occlusion will be the area adjacent to the infarct (the "penumbra"). Here, the circulation is dependent more on collateral rather than direct supply making it susceptible to falls in mean blood pressure.

To test these hypotheses, a rat model of focal cerebral ischaemia has been used, and a 25-30% reduction in mean arterial blood pressure induced after vessel occlusion. Treatment (hypotensive) groups have been compared with control (normotensive) animals by measuring cerebral oedema and cerebral blood flow within the ischaemic penumbra, and total infarct volume, after proximal middle cerebral artery occlusion. In support of the level of induced hypotension used in this thesis (70 mmHg), a separate study has been designed to confirm the lower limit of blood pressure at which cerebral autoregulation fails, and to assess how this is affected after MCA occlusion. The hypothesis is that cerebral autoregulation is still intact at a mean arterial blood pressure of 70 mmHg, and after proximal MCA occlusion is still functional (albeit impaired) in the cortical area where blood flow is being measured in the experimental model used in this thesis.

1.7.2. Such studies depend on maintaining a period of induced hypotension after the ischaemic insult that is precise and reproducible. A new method of hypovolaemic blood pressure control for the experimental model used in this thesis is presented and evaluated. Although established methods of measuring cerebral oedema by tissue specific gravity and cerebral blood flow by hydrogen clearance have been adopted, the background to these methods has been critically reviewed to justify their use. With respect to hydrogen clearance, a computer-derived method of on-line data acquisition has been evaluated and compared to traditional methods of manual data analysis.

1.7.3. There is a growing interest in the role of peripheral blood components in the generation of cerebral oedema. The author has been involved in other studies assessing the effect of immunosuppression on the development of cerebral oedema in a different model of cerebral ischaemia (Kane et al 1992). This has prompted him to independently study the effect of immunosuppression by whole-body irradiation on the development of cerebral oedema after proximal middle cerebral artery occlusion. The hypothesis to be tested is that immunosuppression will reduce the extent of peri-infarct oedema formation after middle cerebral artery occlusion.

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Chapter 2

METHODOLOGY

Outline of the basic experimental methods

2.1 PLAN OF INVESTIGATION

2.1.1. All anaesthetic and surgical procedures in this thesis have been conducted by the author in accordance with the Animals (Scientific Procedures) Act (1986), and under Home Office licence. Adult male Wistar rats (weight range 297 g - 562 g) were divided into 14 experimental groups, outlined in the flow-chart on the next page. The study protocol has been designed to assess the following:

(1) The effect of middle cerebral artery occlusion (MCAO) on the lower limit of cerebral autoregulation (2 groups; n = 10).

(2) The effect of 30 minutes of modest hypotension (mean arterial blood pressure = 70 mmHg) on:

  (a) infarct size after MCAO (5 groups; n = 34).

  (b) cerebral oedema and cerebral blood flow (CBF) after MCAO (3 groups; n = 20).

(3) The degree of brain oedema at 24 and 72 hours after MCAO (2 groups; n = 14).

(4) The effect of immunosuppression by irradiation on the degree of brain oedema 24 hours after MCAO (2 groups; n = 15).

2.2 OUTLINE OF METHODS.

2.2.1. Proximal MCA occlusion was adopted as an established method of producing focal cerebral ischaemia in the rat (Tamura et al 1981a). Anaesthesia was either induced and maintained in non-survivor experiments by inhalation of $N_2O/0_2$ (70:30%) and halothane with mechanical ventilation via a tracheostomy, or in survivor experiments by intraperitoneal anaesthesia using midazolam/fluanisone/fentanyl (MFF). Hypovolaemic hypotension was induced by exsanguination alone and maintained using a new method of computer-managed closed-loop blood pressure control. Infarct size was assessed at 4 hours post-MCAO by perfusion-fixation with 2,3,5-triphenyl tetrazolium chloride (TTC), and histologically at 6 hours post-MCAO by perfusion fixation by 10% buffered formalin. Histological studies were carried out in separate experimental groups because (i) brain slice preparation was different

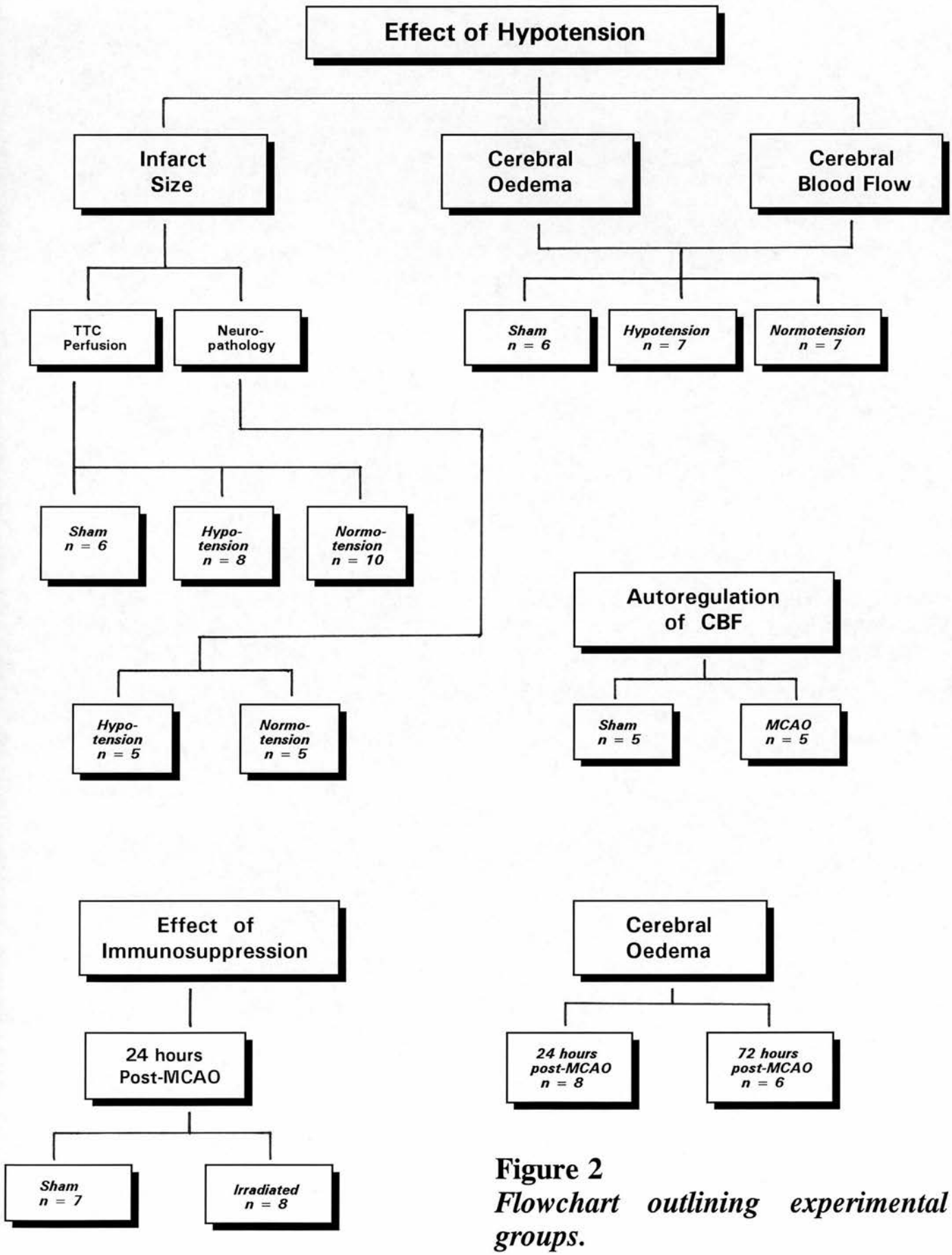

Figure 2
Flowchart outlining experimental groups.

between the two groups; (ii) the post-occlusion time period in the pathological studies was extended to guarantee clear infarct delineation; (iii) the methods of infarct quantification differed between the studies. Cerebral oedema was assessed using microgravimetric techniques. Cerebral blood flow was measured using Hydrogen clearance. Whole-body immunosuppression was induced by sub-lethal irradiation. The experimental groups are described below and a detailed description of techniques follows.

NUMBER	PROTOCOL
Group 1 *Sham n = 5*	Assessment of lower limit of autoregulation; Sham Occlusion; Insertion of cortical H_2 hydrogen electrodes for measurement of CBF; Incremental fall in mean arterial blood pressure in 10 mmHg steps from 90 mmHg to 40 mmHg, and estimation of CBF at each level; Reperfusion blood flow.
Group 2 *MCAO n = 5*	As Group 1; Middle cerebral artery occluded, with baseline CBF prior to occlusion.
Group 3 *Sham n = 6*	Assessment of Infarct size; Sham occlusion; perfusion fixation at 4 hours with TTC.
Group 4 *Normotension n = 10*	As Group 3; Middle cerebral artery (MCA) occlusion.
Group 5 *Hypotension n = 8*	As Group 4; Hypotension (70 mmHg) induced for 30 minutes post-occlusion.
Group 6 *Normotension n = 5*	Histological assessment of Infarct size; MCA occlusion and perfusion fixation with 10% formalin at 6 hours post-occlusion.
Group 7 *Hypotension n = 5*	As Group 6; Hypotension (70 mmHg) induced for 30 minutes post-occlusion.
Group 8 *Sham n = 6*	Assessment of CBF and Specific Gravity; Insertion of intracortical H_2 electrodes; Sham occlusion; baseline measurement of CBF, and every 30 minutes to 4 hours; brain removed and assessment of brain specific gravities.
Group 9 *Normotension n = 7*	As Group 8; MCA occlusion after baseline CBF.
Group 10 *Hypotension n = 7*	As Group 9; Hypotension (70 mmHg) induced for 30 minutes post-occlusion; CBF measured at 10 minutes post-occlusion; reperfusion at 30 minutes post-occlusion.
Group 11 *24 hours n = 8*	Intraperitoneal anaesthesia and survival to 24 hours; MCA occlusion; assessment of brain specific gravity.
Group 12 *72 hours n = 6*	As group 11, except survival to 72 hours.
Group 13 *Control n = 7*	No preoperative irradiation; Intraperitoneal anaesthesia and survival to 24 hours; MCA occlusion; assessment of brain specific gravity.
Group 14 *Irradiated n = 8*	As Group 13, except immunosuppression by whole-body sub-lethal X-irradiation 7 days preoperatively.

2.3 GENERAL PREPARATION & ANAESTHESIA

2.3.1. Two methods of anaesthesia have been adopted. In 4 and 6 hour non-survivor experiments (Groups 3 - 10), the rodent was anaesthetized within an enclosed perspex box in an atmosphere of nitrous oxide/oxygen (600:400 ml/min) and 3-4% halothane. After induction, a tracheostomy is performed while the anaesthetic is continued by mask. The halothane concentration is reduced to 2%. Through a small tracheotomy, a size 14 Fr. gauge venflon is inserted, and held firmly with proximal and distal 3/0 silk ties. This is attached via a Y-connector to the anaesthetic circuit of a Harvard small-rodent ventilator, through which is delivered N_2O/O_2 at 700:300 ml/min., and halothane 0.5-1%. The inspired halothane concentration is usually maintained in the 0.6-0.8% range depending on blood pressure and depth of anaesthesia required. The N_2O/O_2 concentrations are generally left unaltered, unless the oxygenation of the animal is tending to fall, as monitored by regular arterial blood gas estimations (the paO_2 was always maintained above 100 mmHg). The expiratory CO_2 concentration is continuously monitored by a Gould capnograph, and a permanent tracing made on a Grass Polygraph. A linear regression analysis between the end-tidal capnographic CO_2 and $paCO_2$ was not particularly good, but changes in the former mirrored the trend in $paCO_2$, and allowed appropriate measures to be taken to maintain $paCO_2$.

2.3.2. In recovery experiments (Groups 11-14), where the animal is allowed to survive for up to 72 hours, an intraperitoneal method of anaesthesia was used. 2 ml *hypnovel* (5 mg/ml midazolam) and 2 ml *hypnorm* (10 mg/ml fluanisone + 0.315 mg/ml fentanyl citrate) are mixed with equal amounts of water for injection, which prevents precipitation of the midazolam on mixing with the hypnorm. This is given by the intraperitoneal route at a dose of 3 ml/kg (1.25 mg midazolam / 2.5 mg fluanisone / 0.079 mg fentanyl citrate per ml). A small close-fitting mask constructed from the end of a 20 ml syringe allows the continuous delivery of oxygen at 300-500 ml/min throughout the procedure. A laboratory study with full monitoring via femoral arterial catheters was carried out to assess the physiological effects of this method of anaesthesia (n = 4; table 20, page 129). Continuous monitoring with a pulse oximeter (*Novametrix*) demonstrated a satisfactory oxygen profile (table 20).

The animal is recovered in an oxygenated incubator, given naloxone to help reverse the anaesthetic and provide some postoperative analgesia, and later returned to its cage. It is allowed free access to food and water, but fluid intake is occasionally supplemented by subcutaneous injection of 3 - 5 ml of dextrose/saline.

2.3.3. In all experiments, a full blood count is taken from every animal to ensure that haemoglobin, total white blood count, platelet count, and haematocrit are similar between experimental groups. In 4 hour non-recovery experiments, where regular monitoring is undertaken, blood samples are taken for full blood count (*ca* 0.5 ml), haematocrit (0.08 ml), glucose (*ca* 0.1 ml), and blood gas estimations (0.125 ml) after insertion of the first arterial line. Haematocrit and serum glucose concentration are also checked just prior to the MCA occlusion and before the experiment is terminated at 4 hours. In 6 hour experiments for histological examination, further blood specimens are taken for haematocrit and glucose estimations 2 hours post-occlusion. Haematocrits are measured using a Hawksley microhaematocrit centrifuge and reader. Glucose samples are kept on ice in small fluoride oxalate tubes, centrifuged in a high speed centrifuge at 5000 rpm, and serum glucose concentrations made using a Beckman Glucose analyzer. Arterial blood gases are checked before the MCA occlusion, during any period of controlled hypotension, and regularly at 30 minute intervals in all cases.

2.3.4. In non-recovery experiments terminated at 4 - 6 hours, bilateral femoral arterial catheterisation is used to monitor blood pressure, permit regular sampling for arterial blood gas, glucose and haematocrit estimations, and to allow infusion of fluids. Both femoral arteries are cannulated with fine portex tubing, connected to small, blunted needles (Fr. gauge 25). The right side is connected via a Bell and Howell strain gauge transducer to the Grass Polygraph recorder. The transducer is independently linked via an RS232 interface to an Amstrad PC 1640 computer, so that the systolic and diastolic pressures are monitored every 2 seconds, a mean calculated, and the information graphically displayed on the computer VDU. This system drives the syringe pump used in hypotensive experiments, and is described more fully in the next section. Although femoral venous cannulation can be used for the routine infusion of fluids, many experiments require bilateral arterial lines for

combined monitoring/blood gas analysis and closed-loop blood pressure control. In other experiments, the second arterial line may be utilised for monitoring should the first line fail. Fluid replacement involves the continuous infusion of 1 - 1.5 ml colloid (haemaccel or gelfusin) per hour, supplemented with 0.5 - 1 ml of saline per hour, used to clear the arterial line after blood sampling.

2.4 HYPOVOLAEMIC BLOOD PRESSURE CONTROL

2.4.1. A new method of hypovolaemic blood pressure control has been introduced into this thesis which allows fully automated continuous monitoring and adjustment of blood pressure using a computer-driven syringe driver (Chambers et al 1991) (Figure 3). This has provided accurate control of blood pressure in hypotensive and autoregulation experiments (Groups 1, 2, 5, 7 and 10). Preparation of the rodent involves cannulation of both femoral arteries the right with fine Portex polyethylene tubing (ref. 800/100/140/100, i.d. = 0.4mm, o.d. = 0.8mm), and the left with the proximal 3 - 4 cms of a Portex intravenous cannula (ref. 200/300/020, i.d. = 0.51mm, o.d. = 0.75mm), which incorporates a luer mount. The luer mount is connected via a 27 cm length of extension tubing (capacity = 0.5 ml) and a 3-way tap to a 5 ml Plastipak syringe. The extension tubing and 3-way tap are filled with 5000 units of heparin, left overnight, and washed out with saline before use. The syringe is mounted in a purpose built syringe driver capable of both withdrawing and infusing fluids, which is designed to hold 5, 10 or 20 ml syringes. In this model, a 5 ml syringe provides the optimum level of control. Prior to use, the syringe is filled with 2 ml of saline and 150 units of Heparin, which prevents blood clotting within the system during operation.

2.4.2. Blood pressure is monitored through the right femoral catheter using a Bell and Howell strain gauge pressure transducer and a Grass polygraph recording system with a low level dc amplifier. The arterial pressure waveform output is coupled to an analogue peak and trough detector which extracts the systolic and diastolic values of the blood pressure signal. These signals are then linked to an eight channel analogue to digital convertor with a 10 bit accuracy housed in a personal computer. The syringe driver is linked to the computer via

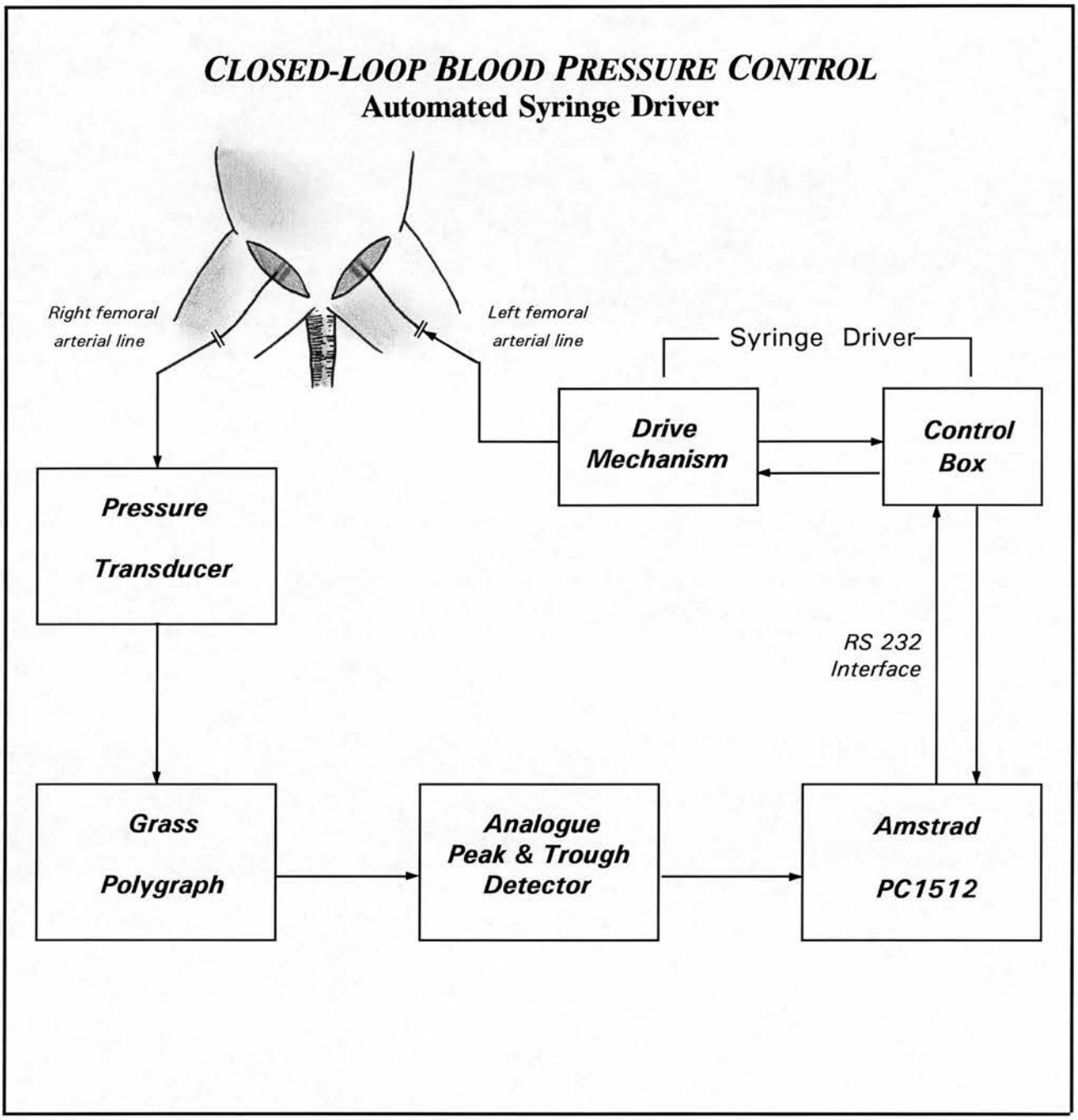

Figure 3

Schematic diagram illustrating the hardware layout used in hypovolaemic blood pressure control. The method is detailed in the adjacent text. The control box contains the microprocessor, EPROM memory, RS232 interface, switches, front-panel indicators, and the electronics required to control the syringe driver.

a RS232 serial interface, and consists of two parts, a drive mechanism and a control box. The drive mechanism comprises a yoke driven along two horizontally mounted guide rods by a linear actuator. Energising the actuator causes fluid to be infused or withdrawn. Limit switches detect when the plunger has reached the end of its travel in either direction. The control box houses a 6809 microprocessor, EPROM memory storing the operating program, RS232 interface, drive electronics for the linear actuator, input switches and status indicators. The syringe driver can be operated either remotely through a serial interface or directly from the front panel of the instrument. Normally the driver is controlled from the serial interface but this can be interrupted at any time to allow an immediate infusion or withdrawal of fluid. Flow rates can be varied from 10.3 ml/min to 0.14 ml/min in 8 steps, each step approximately half the previous rate. Precise rates depend on the exact dimensions of the syringe in use.

2.4.3. A computer program written in Pascal controls the syringe driver from the computer. Every two seconds the systolic and diastolic values of the blood pressure are sampled, and a mean arterial pressure is calculated (diastolic + 1/3rd pulse pressure). This is then displayed in a graphic format on the computer display. A simple algorithm is used to control the syringe driver. Using the computer keyboard, the chosen level of mean arterial blood pressure to be maintained (i.e. the target pressure) is selected. The syringe driver will be set to infuse or withdraw depending on whether the measured value is less than or greater than the target pressure. The absolute difference between the values determines the flow rate which is set. Although there are 8 possible flow rates, the computer utilises 3 of these, so that it adopts a rapid, intermediate or slow mode, depending on the pressure difference. The precise flow rates have been determined experimentally using a 5 ml Plastipak syringe, and these are shown in Table 9. The computer sends the appropriate instruction to the syringe driver every 2 seconds based on the sampled mean pressure, and is continually active at the set flow rate until the next calculation is made 2 seconds later.

2.4.4. Stability of control depends on the integrity of the arterial line measuring the blood pressure. Arterial blood is taken intermittently from the right femoral catheter, which is used to record blood pressure, for blood gas estimations ("ABG" in figure 7, page 47). During

disconnection of the line, blood pressure is detected as zero by the pressure transducer. The pump is switched temporarily to an inactive mode, preventing inappropriate infusion of fluid via the left femoral catheter. Initial problems with over-correction of the blood pressure have been overcome by making variations in flow rate smaller. A 3-way tap is included in the system to allow a rapid reduction manually in the blood pressure to the target pressure (if desired), or can be used as a reservoir to rapidly empty or fill the pump syringe, minimising delays in effective blood pressure control.

2.5 MIDDLE CEREBRAL ARTERY (MCA) OCCLUSION

2.5.1. A subtemporal approach is used, similar to that originally described by Tamura et al (1981a) but with a number of modifications developed by the author. The animal is positioned supine with the thorax and head tilted to the left, lifting the right zygoma into an oblique position. A suture can be placed through the right ear, to help in holding the head over to the left. Although a full left lateral position is used in survival experiments, this is avoided where both femoral arteries are cannulated to prevent possible line failure. A right tarsorrhaphy is done with a single 3/0 suture to prevent extrusion of the eye during operative retraction and corneal exposure post-operatively in the survival animals. A right-angled incision is made adjacent to the corner of the right eye over the zygoma and extending across the temporalis origin to the right ear. In non-recovery experiments, the skin flap may be excised completely. The temporalis muscle is divided vertically from the zygoma to the temporal line and the origin of muscle is stripped from the floor of the temporal fossa. The zygoma is left intact (Shigeno et al 1985a). The muscle is retracted posteriorly, by placing a custom-made malleable 2mm tantalum retractor around and behind the origin of the zygoma. The anterior aspect of the muscle and right eye are retracted in the opposite direction using a slightly larger retractor. This exposes the subtemporal fossa, with the foramen ovale posteriorly and the optic foramen anteriorly. Using a small 2mm dental burr, a craniectomy is made as far medially and posteriorly as possible, lying just in front the foramen ovale (figure 4a). With experience the craniectomy can be sited precisely over the proximal middle cerebral artery. The dura is opened carefully in the line of the artery using

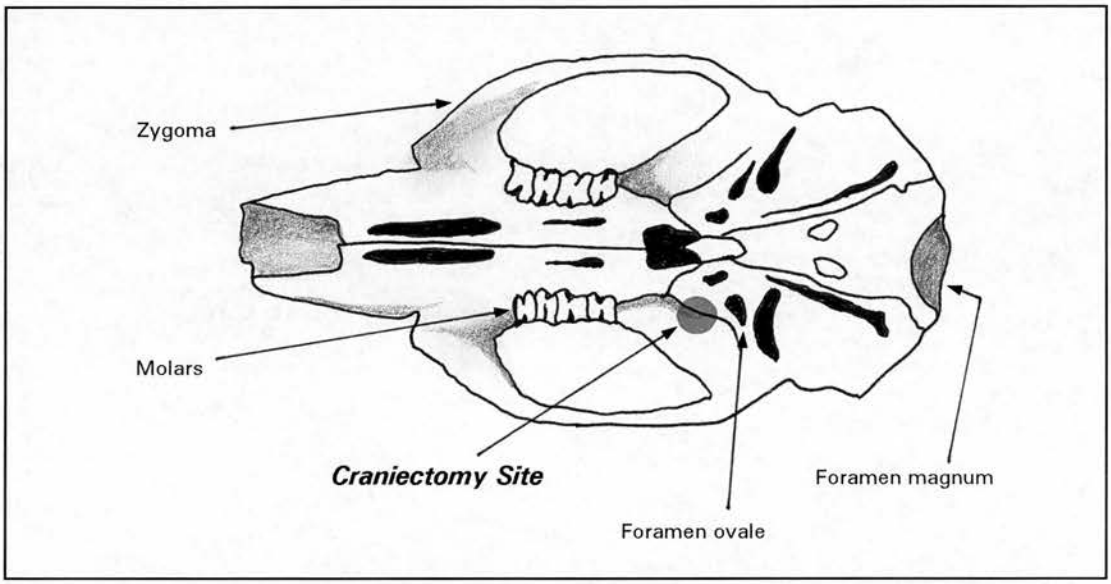

Figure 4a: Inferior view of rat skull base, showing craniectomy site (red).

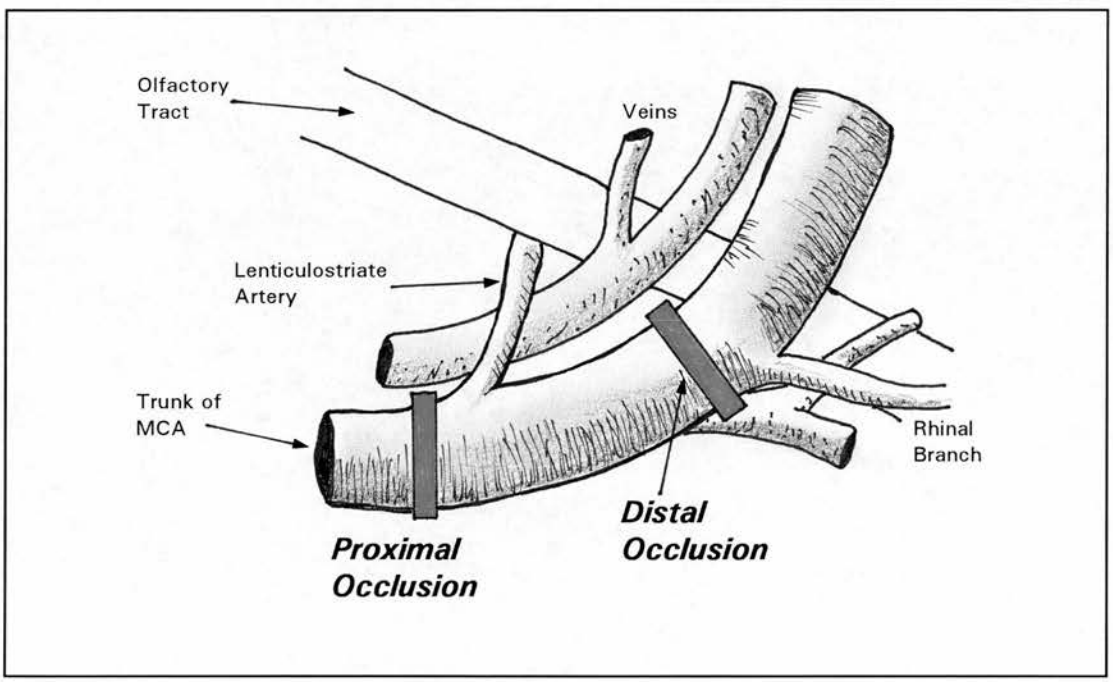

Figure 4b: Line diagram illustrating position of proximal occlusion (adapted from Shigeno et al 1985).

FIGURE 4

The position of the craniectomy and the sites of proximal and distal middle cerebral artery occlusion. The proximal occlusion includes the lenticulostriate artery, leading to ischaemia of the lateral caudate.

an orange needle with the tip bent at 90°. Great care is required at this stage to prevent haemorrhage from small veins which obliterates the operative view, and contact with the artery which may cause spasm or haemorrhage. The arachnoid around the artery needs to be opened, to allow the tips of the microbipolar forceps to pass either side of it. After identifying the lenticulostriate branches running behind and below the olfactory tract, the artery is coagulated proximal to this point (figure 4b). Coagulation is repeated several times, until the artery is divided. The forceps need to be cleaned and polished regularly, to prevent tissue adherence. Where recovery is planned, the wound is sprayed with topical antibiotic spray, and closed with interrupted 3/0 sutures.

2.6 PERFUSION FIXATION

2.6.1. 10 minutes prior to fixation, the animal is given 1000 units of heparin. All perfusion fluids are prewarmed to 37 deg C. With the animal deeply anaesthetized, the thorax is opened bilaterally, the diaphragm is split and the oesophagus divided to allow direct vision of the descending aorta. 1000 units of heparin is injected into the right atrium. The aorta is clamped, and the left ventricle cannulated. 120 ml of heparinised saline (20,000 units /litre) is infused via the ascending aorta in a pulsatile fashion at a mean pressure of 150 mmHg. The infusion is done by hand, via a 3-way tap, to which is attached the Bell and Howell transducer so that the infusion pressure is verified. After infusion of 30 - 40 ml of saline, the right atrium is opened. Once the effusate is clear (usually after 120 - 150 ml saline), 30 ml of 2% TTC is infused manually in a pulsatile fashion at a mean pressure of 150 mmHg. This is followed 5 minutes later with infusion of 60 ml 10% buffered formalin. The catheter is removed, and the rat decapitated 10 - 15 minutes later. The head is immersed for 24-48 hours in 4% formaldehyde, the brain removed and immersed for a further 48-72 hours in the same fixative.

2.6.2. Perfusion fixation for histology is carried out in a similar fashion, also using 10% buffered formalin. Immersion of the whole head for 24 - 48 hours in formalin facilitates easier removal of the brain. 4% formaldehyde solution, with marble chips which maintain

alkalinity, allows adequate fixation with less shrinkage, especially if the specimens are left for some time before histological examination. Experience with the TTC perfusion experiments suggested that if perfusion fixation is carried out manually using a pulsatile method, results are better if the mean perfusing pressure (130 - 150 mmHg) is greater than normal mean blood pressure. The reason for this is not clear, but the author suggests that cerebrovascular resistance is greater and brain compliance reduced soon after death of the animal. Traditional methods of perfusion fixation using formaldehyde, glacial acetic acid and methanol (FAM) are better if detailed histological evaluation is required, as it is suggested that this leads to less artifactual damage (Brierley et al 1973). However, it causes greater shrinkage of the tissue, and as the author was interested only in the estimation of infarct size, he was advised by the Neuropathology department (Newcastle General Hospital) to use formaldehyde alone for perfusion fixation. The fixed brains were stored at 4°C, and subsequently imbedded, sliced and stained at the Department of Neuropathology, Newcastle General Hospital.

2.7 VOLUMETRIC ANALYSIS

2.7.1. After 48 - 72 hours in formaldehyde, the TTC-perfused brains are sliced in a custom-made brass block (figure 5), using a hand held razor blade. 9 passes are possible through both hemispheres, giving 10 slices with an average slice thickness of 1.49mm. The frontal poles are discarded, leaving 9 slices for volumetric analysis. These are laid rostral surface uppermost in rows of 3, and photographed with a Nikon camera using Ilford Pan F (ASA 50) film, and fitted with a Tamron 2.8 mm Macro lens plus 12mm and 20mm extension tubes. A numbered identification mark and millimetre scale is photographed with each set of 3 slices. Each animal was assigned its own specific number, but this did not identify to which group the animal belonged, so that to some extent the volumetric analysis was "blind". The author has used a Hoya green-yellow filter to enhance contrast between the white infarct and the pink-stained non-infarcted brain. Although the film is developed professionally, the author carried out his own printing on 8" x 6" Ilford multigrade paper, using an Axomat S Standard enlarger and a grade 3 filter.

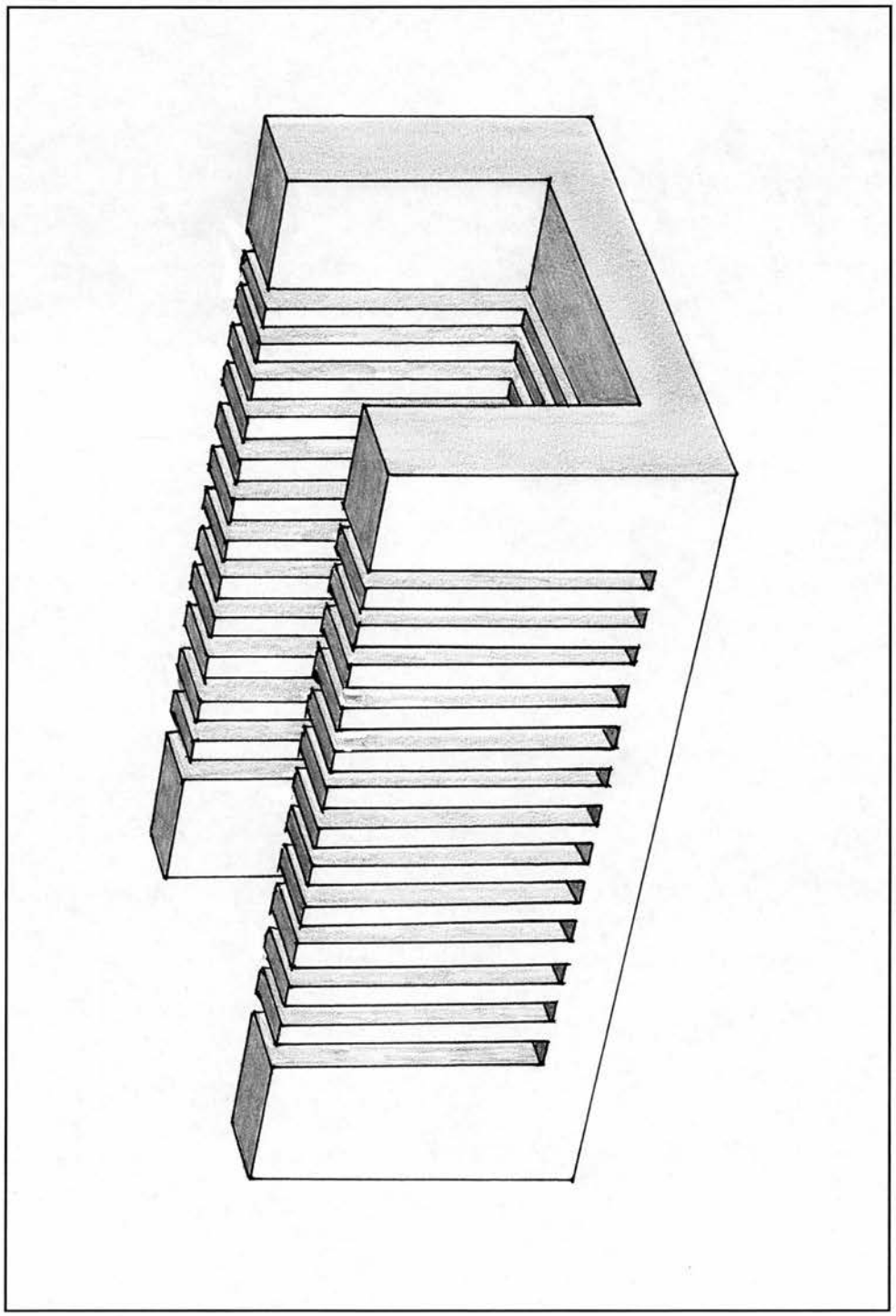

FIGURE 5

Line drawing of the custom-built brass block used in sectioning for volumetric analysis. The slots measured 0.025 inches (the narrowest saw drift possible) and the "land" between each slot 0.034 inches, giving a slice thickness of 0.059 inches or 1.5 mm.

2.7.2. Image analysis was carried out using a Kontron MOP-videoplan image analysis system. Nine areas were measured on each slice i.e. a total of 81 areas per animal. These areas are listed in the adjoining table. The hemisphere was arbitrarily divided into two areas : the "cortex", which included the thin area of white matter; and the "caudate" which included the whole area below the white matter i.e. caudate nucleus plus other basal ganglia. Although the ventricular areas tended to be small, especially if the hemisphere was swollen, these areas (6 and 9) were calculated and subtracted from the hemisphere areas (1 and 7) to give the true area of brain tissue in each hemisphere. The size of the right and left "caudate" areas were calculated by subtracting the cortical areas (3 and 8) from the hemisphere values. Thus, right caudate = (1 - 6) -3, left caudate = (7 - 9) - 8. If a particular area was not appropriate to a given slice, then no value was entered. All the results were entered on a custom-designed form, and the areas summated and multiplied by 1.49 (the slice thickness) to give a volume. A number of differences and ratios between areas were calculated, outlined in table 11.

1.	*Right*	*Hemisphere*
2.	*"*	*Lesion (infarct)*
3.	*"*	*Cortex*
4.	*"*	*Cortical Lesion*
5.	*"*	*Caudate Lesion*
6.	*"*	*Ventricle*
7.	*Left*	*Hemisphere*
8.	*"*	*Cortex*
9.	*"*	*Ventricle*

In the neuropathology groups, volumetric analysis was performed independently at the Institute of Neurological Sciences and Wellcome Institute, Glasgow, using a Quantimet 970 image analysis system. This method is described in a previous study quantifying early brain damage after MCA occlusion in the rat (Osborne et al 1987). Areas of infarction are delineated on paraffin sections (D.I. Graham) taken from 8 preselected coronal stereotactic planes, transcribed on to scale diagrams (cf. figure 9, page 67). Using computer software, infarct volumes are estimated by generating an idealised trapezoid shape derived from the integration of these areas.

2.8 MEASUREMENT OF BRAIN SPECIFIC GRAVITY

2.8.1. Oedema measurements are carried out using a microgravimetric technique (Nelson et al 1971; Shigeno et al 1982). At the end of the experimental period, the animal is rapidly

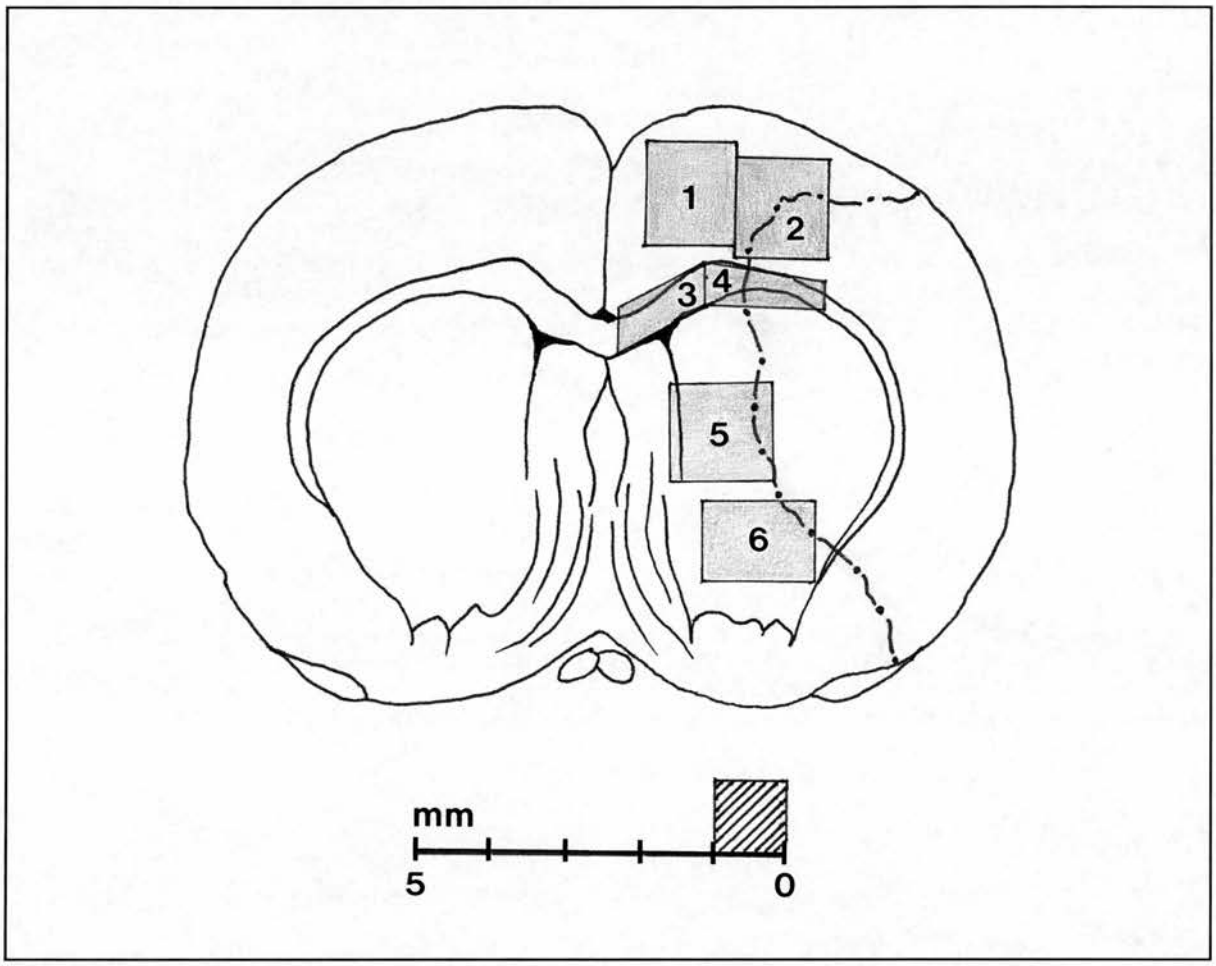

Figure 6. *Line drawing designed to indicate the tissue sampling sites of cortex (1 & 2), white matter (3 & 4) and caudate (5 & 6) relative to the area of infarction (within the dotted line).*

killed by an intracardiac injection of pentobarbitone (*Expiral*), and the brain is removed immediately. A 1.5 mm slice is taken through both hemispheres at the level of the Bregma, which is known to coincide with the largest area of infarction as estimated in previous experiments using perfusion fixation with TTC. Paired 1.5-2 mm^3 samples are taken from the cortex, white matter, and caudate nucleus, selected from areas which are known to be close to or within the penumbra of the infarct (figure 6, above). Paired control samples are also taken from each cerebellar cortex. The samples are dropped into a layered bromobenzene-kerosene column, calibrated with potassium chloride droplets of known specific gravity, and the point of descent measured at exactly 1 minute. An average result

is taken between the paired specimens. Specific gravity is determined using a linear regression analysis calculated from calibration of the column. The correlation coefficients of all the columns in this project are listed in appendix 5. A critical review of the gravimetric technique is discussed in the next chapter.

2.8.2. The specific gravity of the bromobenzene/kerosene solution, and linearity of the column, will be affected by evaporation of the volatile solvents. Problems related to this can be minimised by making up large quantities of stock solutions, checking and correcting their density by adding bromobenzene (to increase density) or kerosene (to decrease density) prior to use, using freshly prepared columns, and always recalibrating any column immediately before use. It is usually best to leave the columns for 24 hours before use to allow the gradient to settle and equilibrate, and to discard them after 72 hours when the linearity begins to fail. Although a 7-point calibration was initially adopted, the author noted that better correlations were usually obtained (but not always) if the top 2 points were excluded, and a 5-point calibration adopted. This was thought to be due to early evaporation from the top of the column, or alterations in the linearity as the last of the solutions are run together. Since the top two calibration points were 1.03 and 1.035, which was generally outwith the range of brain specific gravity measurements, i.e. *above* the "working" area of the column, it was considered correct to exclude them from the calibration.

2.9 MEASUREMENT OF CEREBRAL BLOOD FLOW (CBF)

2.9.1. CBF was measured using the hydrogen clearance technique (Aukland 1965; Haining et al 1968; Pasztor 1973; Symon et al 1979; Young 1980). While the basic principles remain the same, new techniques have been adopted for electrode fixation, and use of an Apple II computer for on-line data acquisition and analysis. Preparation involves making a mid-line sagittal incision, and reflecting skin and periosteum laterally. Using the operating microscope, 4 burr holes are made with a 2 mm dental burr, and the dura opened carefully to avoid causing haemorrhage or lacerating the brain. The burr holes are positioned 3 mm anterior and posterior, and 2.5 mm lateral (right and left), to the bregma. These positions

were chosen after careful assessment of the configuration of the infarct using the volumetric studies.

2.9.2. Electrodes are pre-constructed using 1.5 cm lengths of 0.125 mm non-coated platinum wire (99.95% pure), with a small 2-3 mm bleb of acrylic cement placed about 1 mm from one end. These electrodes are trimmed to the same length after the acrylic has hardened, so that no more than 1 mm of platinum wire will project into the cortex. In rats over 10 days old, the cortex measures 1.5 - 2 mm thick (Craigie 1963). Longer electrodes may reach the white matter leading to biphasic desaturation curves making analysis difficult. This is discussed more fully in the next chapter. The electrodes are seated into the burr holes, supported by the blebs of acrylic, and glued in placed by superglue (*Loctite*). Superglue hardens rapidly without significant heat production, and it ensures a sound fixation providing the skull vault is dry and free from blood at the time of fixation. This allows the animal to be moved or positioned appropriately to carry out the surgery, without disturbing the position of the electrodes. A silver chloride reference electrode is fixed into an intramuscular pocket between the scapulae.

2.9.3. Small crocodile clips are attached to each intracortical electrode, and separated from each other by small pieces of foam to prevent inadvertent contact between them during experiments. Changes in electrical impedance resulting from oxidation/reduction of H_2 are detected and displayed on a Rikadenki chart recorder. Different coloured inks are used for each channel, and the whole system is colour-coded so that it is clear from which electrode each recording has been generated. The animal is left undisturbed for 20 minutes so that it is physiologically stable, arterial blood gases are satisfactory, and the electrode recordings have levelled out at their baselines. Hydrogen is delivered to the animal via the inspiratory side of the ventilation circuit at a rate of between 5 and 10% (about 50 to 100 ml per minute) (Bell et al 1985). The precise rate does not affect the calculation of the CBF (Symon et al 1979), but the same rate is maintained for each experiment. Saturation of the animal occurs within 3 to 4 minutes, and when the curves begin to plateau the hydrogen is switch off. A baseline recording is made; if the system is recording satisfactorily, the MCA is coagulated and divided. In the sham and normotensive groups, the first clearance is made

at 10 minutes, 30 minutes and every 30 minutes thereafter until the animals are killed after the last clearance is recorded at 4 hours. In the hypotensive group, the first clearance is made at 10 minutes (as soon as the blood pressure has stabilised at 70 mmHg), at 60 minutes, and thereafter every 30 minutes until 4 hours. As the animals in this group are reperfused at 30 minutes, no blood flow estimation is made at that time. Arterial blood gases are checked 10 minutes before each clearance, and readjusted and checked again if necessary before any blood flow recording is made.

2.9.4. On-line data acquisition is carried out via an Apple II computer and a program written in Pascal. Minimum (baseline) and maximum (fully saturated) recordings are detected by the program after suitable prompts (spacebar), so that the displayed image can be adjusted appropriately. Data is acquired every 2 seconds from all four electrodes, and displayed on screen in real time. Data acquisition ceases automatically at 5 minutes or can be aborted before this if the clearance curve has levelled out (usually at 4-6 minutes, depending on the blood flow). The information is stored on disc for later analysis, or the clearance curves can be recalled for immediate analysis. The computer performs a logplot for each channel, and displays this on request. Left and right cursors can be positioned at the area of the line where blood flow estimation is most appropriate (usually between the end of the first and third minutes). If the line is straight, as is often achieved with monoexponential clearance curves, the slope of the line is constant, so the blood flow will be the same whichever part of the line is selected for analysis. The computer calculates and displays the blood flow in ml/100g/min.

2.9.5. Evaluation of the computer analysis of the H_2 clearance curves is an integral part of this thesis, so hand-analysis has been carried out on all H_2 clearances to compare the two methods. The accuracy of the on-line data acquisition and computer-assisted calculation of the blood flows was established only when all experiments were completed (MacDonald et al 1993). Therefore, the traditional hand derived results were analyzed and compared in this thesis. The clearance curves are simultaneously plotted on paper using the 6-channel Rikadenki chart recorder. Data points are later selected from each curve at 15 second intervals and plotted on logarithmic graph paper. Best-fit lines are hand drawn, $t_{½}$ times

established, and CBF calculated using the appropriate formula (see appendices 1, 2 and 3). The background to hydrogen clearance is discussed fully in the next chapter.

2.10 IMMUNOSUPPRESSION BY IRRADIATION

2.10.1. In experiments designed to assess the effect of immunosuppression on the development of cerebral oedema, irradiation was carried out by placing the animals, including the tail, into a perspex restraining tube and exposing them to an X-ray dose of 5 Gray over 15 minutes. The X-ray quality specification was 240 kV constant potential and 3.3 mm Cu half value thickness. The animals were treated equally through each flank so that the dose was uniformly distributed within a range of 5%. Control animals were also placed into the perspex tube for 15 minutes but not irradiated. The animals were treated without the knowledge of the author, and at the time of surgery the author was blind to which animals had been irradiated. Animals were randomly assigned to each experimental group, and control and irradiated animals were operated on contemporaneously in mixed groups of 4 or 5 animals. Surgery was carried out 7 days post-irradiation, and specific gravity measurements 24 hours post-surgery. The use of the term "immunosuppression" is clarified in paragraph 3.8.2., page 61.

2.11 STATISTICAL VALIDATION

2.11.1. Comparisons made between normotensive and hypotensive groups are the most relevant, but comparisons between each of the treatment groups and sham operated animals have also been made where appropriate. The Student's t-test has been used throughout to compare one group with another, using standard errors calculated as a function of the standard deviation ($SE = SD/\sqrt{n}$). If the standard errors were markedly different, an F-test (analysis of variance) was done to establish significance. The author has used Minitab Release 1.2 (Sirius version 1.0; © 1981), a computer software package originally formatted for an Apricot computer but made IBM-compatible using an Apricot emulator program. All

data is expressed throughout as means ± SE; in the interests of clarity, standard error bars have been omitted from the CBF graphs (figures 8, 12, and 13), but all the equivalent data listed in tables 15, 16, and 17 includes the standard errors.

2.12 MATERIALS

Anaesthetics

Boyle's Apparatus	
N_2O; O_2; (H_2 for hydrogen clearance)	*BOC Ltd, Guildford, UK*
Halothane BP	*May & Baker Ltd, Dagenham, England*
Fluovac & Fluosorber	*IMS, Chartan-Aldred Ltd, Worksop, UK*
Midazolam (Hypnovel)	*Roche Products Ltd., Welwyn Garden City, Herts.*
Fentanyl/fluanisone (Hypnorm)	*Janssen Pharmaceutical Ltd., Wantage, Oxford.*

Monitoring

Corning 170 Blood Gas Analyzer	*Corning Medical & Scientific Ltd, Halsted, England*
Gould-Godart Capnograph Mk II	
Statham P23G blood pressure transducer	*Statham Laboratories Inc., Hato Rey, Puerto Rico*
Amstrad PC1640 (Blood Pressure)	
Hawksley micro-haematocrit centrifuge + reader	
Beckman Glucose Analyzer 2	*Beckman Instruments Inc., Fullerton, USA*
Harvard Homeothermic Animal Blanket control unit	
Novametrix pulse oximeter	

Recording

Grass 7D polygraph	*Grass Instruments Co., Quincy, Mass., USA*
Rikadenki DBE-6 6-channel recorder	*Rikadenki Mitsui Electronics (UK) Ltd*
Apple II Europlus PC + Apple Silentype printer (Hydrogen Clearance)	
Axiom EX801 microprinter (arterial blood gases)	

Surgery

Zeiss Operating Microscope

Downs microbipolar coagulator

Microbipolar forceps	*Codman Ltd., High St., Maidenhead, Berks.*
Portex intravenous cannula	*Portex Ltd., Hythe, Kent, England*
Portex tubing	
Terumo needles (Fr. gauge 25, orange)	*Terumo Europe NV, 3030 Leuven, Belgium*
Sutures: Dexon 3/0	*Davis & Geck, Faveham Road, Gosport, Hamps*
Mersilk 3/0	*Surgicon Ltd., Wakefield Road, Brighouse, W. Yorks*
Haemaccel	*Hoechst UK Ltd, Hounslow, Middlesex*

Hydrogen Clearance Electrodes

Platinum wire (99.95% pure), 0.125 mm	*Goodfellow, Cambridge Science Park, Cambridge*
Surgical Simplex P bone cement	*Howmedica International Ltd, Grasling Rd, London*
Loctite Superglue	*Loctite UK Ltd, Welwyn Garden City, Herts*

Reagents

2,3,5-Triphenyltetrazolium chloride (TTC)	*Sigma Chemical Company, St. Louis, USA*
10% buffered formaldehyde solution	*Pharmaceuticals Ltd, Wetherby, Yorks., England*
4% formaldehyde solution (unbuffered)	
Bromobenzene; Kerosene (Heavy Distillate)	*BDH Ltd, Poole, England*

Photography

Contax RTS 35mm SLR camera

Tamron 44A 28-70mm f/3.5-4.5 macrolens

Jessops Extension tubes, 36,20 and 12mm

Hoya G(X1) 62mm green-yellow filter

Axomat S Standard enlarger

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Chapter 3

JUSTIFICATION OF METHODS

A critical review of the experimental methods with particular reference to gravimetry and hydrogen clearance

3.1 THE EXPERIMENTAL MODEL

3.1.1. In the study of the pathophysiology of cerebral ischaemia, animal models are essential because in the human situation the manifestations of stroke are so diverse that precise analysis and experimental control are impossible (Ginsberg & Busto, 1989). Moreover, rigorous investigation often requires invasive techniques and direct access to brain tissue, especially around the time of the ischaemic insult; this is clearly not possible in the human setting. In vitro models, such as isolated arterial preparations, cannot replicate the dynamic and variable physiology that is an integral part of living vasculature. Larger animal species eg cats, dogs and primates have been extensively used to study cerebral ischaemia (Molinari & Laurent 1976). Nevertheless, rodent models may be far more commonly used for several reasons: low cost, homogeneity within strains by inbreeding, similarities in cerebrovascular anatomy with man (Yamori et al 1976), and greater ethical acceptability (Ginsberg & Busto 1989). Further, certain procedures such as brain fixation are easier with small brain size, and because of the low cost experimental numbers can be higher. Rodent models of cerebral ischaemia are generally divided into *global*, often transient affecting widespread areas of the brain, or *focal* ($\pm$ reperfusion) leading to localized infarction (Molinari & Laurent 1976). Middle cerebral artery (MCA) occlusion in one of its many forms has now become the best recognised method of inducing focal ischaemia.

3.1.2. Experimental middle cerebral artery occlusion is not a recent technique. In 1951, Harvey and Rasmussen described occlusion of the MCA in monkeys using a craniotomy, and later a subtemporal craniectomy (Ralston et al 1954). More recently, Sundt & Waltz in 1966 carried out a retro-orbital, extradural occlusion of the MCA in dogs. Since then, using variations of this approach, it has been used in cats (eg. O'Brien & Waltz 1973; Hossmann & Schuier 1980; Bose et al 1984), dogs (eg. Heilbrun & Goldring 1968; Lawner et al 1981) and primates (eg. Hudgins & Garcia 1970; Sundt & Michenfelder 1972; Symon et al 1974a, 1974b, 1979). A transorbital approach is not feasible in rats, because the orientation of the middle cerebral artery behind the orbit is anatomically different. An alternative approach was ultimately introduced in rats using a subtemporal craniectomy (Albanese et al 1980;

Tamura et al 1981a; Coyle 1982). Several modifications have since been adopted in the quest for pathophysiological reproducibility (Bedersen et al 1986a; Chen et al 1986; Brint et al 1988; Shiraishi & Simon 1989).

3.1.3. An important criterion for a good model of cerebral ischaemia is its high degree of reproducibility in producing infarcts of a predictable size (Bose et al 1984). Variability of infarct size has been recognised as a confounding factor in many studies (eg. Crowell et al 1981; Duverger et al 1985; Coyle 1982, 1986). Efforts to develop a consistent model of cerebral infarction in the rat have been intense (Tamura et al 1981a; Graham et al 1985; Chen et al 1986; Bedersen et al 1986b; Brint et al 1988; Shiraishi & Simon 1989). Proximal MCA occlusion with inclusion of the lenticulostriate arteries is described as producing consistent infarcts (Tamura et al 1981a; Tyson et al 1985), but even in this model considerable variability has been reported (Shigeno et al 1985a; Graham 1988). However, validation of this model has been more extensive than others (Tyson et al 1985; Bedersen et al 1986b; Osborne et al 1987), and it has been adopted by the author. Recent studies have demonstrated the significant influence of animal strain on models of focal ischaemia, specifically infarct variability (Brint et al 1988; Jacewicz et al 1990). Several reports have suggested that Wistar rats are not the best choice for consistent results (Brint et al 1988; Duverger & MacKenzie 1988; Ginsberg & Busto 1989). The factors responsible for this variability in infarct size between animals are not clear, but Brint and co-workers (1988) suggest that differences in collateral blood supply between strains may be the major factor. Variability *still* occurs whatever model or animal strain is used, even in spontaneously hypertensive rats (SHR). The author would argue that although the most consistent results are obtained in SHR strains (Brint et al 1988), these animals have been deliberately bred to make them "stroke-prone". This may not be appropriate in experimental studies that examine the effects of focal ischaemia on normal cerebral vasculature.

3.2 HYPOVOLAEMIC BLOOD PRESSURE CONTROL

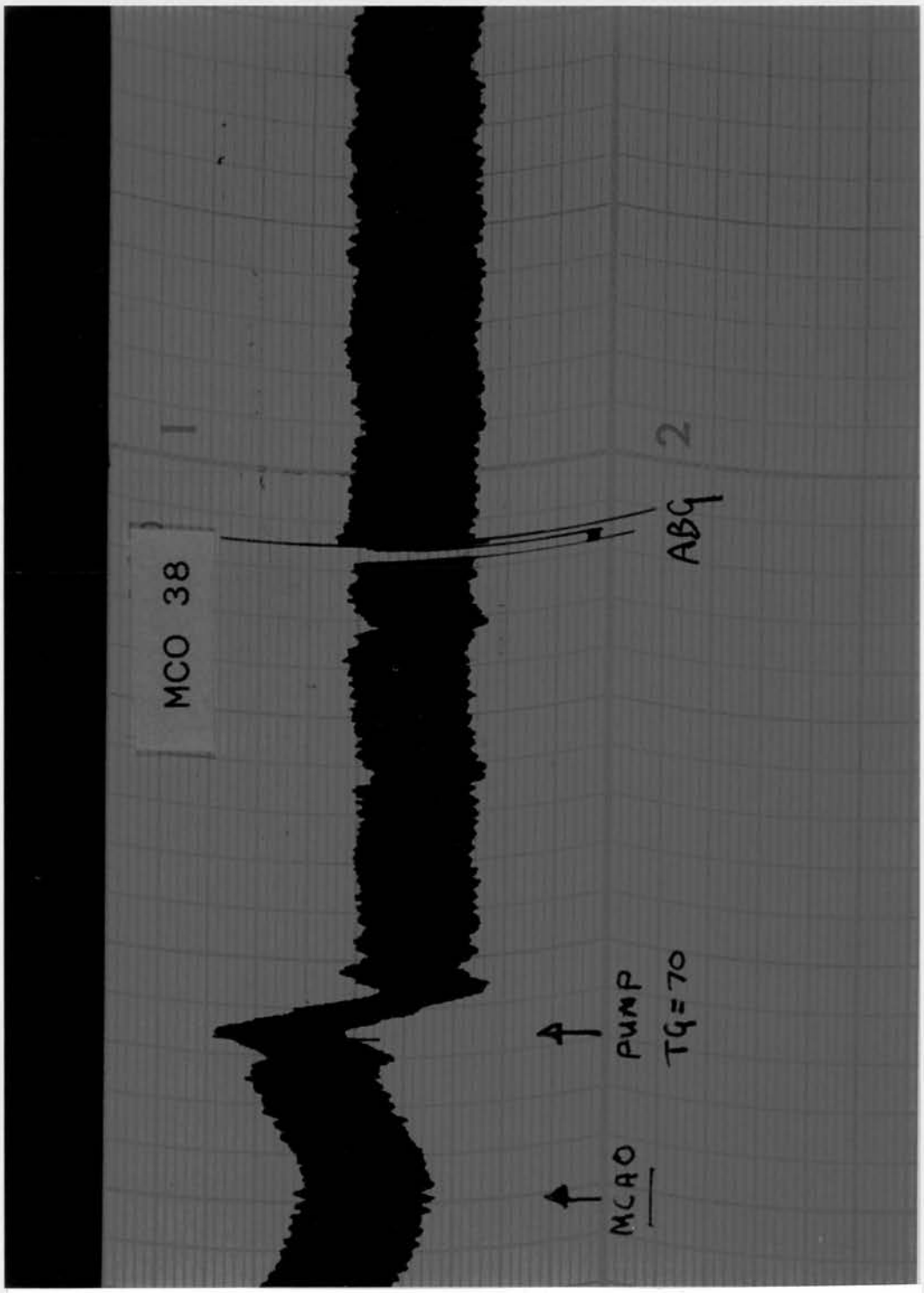
3.2.1. Studies that assess the effect of hypotension on cerebral ischaemia require a method

of maintaining the mean arterial blood pressure that is consistent and reproducible. To properly evaluate the precision and reliability of the syringe driver system used in this thesis, the polygraph tracings from 5 randomly selected group 7 rats were studied. An example of one of the systolic/diastolic waveform tracings is shown in figure 7 (next page). A line was drawn at the target pressure (70 mmHg) through the polygraph tracing of the *mean* blood pressure over the 30 minutes the syringe driver was operational. From each minute, the maximum or minimum pressure recorded was selected, and the 30 results summated and averaged. The deflection of each data point (positive or negative) from the target pressure was also recorded, and an average deflection calculated. Over 30 minutes, the average variation from the mean pressure was only 0.95 mmHg to 1.21 mmHg, or a deflection of +1.36% to -1.73%. Using all 150 data points, the mean pressure was 69.78 mmHg, with a standard deviation of 2.38 (see table 10).

3.2.2. The syringe driver proved to be less useful in maintaining blood pressure stability in normotensive animals, or during operative preparation. Short-term fluctuations in blood pressure may occur when the animal is stimulated (eg. tracheal suction, drilling of burr holes, or retraction during operative exposure), and a hypertensive response to the occlusion was often noted. Such temporary swings in MABP can cause the pump mechanism to make inappropriate or excessive efforts to restore the blood pressure to the chosen target pressure. However, once an animal was settled and physiological stable, the system was highly effective at maintaining the blood pressure within fairly precise limits following hypovolaemia (cf. figure 7). In autoregulation studies involving the measurement of cerebral blood flow at different levels of blood pressure, the system functioned satisfactorily for over 3 hours without line failure, at pressures as low as 40 mmHg.

3.3 ANAESTHETIC METHODS

3.3.1. The anaesthetic methods used in this project are standard for small rodent anaesthesia. The use of halothane can be criticised as it affects blood pressure, and is known to alter cerebral vascular reactivity causing vasodilatation and increased cerebral blood flow



SCALE Magnification = x1.5. Each major line on y-axis (5mm) = 20 mmHg.
Each major line on x-axis (5mm) = 1 minute.

Figure 7 **Photograph taken from the polygraph tracing of experiment MCO 38 (hypotensive group) demonstrating how effective the syringe driver is at maintaining the blood pressure at the target pressure (TG) of 70 mmHg. ABG = Arterial Blood Gas estimation taken from the same line that monitors blood pressure.**

in normal and ischaemic brain tissue (Smith & Wollman 1972; Smith et al 1973; Anderson et al 1980). While concentrations of halothane in excess of 1% are known to affect autoregulation (Okuda & Mcdowell 1975), autoregulation is intact during anaesthesia with 0.5% halothane (Morita et al 1977; Dirnagl & Pulsinelli 1990). In this thesis, inspired halothane concentrations remained between 0.5 and 1%, and were generally within the 0.6 to 0.8% range after surgery, when the animal was physiologically settled. Moreover, in non-recovery experiments, the inspired concentration was left fixed during any periods of hypotension or data acquisition.

3.3.2. In recovery experiments, the anaesthetic and analgesic efficacy of the midazolam/fluanisone/fentanyl (MFF) combination is well established (Flecknell & Mitchell 1984), producing neuroleptanalgesia and good muscle relaxation. Although it produces a degree of respiratory depression, it causes significantly greater cardiac output, low peripheral resistance, and adequate tissue perfusion, unlike inhalation anaesthetics (eg. halothane) or pentobarbital which can cause increased total peripheral resistance and decreased cardiac output (Skolleborg et al 1990). Blood pH, pCO_2 and PO_2 are known to remain stable with this method of anaesthesia (Skolleborg et al 1990). Another potential advantage of this anaesthetic combination is the rapid reversibility of respiratory depression and sedation caused by MFF by the immediate post-operative injection of 0.01 mg/kg naloxone (narcan) (Flecknell et al 1989).

3.3.3. In survival experiments using an intraperitoneal anaesthetic and oxygen delivery by mask, blood pressure was not continuously monitored during the operative period. This would have required a second procedure (insertion of an arterial line), and a longer anaesthetic time may have lead to increased morbidity. Moreover, theatre facilities for blood pressure monitoring and blood gas analysis were initially unavailable. To overcome some of these difficulties: (1) continuous monitoring with a pulse oximeter confirmed that the technique allowed satisfactory arterial oxygenation; (2) the operative time was reduced to 30 - 40 minutes; (3) a series of 4 animals were studied independently in the laboratory using the same anaesthetic technique with continuous blood pressure monitoring and regular blood gas analysis. The results are shown in table 20. Blood pressure was well maintained in the

normotensive range; oxygenation was satisfactory; pCO_2 tended to be high in the 40 to 50 mmHg range, as the animals tend to hypoventilate. The author suggests that normocapnia can only be maintained by tracheostomy or endotracheal intubation.

3.4 ESTIMATION OF PHYSIOLOGICAL VARIABLES

3.4.1. *Arterial Blood Gases*

The pH values tended to fall slightly towards the end of the experimental period in most study groups, perhaps reflecting a general deterioration in the animals metabolic condition. This may be due to lactic acidosis generated by poor peripheral circulation eg. bilateral lower limb ischaemia. Occasionally, an incipient acidosis was averted by the judicious infusion of small quantities of 8.4% sodium bicarbonate (0.1 - 0.2ml), but this was rarely necessary. The lower pH values seen in group 10 animals (post-occlusion hypotension) probably reflects a systemic effect of the period of hypotension, with peripheral vasoconstriction and greater lactic acidosis. This effect is seen to a lesser degree in group 5 animals, as the duration of anaesthesia up to the time of the MCA occlusion is less - an additional hour is required in group 10 animals for placement of the intracortical hydrogen electrodes. Providing pCO_2 is kept constant, changes in the H^+ concentration of arterial blood does not significantly alter cerebral blood flow (Harper & Bell 1963).

PO_2 levels in all groups varied over a wide range (113.3 $\pm$4.6 to 173 $\pm$6.0 mmHg) leading to some statistically significant differences between groups (tables 5, 6, and 8). Significant elevations in oxygen tension are associated with only a minor decrease in CBF, and modest swings in pO_2 above and below normal levels do not affect blood flow (McHenry 1983). Hypoxia is associated with an increase in cerebral blood flow (increased lactic acid production); no animal however was ever hypoxic ($pO_2 \leq 100$ mmHg) at any time during the experimental period. PCO_2 values were rigorously controlled. Carbon dioxide is the most potent vasodilator known, and changes in the pCO_2 have a marked affect on CBF (Sokoloff 1960; McHenry 1983). Although levels within the 32 to 38 mmHg range were considered acceptable, efforts were made to adjust the ventilation so that pCO_2 remained between 33 and 36 mmHg. This is reflected in consistent mean values with small

standard errors.

3.4.2. ***Body Temperature.***

Core body temperature was effectively maintained by the heating blanket, although there was a tendency for temperature regulation to be more labile in midsummer when the laboratory temperature exceeded 32°C. This problem was corrected by judicious cooling of the animals using damp towels and/or fanning which optimised conditions for temperature control. The author is aware of recent studies which suggest that brain temperature in rats, particularly in situations of ischaemia, may not correlate with the measured rectal temperature (Busto et al 1987; Ginsberg & Busto 1989). They report that in global ischaemia, injury to hippocampal pyramidal neurons was markedly reduced by modest reductions in brain temperature. However, in this thesis rectal temperature alone has been monitored in keeping with most other current studies of cerebral ischaemia. It is likely in any case that the high room temperature within the laboratory would minimise reductions in cranial temperature.

3.4.3 ***Haematocrit.***

Estimation of the haematocrit in cerebral blood flow studies is important because CBF is inversely related to whole blood viscosity, which is largely determined by the haematocrit. In blood, the red cells are the most numerous of all cell types, and contribute most to whole blood viscosity. Where circulation is very sluggish eg. beyond an arterial occlusion, the viscosity at a haematocrit of 48% is approximately twice that at 38% (Harrison 1989). If the haematocrit is elevated, a much higher perfusion pressure will be required to open up collateral channels around an area affected by a vascular occlusion, because the force needed to restart flow is proportional to the third power of the haematocrit (Harrison 1989).

The difference between "derived" and "actual" haematocrit results outlined in tables 1 - 4 is of passing interest. The haematology department value is always significantly lower than the value obtained in the laboratory using the Hawksley microhaematocrit centrifuge. A linear regression analysis between the two groups gives an r value of 0.564, so the correlation is not particularly good. It is the latter centrifuged specimens which are

considered more accurate: they have been used for statistical analysis.

3.4.4. ***Serum Glucose.***

The importance of maintaining normal serum glucose concentrations in studies of cerebral ischaemia is well established (Crockard et al 1992). Hyperglycaemia at the time of an ischaemic insult significantly exacerbates brain injury in models of cerebral ischaemia (Siemkowicz & Hansen 1978; Nedergaard 1987; Nedergaard & Diemer 1987; Prado et al 1988). Depletion of glucose reserves as a result of anaesthetic and operative stress can occur, and it may be more appropriate to use dextrose-saline rather than saline as the maintenance infusion fluid. However, none of the animals were ever hypoglycaemic. In the group of 15 animals sacrificed to assess normal brain weight and volume (appendix 4), serum glucose levels were measured to ascertain the "normal" concentration in the rat (following induction of anaesthesia). The range was 131 to 194 mg% (7.27 - 10.77 mmols/l) with a mean concentration of 153.7 $\pm$ 4.5 mg% (8.53 $\pm$ 0.25 mmols/l). These ranges compare favourably with the experimental results, and with normoglycaemic ranges quoted in other series (eg. Siemkowicz & Hansen 1978; Nedergaard & Diemer 1987).

3.5 ASSESSMENT OF INFARCT SIZE

3.5.1. Whatever method is used to assess infarct size after acute focal ischaemia, methodological problems arise specifically related to the early assessment of neuronal death, and reproducibility of the results. Neuropathological changes have been described as early as 2 hours post-occlusion (Tamura et al 1981a), based on the changes described by Brown and Brierley (1968). They stated that as early as 30 minutes following *profound* ischaemia using the "Levine" model, shrinkage of the cell body and microvaculation in the cell cytoplasm represented "the earliest anoxic-ischaemic neuronal alteration recognizable with the optical microscope". Histopathological assessment requires an experienced neuropathologist to interpret correctly the early changes of cerebral ischaemic injury, and is costly in terms of time and materials (fixation, imbedding, slicing, staining, microscopic sectional analysis, chart construction and data processing). Delineation of the infarct by

perfusion techniques is based on methods that stain normally perfused tissue (eg. perfusion with carbon black)(Hossman & Schuier 1980) or tissues which are still physiological active eg. perfusion with 2,3,5-Triphenyltetrazolium chloride (TTC), or nitro-blue tetrazolium (Hamaya & Doi 1987). Unlike histopathological techniques, staining methods may relate more to a perfusion deficit rather than a true physiologically "inert" area that reflects irretrievable ischaemia. This is especially important in situations of complete ischaemia or "no-reflow". Staining techniques may differ from one author to another, detracting from their reproducibility. However, they are easier and cheaper to develop and learn, and have thus gained in popularity.

3.5.2. In this thesis, one series of experiments has adopted perfusion-fixation with TTC, and an independent study has assessed infarct size histologically. Tetrazolium salts have been used to detect infarcts in myocardium (Lie et al 1974; Boor and Reynolds 1977; Fishbein et al 1981). Irreversible physiological dysfunction exists when cells can no longer regulate volume, maintain lactate and inorganic phosphate levels, or support mitochondrial oxidative phosphorylation (Liszczak et al 1984). Mitochondrial integrity is essential for oxidative phosphorylation to occur, as dehydrogenase enzymes essential for electron transfer are associated with the inner mitochondrial membrane. TTC reacts with intact oxidative enzyme systems within mitochondria, accepts electrons, and becomes reduced to a red formazan dye. Tissues with intact mitochondria are stained pink, while the infarcted area where mitochondria are irreversibly damaged fail to stain. Previous studies have shown good correlations between TTC staining techniques and histopathology (Lundy et al 1986; Bederson et al 1986a; Park et al 1988).

3.5.3. Despite the simplicity of perfusion techniques to delineate the infarcted area, no study of experimental cerebral ischaemia would be complete without some histological confirmation. In this thesis, it was not possible to use the material generated from the TTC perfusion experiments for concurrent histological analysis. The slice thickness (1.5 mm) in the TTC experiments was considered too thin for adequate imbedding, which used 3 mm slices, and to ensure adequate infarct delineation, a time-course of 6 hours was adopted for histological analysis. A separate study was therefore set up to assess the effects of

hypotension on the histological size of the infarct in the same experimental model. No attempt has been made to evaluate the specific histological or ultrastructural changes seen as a result of cerebral ischaemia (with and without hypotension), as this has been extensively described in many other studies (eg. Brown & Brierley 1968; Brierley et al 1969; Garcia et al 1971; Ginsberg et al 1978; Tyson et al 1981; Osborne et al 1987).

3.5.4. The calculation of infarct volume usually involves measuring the cross-sectional area of the infarct on individual slices of the brain (cross-sectional planimetry), and extrapolating or summating the results to estimate a total volume. Simpler methods to assess infarct size may involve comparison of a single cross-sectional area on comparable sections. This can even be as straightforward as cutting out areas from photographs or tracings and weighing them (Bedersen et al 1986a). More elaborate methods involve histological examination of many slices, the calculation of areas on each slice (after transcription on to selected stereotactic maps of the rat brain), and integration of these areas to calculate a volume (Graham et al 1985; Osborne et al 1987). Technical advances with computer-assisted image analysis systems have lead to more accurate and reproducible studies. The differential effect of brain swelling between groups, or the effect of minor differences in brain sizes between the experimental groups, can be eliminated by using fixed-size image magnification and\or projection (eg. Lundy et al 1986), or transcription on to outline charts of a fixed size (eg. Graham et al 1985). Alternatively, areas and sizes can be expressed as percentages or ratios of the whole (eg. Bedersen et al 1986a; Swanson et al 1990). In this thesis, estimation of infarct volume has used both cross-sectional planimetry and summation of areas (TTC perfusion) and integration of areas traced on to selected stereotactic sections (Histology; cf Graham et al 1985; Osborne et al 1987). In the former analysis (TTC perfusion), the author has calculated results both as absolute values and as percentage ratios (proportion of the whole hemisphere) to counter the effect of differential brain swelling.

3.6 ESTIMATION OF OEDEMA BY GRAVIMETRY

3.6.1. Cerebral oedema was indirectly quantified in this study by micrograviметry. The

principle of brain gravimetry is not new: Ferszt et al (1980) in their critical examination of the methods of assessing brain oedema by gravimetry cited a reference dating back to 1762. The use of specific gravity estimations to indirectly measure brain oedema has gained in popularity over conventional wet/dry weighing methods because of its ability to analyze small pieces of tissue. Marmarou et al (1978) commented that conventional techniques for assessing brain water content using weighing and drying methods were "time consuming and of limited precision". The concept of tissue suspension in a solution of known specific gravity was introduced by Lowry and Hunter in 1945 to measure serum protein concentrations. Their methods for preparing and using a gradient column was adopted by Nelson et al (1971) to assess specific gravity of cerebral tissue in mice.

3.6.2. The basic principles involve constructing a gradient column using the organic solvents bromobenzene and kerosene. These solvents are miscible but have different densities, so that a solution of a particular density can be manufactured by mixing together known quantities of each solvent. Two solutions are made with specific gravities of 1.03 and 1.06. The most important part of the process then involves the careful, controlled mixing of the two solutions to create a fluid column with a specific gravity gradient. The column is then calibrated by measuring the precise point of descent of droplets of potassium sulphate of known specific gravity. Linear regression analysis allows a correlation coefficient to be calculated - the better the r value, the more even and precise is the column. The specific gravity of fragments of tissue dropped into the column can be calculated from the regression line. Ferszt et al (1980) advised using rectangular-shaped containers measuring 2 × 20 × 25 cm for the columns rather than the hitherto conventional cylindrical vessels, as these proved more accurate, and this container style is used in this study.

3.6.3. Marmarou et al (1978) described an automated method of generating linear and reproducible gradient columns of high precision, which has made gravimetric methods more accurate and columns easier to generate. This was developed further by Shigeno et al (1982), and it is their method for producing the gradient columns that is used in this study. Although gravimetric methods are technically simple and allow the indirect estimation of the water content of small tissue fragments only a few milligrams in size, they are not without

practical and theoretical difficulties. Nelson et al (1971) commented that the use of small samples increases the possibility of error due to drying of the tissue on exposure to air. They calculated that water loss occurred at a rate of 1.5-2.5% per minute at a temperature of 21°C and 55% humidity. This loss could be minimised by cooling and increasing humidity. To reduce this error, the author removes the brain as rapidly as possible, takes a single slice at the level of the bregma, and cuts out small paired samples always in the same sequence (right hemisphere before left; cortex first, cerebellum last). These samples are immersed in a thin film of the bromobenzene/kerosene solution to prevent drying (cf. Fujimoto et al 1976; Symon et al 1979; Hatashita & Hoff 1986a) and are processed as soon as they are all collected. Specimens are taken from the deeper layers of each area under study, where surface drying is less likely (Figure 6, page 37).

3.6.4. The specific gravity of the tissue (SG_t) will include the specific gravity of both fluid and solid components. The specific gravity of the tissue solids (SG_s) can be determined from the dry tissue, and knowledge of this is necessary in the theoretical calculation of tissue water content as percentage grams water per grams tissue (% g H_2O/g tissue) (Nelson et al 1971; Marmarou et al 1978; Galbraith et al 1984). The SG_s will vary from species to species, and possibly even from strain to strain. A number of theoretical assumptions have to be made in the mathematical derivation of tissue water percentages from specific gravity calculations, to compensated for changes in tissue blood volume or protein content of oedema fluid (Bell et al 1987).

Earlier studies suggested that changes in brain water content did not alter the specific gravity of the tissue solids (Nelson et al 1971; Takagi et al 1981). However, later observations with different experimental models suggested that if the oedema fluid contained protein, as occurs in vasogenic oedema, a significant error is introduced in the calculation of tissue water (Marmarou et al 1982; Galbraith et al 1984; Bell et al 1987). With respect to peritumoral oedema in man, Bell and co-workers (1987) have demonstrated that Marmarou's derived equations (Marmarou et al 1982) overestimate tissue water content. The effect of plasma protein in the oedema fluid may non-validate the interpretation that a fall in SG is directly proportional to the extent of cerebral oedema (Bell et al 1987). However, Bothe et al (1984) suggest that the error induced by the protein content of oedema fluid was

small in comparison to the water induced changes. Moreover, in the current context of an ischaemic model of cerebral oedema, where specific gravity measurements are made at 4 hours post-MCA occlusion and presumably before BBB breakdown is established, the author anticipates that the effect of serum proteins in the oedema fluid is much less marked. The author therefore considers it legitimate to express his results as specific gravities.

Clinically, it may be more relevant to express oedema in the recognisable terms of % g H_2O/ g tissue, or express the degree of oedematization as changes in tissue *volume* (eg Elliot & Jasper 1949). However, it is not helpful to introduce further potential errors into the calculations. Even Marmarou et al (1978) commented in their conclusions that it was essential that both normal % H_2O values using the dry/wet ratio and normal SG_t calculated from the gradient column were accurate, as constants in their conversion equations and SG_s were derived from them.

3.6.5. Ferszt and his colleagues (1980) eloquently demonstrated that the use of tissue fragments as small as 5mg and less can lead to a number of problems. Because of their relatively large surface area, they will tend to dry much more rapidly if exposed to air. Even temporarily maintaining the samples by immersion in kerosene appeared to cause discrepancies in subsequent SG measurement, possibly because the kerosene tends to dissolve the lipids within the tissue block (also Shigeno et al 1982). They have recommended transfer of the specimens in a high humidity environment (100%) and rapid processing. In studies involving rat brain, tissue samples have to be small, especially where the availability of tissue is limited eg. white matter. The author estimates that samples will weigh between 3mg and 8mg. However, these sources of error are less significant if volume changes are *not* calculated using the specific gravity data (which multiplies the errors substantially), and this has been avoided.

The "flotation kinetics" of small tissue samples as they descend through the column, compared to large tissue fragments which equilibrate rapidly and have a clear fast/slow phase interface, suggests that the use of small tissue specimens may be suboptimal (Ferszt et al 1980; Shigeno et al 1982). Small samples continue to descend even after several minutes, and it is difficult to identify a fast/slow point at which the specific gravity can be measured. The accepted time of descent at which measurements are estimated varies from

one study to another (Shigeno et al 1982 - 1 min; Marmarou et al 1978, 1982; Ferszt et al 1980 - 2 min; Picozzi et al 1985 - 3 min). This thesis uses the point of descent at 1 minute, based on the methodology used by Shigeno et al (1982). Ferszt et al (1980) suggest that the poor equilibration characteristics demonstrated by small tissue fragments, especially white matter, is due to the higher friction or drag coefficient related to their larger surface area and a reaction between the fragments and the organic solvents i.e. changes in specific gravity related to tissue lipid solubility. They maintain that this significantly affects reproducibility of results. To test this possible source of error, the author estimated tissue specific gravities in 7 control animals using identical experimental methods, and found that results were consistent with small standard errors, contrary to Ferszt and colleagues' opinion (appendix 4, q.v.).

Despite many technical pitfalls, microgravimetry remains a useful tool in brain oedema estimations, providing sources of error are kept to a minimum. It cannot be applied to the exclusion of other techniques, but should be considered an adjunct to them.

3.7 MEASURING CBF BY HYDROGEN CLEARANCE

3.7.1. Current techniques for measuring CBF took origin in the mid-1940's after methods were developed by Kety and Schmidt (1945) using the inert gas nitrous oxide. They measured cerebral blood flow in man by utilizing cerebral arteriovenous differences in the concentration of nitrous oxide, a method based on the Fick† principle. This restates the law of conservation of mass i.e. the quantity of a given substance carried to an organ in a given time equals the amount of that substance carried to the organ minus the amount removed (the arteriovenous concentration difference). The equation $F = Q_b(A - V)$ is derived from this, where F = blood flow, Q_b = quantity of the substance taken up by the brain, and A - V = arteriovenous concentration difference. If the partition coefficient of the inert gas is known, Q_b can be calculated, and therefore blood flow established.

3.7.2. Several modifications were developed to improve on the original Kety-Schmidt technique, including the introduction of krypton^{85}, a radioactive inert tracer, by Lassen & Munck (1955). In clinical studies, Ingvar and Lassen (1962) introduced a technique that

† *Adolph Eugen Fick, German physiologist, 1829 - 1901.*

allowed the study of *regional* blood flow in the brain, which involved the rapid injection of xenon^{133} into the internal carotid artery, and measuring the clearance of the isotope using scintillation counters. Modifications of this method are still used in clinical practice, although it is invasive requiring carotid artery puncture. Measurements of regional cerebral blood volume or total (but not regional) blood flow can be made using non-diffusible indicators eg. technetium^{99}-labelled sodium pertechnetate, which can be injected intravenously or inhaled and their passage through the brain monitored by scintigraphic scanning. Modern techniques using positron emission tomography (PET) and nuclear magnetic resonance are being developed to assess cerebral metabolism by non-invasive means, but a discussion of these is beyond the remit of this study.

3.7.3. Experimental measurement of cerebral blood flow does not need to depend on non-invasive techniques. The commonest experimental techniques use either autoradiography, or modifications of inert gas methods eg. the use of hydrogen gas clearance. Autoradiography involves the intra-arterial injection or intravenous infusion of radioactive tracers (eg. ^{14}C-iodoantipyrine), immediate circulatory arrest, and the subsequent assessment of radiographic density after exposing the tissue to X-ray sensitive plates. The radiographic density will be proportional to the blood flow, and is calculated by comparison with carbon-14 standards of known radiographic density. Autoradiography has the advantage of assessing the regional blood flow to many areas at the same instant, but is expensive in terms of equipment and often requires lengthy exposure times of many days before the results are available. It assesses the *average* CBF of an entire cross-sectional area of any particular region under examination (Haining et al 1968). Moreover, as Kety points out, " one cannot decapitate the same animal more than once" (Kety 1965). Hydrogen clearance techniques measure flow only within the immediate vicinity of the electrode, but allow the repeated estimation of blood flow simultaneously in specified areas over a period of time, even in conscious animals (i.e. chronically implanted electrodes eg. Haining et al 1968; Heiss et al 1976). H_2 clearance is economical to implement, the electronic equipment is simple in design and easy to install, and electrodes are easily manufactured (Young 1980).

3.7.4. Aukland introduced the use of hydrogen gas clearance for the estimation of local

blood flow in 1965. His methods were quickly applied to the measurement of cerebral blood flow (Fieschi et al 1965; Gotoh et al 1966). Fieschi and co-workers compared H_2 clearance with ^{14}C-antipyrine autoradiography, and found good correlation between the techniques (Fieschi et al 1969). Excellent correlation has been reported when H_2 clearance is compared with ^{133}xenon gas clearance and radioactive microsphere injection techniques (Rowan et al 1975; La Morgese et al 1975). Aukland commented that hydrogen gas was more suitable than other inert gases because the molecules are small and the diffusion equilibrium between tissue and blood is very rapid. The tissue:blood partition coefficient for hydrogen is 1.0, which simplifies the mathematical derivation of the cerebral blood flow (see appendix 1).

3.7.5. The technique of hydrogen clearance involves the use of tissue platinum electrodes for the polarographic recording of changes in tissue electrical impedance associated with alterations in H^+. Briefly, the concept of polarography entails the oxidation of a substance at the electrode surface (in this case, $H_2 \rightarrow 2H^+ + 2e^-$) in response to the electrode voltage. The acceptance of the electrons by the electrode is improved when it is polarized to a positive voltage (usually +250 mV), which also reduces the sensitivity of the electrode to O_2. The current generated by this oxidative process is proportional to the concentration gradient of hydrogen at the surface of the electrode, and is in turn linearly related to the concentration of hydrogen in tissue solution. After the tissues are fully saturated, the rate of desaturation will depend on the "washout" of hydrogen from the tissues, in turn dependent on tissue blood flow. The falling tissue concentration of H_2 is detected as a proportional fall in electrical voltage at the electrode surface, and this voltage change is compared to a silver-chloride subcutaneous reference electrode. The slope of the desaturation curve depends on the rate of change in this voltage difference and is thus a measure of the blood flow.

3.7.6. Aukland pointed out that an electrode introduced into a tissue will be surrounded by a zone of devitalised tissue, and blood or tissue fluid may accumulate around the electrode, creating a layer through which hydrogen is transported by diffusion. However, despite this diffusion layer, the current is still proportional to the concentration of hydrogen in the surrounding undamaged tissue, but a change in the concentration gradient at the surface of

the electrode in response to a change in tissue concentration will be delayed until equilibration has occurred across the diffusion layer. This delay will depend on the thickness of the diffusion layer. In his critical review of the technique, Young (1980) commented that at low flow rates, as might be expected in ischaemic situations, even moderately thick diffusion barriers can be tolerated without significantly distorting the H_2 clearance curve. Betz (1965) studied the proliferation of connective tissue around chronically implanted electrodes over many weeks, and concluded that this factor was of little or no importance in affecting blood flow estimations. Haining et al (1968) also found little change in flow rates with chronically implanted electrodes over 6 weeks, supporting the concept that tissue damage and recovery has little effect on local blood flow estimations.

3.7.7. Other theoretical and practical difficulties with the technique include baseline changes during H_2 clearance, and problems with biexponential or polyexponential clearance curves. Baseline shifts can occur because the electrode is also sensitive to other substances in the tissue fluids such as O_2 and ascorbate. This problem is minimised by polarizing the electrode, so that its voltage is within a range that is more applicable to the oxidation of H_2 rather than other compounds. The problem of polyexponential curves arises because the homogeneity of the environment (ie. evenness of tissue blood supply and H^+ concentration) around the point of the electrode may not be entirely stable. In the first place, the electrode itself may penetrate different areas where blood flow is in fact different, so that fast and slow components arise in the clearance rates. This is further complicated by cross flow between compartments, where one compartment which loads rapidly with hydrogen transfers its gas to a slower loading compartment, probably because hydrogen is so diffusible within brain tissue (Halsey et al 1977). Like many experimental methods, a number of theoretical and practical assumptions have to be made in deriving cerebral blood flow from hydrogen clearance (Aukland 1965; Young 1980). However, the validity of the method has been confirmed experimentally by many studies (eg. Aukland 1965; Gotoh et al 1966; Fieschi et al 1969; Pasztor et al 1973; Young 1980), and hydrogen clearance remains an extremely useful tool for cerebral blood flow studies.

3.8 IMMUNOSUPPRESSION BY WHOLE BODY IRRADIATION

3.8.1. The author has contributed to other studies that assessed the effects of immunosuppression by whole body irradiation on the development of cerebral oedema in a different model of cerebral ischaemia (Kane et al 1992). In these studies, whole body irradiation of male Wistar rats also achieved marked reductions in platelet and white cell counts, which were greater at 7 days than 4 days. This thesis has used a 7 day interval between treatment and surgery. Kane et al (1992) also ascertained the effect of regional radiation, by comparing whole body irradiation with body-only and head-only irradiation. They demonstrated that head-only irradiation *increased* leucocyte counts, and indeed made cortical and caudate oedema significantly worse. This supports the concept that whole body irradiation is acting to reduce cerebral oedema by its immunosuppressive effect, rather than any direct effect of irradiation on brain circulation. Although the *late* effects of brain irradiation on the generation of vasogenic oedema have been studied (Caveness et al 1980), no other current experimental studies in this field have used irradiation as a method of inducing white cell depletion. In this respect, this thesis has pioneered the technique (Strachan et al 1992; Kane et al 1992).

3.8.2. Although whole body irradiation is effective in causing immunosuppression (Coggle 1971), this effect is dose-related. Immune function is not an all-or-none phenomenon; depression of immune function encompasses a continuum of impaired humoral and cell-mediated responses from minor through moderate to severe. A radiation dose of 5 Gray (500 rads) to a rat delays the rejection of kidney grafts, yet has no effect on the survival of skin grafts (Hutchison, personal communication). This dose significantly affects humoral (antibody) responses, because β-lymphocytes are sensitive to the effects of radiation. It has less effect on cell-mediated immunity. The author therefore uses the term "immunosuppression" in its broadest sense throughout this thesis, as a description of the generalised white cell and platelet depleting function of irradiation incorporating dose related effects on humoral and cell-mediated immunity. It does not imply a precise quantified effect on immune function. This would be true of any study that describes the depression in number or function of peripheral blood components in terms of "immunosuppression".

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Chapter 4

RESULTS

Details of all the experimental results

4.1 AUTOREGULATION [Groups 1 & 2]

4.1.1. In the autoregulatory study groups, no significant differences were noted in any physiological variables, except between the baseline haematocrits and an isolated difference in pre-occlusion pO_2 (tables 1 & 5). The blood flow data is listed in table 15, and displayed graphically in figure 8. In the sham-operated group, baseline blood flows in the four electrodes were variable in keeping with the recognised heterogeneity of local cerebral blood flow. Thereafter, blood flows in all four areas were well maintained until mean arterial blood pressure fell to 60 mmHg, when there was a fairly precipitous fall. Mean blood flows at 50 and 40 mmHg were far more consistent in all areas. On reperfusion, cortical blood flow recovered relatively well, except in the left frontal electrode. As a percentage of the baseline blood flows, the recovery blood flows are shown in the adjacent table. It is not clear why the left frontal electrode failed to recover as well as the others. Despite the CBF recovering to over 90% of baseline in the right hemisphere, CBF never attained or exceeded baseline values. There were no statistical differences independently comparing right and left hemisphere electrodes.

REPERFUSION BLOOD FLOW			
% Baseline			
Sham	*RF*	*93.4*	
	RP	*90.4*	
	LF	*67.7*	
	LP	*84.8*	
MCAO	*RF*	*47.0*	*94.1* *
	RP	*55.5*	*98.9* *
	LF	*98.5*	
	LP	*93.3*	

* = *% post-occlusion flow (90 mmHg)*

4.1.2. In the MCA occlusion group, there was a significant fall in post-occlusion blood flow in the right hemisphere. As occurred in the cerebral blood flow studies (para. 4.4.3), post-occlusion blood flow also fell in the non-ischaemic (left) hemisphere, although the reduction in blood flow was less marked. Thereafter, blood flow remained linear, until at a MABP of 70 mmHg when it began to decrease gradually in the left hemisphere, and in the parietal cortex of the right hemisphere. It improved marginally in the right frontal cortex before deteriorating gradually at 60 mmHg. This difference in frontal and parietal electrodes of the ischaemic (right) hemisphere is marginal, although it may be an indication that cortical blood flow is better maintained in the frontal rather than the parietal cortex, perhaps

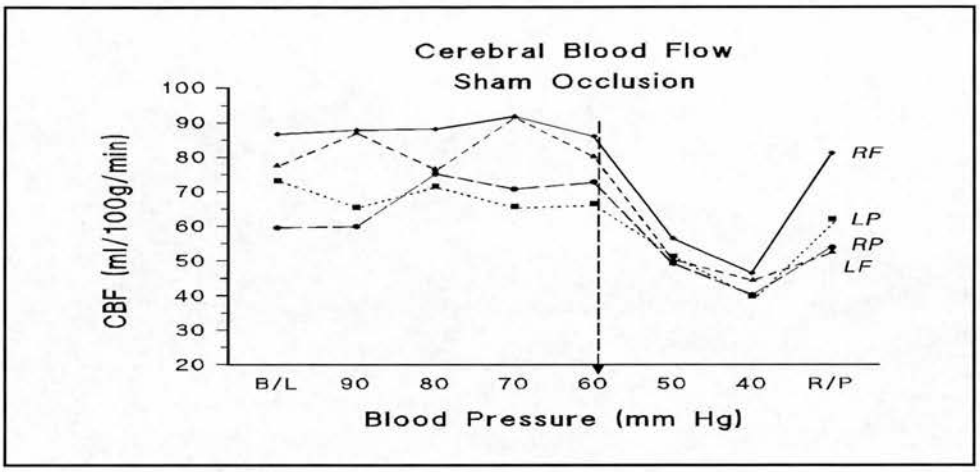


Figure 8a: Cerebral Blood Flow relative to blood pressure in Sham operated animals.

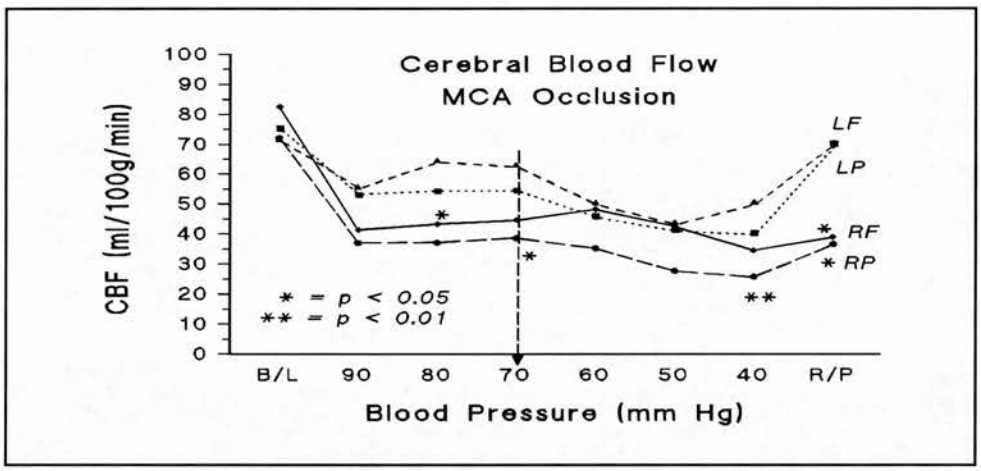


Figure 8b: Cerebral Blood Flow relative to blood pressure in MCA occlusion group.

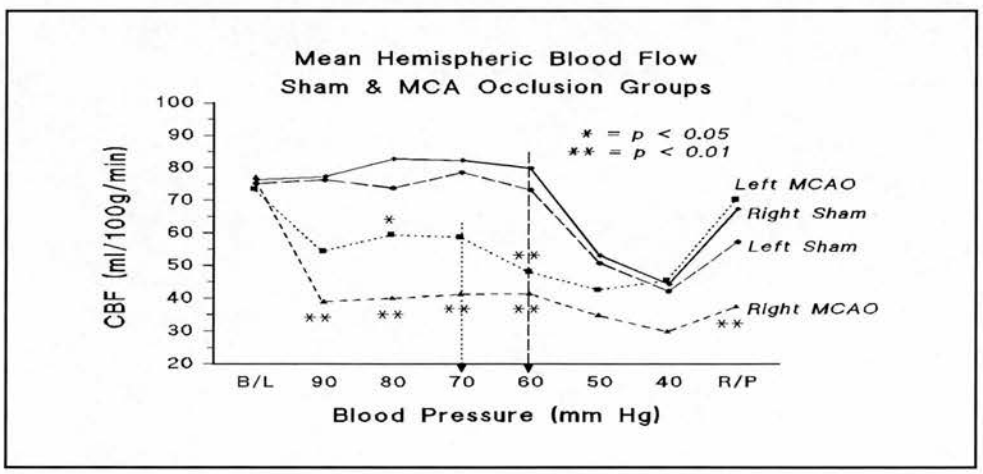


Figure 8c: Comparison of average hemispheric blood flows between sham and MCA occlusion groups.

FIGURE 8

Cerebral blood flow estimated relative to a reduction in MABP. ***B/L*** *= Baseline blood flow prior to occlusion;* ***R/P*** *= Reperfusion blood flow. Electrode position:* ***R,L*** *= Right, Left;* ***F,P*** *= Frontal, Parietal (p values = right v. left sides). The bottom graph compares sham operated with MCA occlusion groups using average hemispheric blood flows calculated as means between frontal and parietal electrodes (p values = MCAO v Sham).*

related to differences in collateral blood flow (ie. autoregulation is less disturbed where the cortex is less ischaemic). Unlike the sham operated group, there was no precipitous fall in CBF at any level of mean blood pressure, perhaps reflecting a dysautoregulatory response. Reperfusion blood flows (listed above) also recovered well compared to baseline blood flows, and in the right hemisphere although the blood flows remained depressed they returned close to the post-occlusion (90 mmHg) level. Because of the MCA occlusion in the right hemisphere, significant differences in blood flow between left and right sides were noted at 80, 70, and 40 mmHg and also on reperfusion (figure 8b).

4.1.3. Average blood flows were calculated for each hemisphere, and both sham and MCA occlusion groups were compared (figure 8c). In the sham operated group, this demonstrated the linearity in the blood flow until a mean pressure of 60 mmHg was reached when CBF falls. In the MCA occlusion group, the apparent gradual reduction in cortical blood flow in the left hemisphere was a more curious finding (see also appendix 6), and this is considered in the discussion (para. 5.1.3). In general terms, the results indicate that the autoregulatory response is different in the MCA occlusion group, but it is not wholly impaired, and appears to be maintained down to a mean systemic pressure of 70 mmHg. This suggests that the electrodes are placed within cortex which is not significantly ischaemic and where autoregulation is at least partially preserved. Linear regression analysis of the mean hemispheric blood flows in the MCA occlusion group also confirmed a dysautoregulatory response, interestingly more obvious in the left (non-lesioned) hemisphere (Appendix 6, page 137).

4.2 INFARCT SIZE [Groups 3, 4, 5, 6, & 7]

4.2.1. Physiological variables in all groups are listed in tables 2,3,6, and 7. In the TTC perfusion studies, pO_2 was marginally higher in the hypotensive group at 60 minutes post-occlusion, and MABP was lower in the hypotensive group at the same period ($p = 0.046$). This difference in MABP has occurred because the mean blood pressure was rather higher in the normotensive group, but still remaining greater than 100 mmHg in the hypotensive

group. There were no differences in full blood counts or serum glucose levels between the groups (table 2). The haematocrit fell in the hypotensive group towards the end of the experimental period, as some blood was lost within the system after reperfusion following the hypotensive period. In the histology study groups, no differences were noted in haematocrits, serum glucose, or full blood counts. A number of differences were noted in the mean blood pressures, but these always exceeded 90 mmHg.

4.2.2. Using this model of focal ischaemia, there is still considerable variability in the sizes of the infarcts, most marked in the hypotensive group (adjacent table). Duverger & MacKenzie (1988) have also noted the variability of infarct volume especially in Wistar rats. However, there is clearly a trend towards larger infarcts in the hypotensive group. The right (lesioned) hemisphere always appeared larger than the left at the time the brain was removed, and this impression was confirmed by volumetric analysis (table 11). In the sham operated animals, hemisphere sizes (right versus left) were almost identical. In the hypotensive group, the difference between the two hemispheres was greater than in the normotensive group, although a comparison between the two groups did not reach statistical significance (figure 17, page 92). The absolute infarct size difference between the two groups (190.2 $\pm$8.8 mm^3 v. 230.7 $\pm$10.1 mm^3) was significant ($p < 0.01$). To correct for differences in the extent of hemispheric swelling that has occurred between normotensive and hypotensive groups, the infarct sizes have also been calculated as percentages of the whole hemisphere. There is still a significant increase in the size of the infarct in the hypotensive group, although the significance is less (25.1 $\pm$1.1 % v. 29.2 $\pm$1.6 %, $p < 0.05$).

NORMOTENSIVE *n = 10*	HYPOTENSIVE *n = 8*
19.0	*27.9*
31.4	*35.1*
23.0	*36.8*
27.8	*29.7*
28.0	*24.7*
21.1	*27.9*
24.1	*24.6*
26.9	*27.4*
25.3	-
24.9	-

Infarct sizes (as a percentage of the hemisphere) in the TTC perfusion groups.

4.2.3. The size of the cortical lesion as a proportion of the cortex (0.39 $\pm$0.02 v 0.42

± 0.03) was not significantly different, although it was larger in the hypotensive group. Interestingly, the size of the caudate lesion as a proportion of the caudate was significantly larger in the hypotensive group (0.10 ± 0.01 v 0.13 ± 0.01; $p < 0.05$). At first sight, it might appear that the fall in blood pressure has affected the caudate more than the cortex, but as the caudate proportion of the whole lesion was about 20% in both groups, the apparent increase in the size of the caudate infarct may be explained simply by variations in the size of the whole caudate between the groups (ie. differences between the size of the right and left caudate was smaller in the hypotensive group; see table 11).

4.2.4. The volumetric data assessed by quantification of the histological studies (groups 6 and 7) is outlined in table 12. Figure 9 (next page) illustrates the configuration of infarcts seen in normotensive and hypotensive groups, and the adjacent table indicates the variability in infarct size. Despite the small number of animals in this series, the infarct areas were significantly larger in the hypotensive group, both absolute sizes (57.9 ± 7.5 mm^3 and 84.9 ± 7.6 mm^3; $p < 0.05$) and where the areas are expressed as a percentage of the hemisphere ($13.3 \pm 1.7\%$ and $19.6 \pm 1.8\%$; $p < 0.05$). There is clearly a trend for the infarcted cortical area to be larger in the hypotensive group, although this did not reach statistical significance ($p = 0.057$). However, the infarcted caudate areas were very similar in both groups, albeit slightly larger in the hypotensive group, which concurs with other reported series (Osborne et al 1987).

NORMOTENSIVE *n = 5*	HYPOTENSIVE *n = 5*
11.3	*15.7*
15.0	*15.9*
19.3	*19.4*
9.7	*22.4*
11.3	*24.5*

Infarct sizes (as a percentage of the hemisphere) in the histology groups.

4.3 CEREBRAL OEDEMA [Groups 8, 9, 10, 11 & 12]

4.3.1. In the 4 hour experiments, haematocrits and glucose results were all similar except the pre-terminal glucose result in the hypotensive group was less (but still within a normal range) than in the normotensive group, perhaps reflecting greater stress in these animals

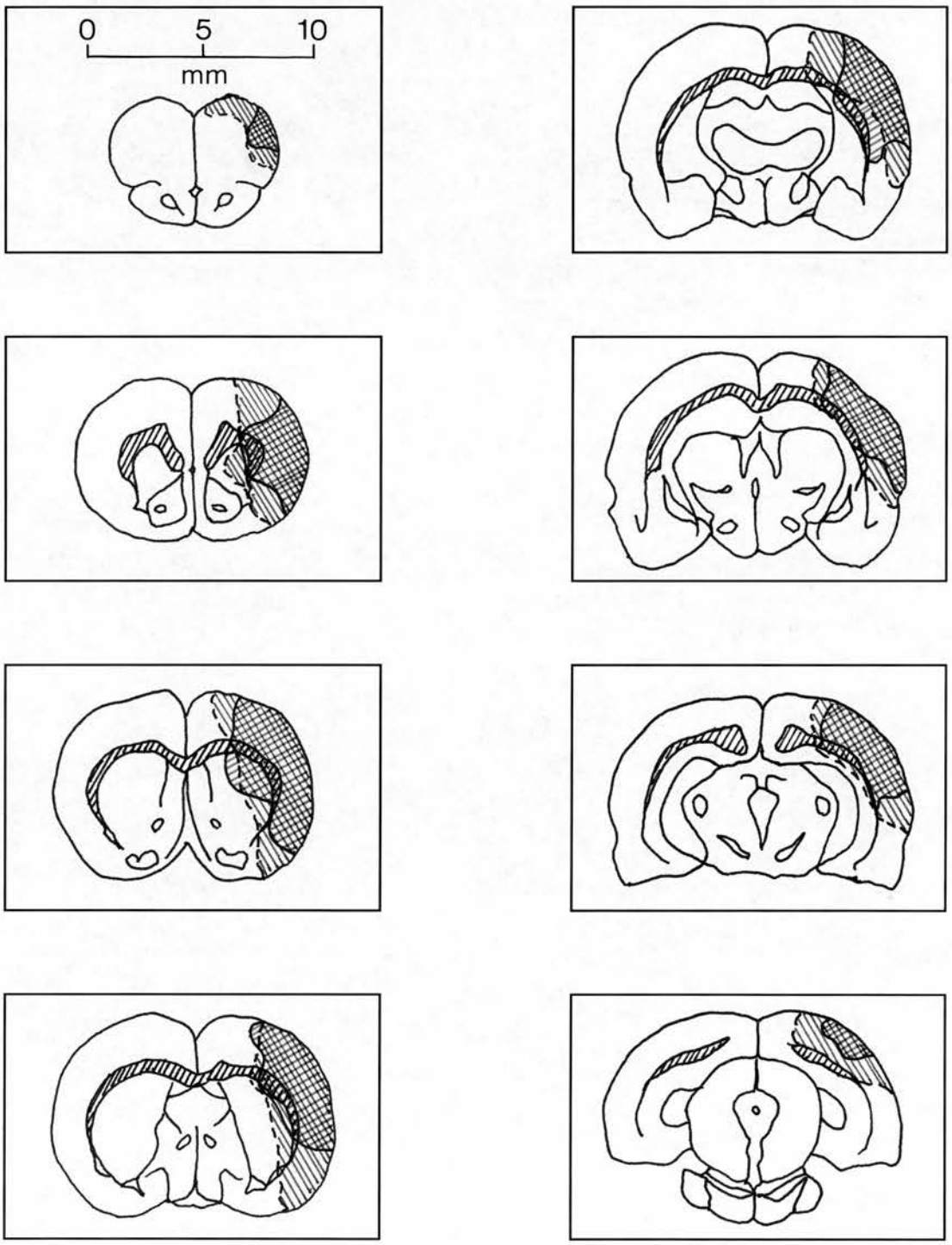


FIGURE 9

Comparison between typical configurations of normotensive (red) and hypotensive (green) infarcts. Scale = 3.5 x 1.

(table 4). No significant differences were noted in the full blood counts (table 4). The other physiological variables are shown in table 8. There were several significant differences noted between the groups in pH, pO_2 and temperature estimations. However, oxygenation was always satisfactory; pH tended to fall in the hypotensive group post-occlusion presumably because of peripheral shut-down followed by reperfusion; and temperature regulation was so precise that the standard errors were very small. A critical examination of these differences suggests that they would not affect experimental outcome. It is important to note that pCO_2 was rigorously controlled, and mean arterial blood pressure was never significantly different (apart from during the hypotensive period!) although it tended to be slightly lower in the hypotensive groups post-occlusion following reperfusion (cf. groups 5 and 7 results).

GROUP	*11* *24 hours* *n = 8*	*12* *72 hours* *n = 6*
WBC $\times 10^9$/l	*8.2 ±0.9*	*8.7 ±0.8*
Hb g/dl	*14.0 ±0.9*	*13.5 ±1.0*
Haem (I) %	*34.4 ±1.8*	*34.3 ±2.1*
Plat $\times 10^9$/l	*778 ±98*	*858 ±104*
Haem (II) %	*44.6 ±1.3*	*44.2 ±1.1*

Full blood count parameters in Groups 11 and 12.

In the 24 and 72 hour groups (11 and 12), full blood counts including haematocrits were very similar in both groups (see adjacent table). The post-operative haematocrits cannot be compared with the pre-operative results, as the former were measured just before the animals were killed using the Hawksley microhaematocrit centrifuge and reader. However, it is noteworthy that the haematocrits were almost identical in both groups just prior to measuring the specific gravities, as this suggests that the fluid status of both groups was similar.

All the specific gravity results are listed in table 14, and several comparisons are displayed graphically in figures 10 and 11 (pages 70 and 71).

4.3.2. *SG - Right versus left hemisphere - 4 hours post-occlusion.*

In the sham operated group at 4 hours, there were no significant differences in specific gravity results between right and left hemispheres. In both the normotensive and hypotensive groups, significant differences were found between the specific gravities of the

right (lesioned) cortex and caudate samples compared to the left (Cortex: normotensive - 1.0493 ± 0.0011 v 1.0526 ±0.0007, $p < 0.05$; hypotensive - 1.0455 ±0.0013 v 1.0508 ±0.0008, $p < 0.05$. Caudate: normotensive - 1.0445 ±0.0010 v 1.0528 ±0.0012, $p < 0.001$; hypotensive - 1.0448 ±0.0017 v 1.0507 ±0.0010, $p < 0.01$). The differences were more significant within the caudate, especially in the normotensive group. A significant difference was noted in the white matter between the hemispheres of the normotensive group (1.0466 ±0.0010 v 1.0499 ±0.0011, $p < 0.05$), but not the hypotensive group (figure 10a/10b, next page).

4.3.3. ***SG - Right versus left hemisphere - 24 and 72 hours post-occlusion.***

As in the 4 hours groups, specific gravity was significantly lower in the cortex and caudate areas within the right hemisphere when compared to the left (24 hours: cortex - 1.0440 ±0.0016 v 1.0495 ±0.0003, $p < 0.005$; caudate - 1.0384 ±0.0017 v 1.0495 ±0.0005, $p < 0.001$. 72 hours: cortex 1.0444 ±0.0011 v 1.0485 ±0.0006, $p < 0.01$; caudate - 1.0403 ±0.0012 v 1.0480 ±0.0004, $p < 0.001$). A significant difference was also noted in the white matter at 72 hours (1.0413 ±0.0007 v 1.0446 ±0.0005, $p < 0.005$), but not at 24 hours (figure 10c/10d, next page).

In all groups, including the 4 hour post-occlusion groups, no significant differences occurred between right and left cerebellar hemispheres. Comparisons between the 24 hour post-occlusion group (group 11) and the irradiation experimental groups (groups 13 and 14) have also been made (see page 80, para. 4.5.3.).

4.3.4. ***SG - Sham versus Normotensive versus Hypotensive groups - 4 hours post-occlusion.***

In the right hemisphere, significant differences were found between the cortex of the hypotensive group and sham operated group (1.0455 ±0.0013 v 1.0525 ±0.0013, $p < 0.005$), but not between the normotensive and sham groups. In the latter comparison (1.0493 ±0.0011 v 1.0525 ±0.0013), the p value was 0.085: the trend was for greater oedema in the normotensive cortex, but this failed to reach statistical significance. Highly significant differences were found when comparing the caudate areas of both normotensive and hypotensive groups with sham animals (1.0445 ±0.0010 & 1.0509 ±0.0017 v 1.0509

Figure 10a: Brain specific gravity at 4 hours post-occlusion (normotensive group).

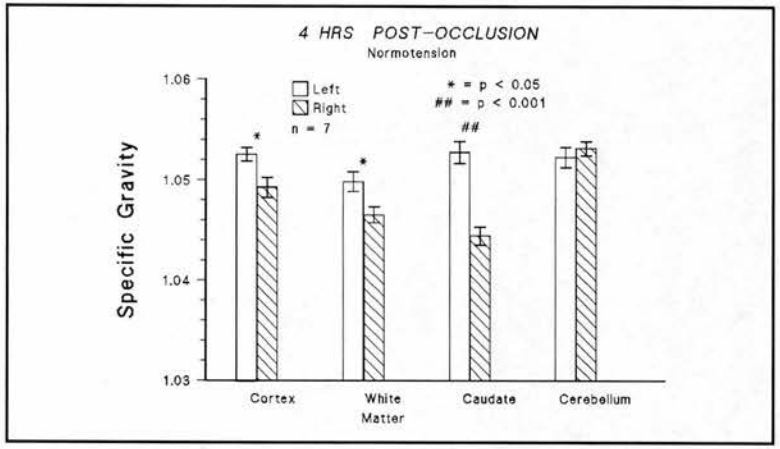


Figure 10b: Brain specific gravity at 4 hours post-occlusion (hypotensive group).

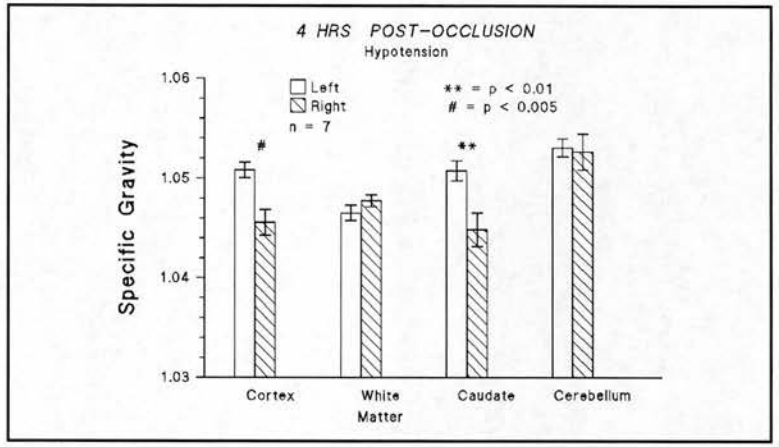


Figure 10c: Brain specific gravity at 24 hours post-occlusion.

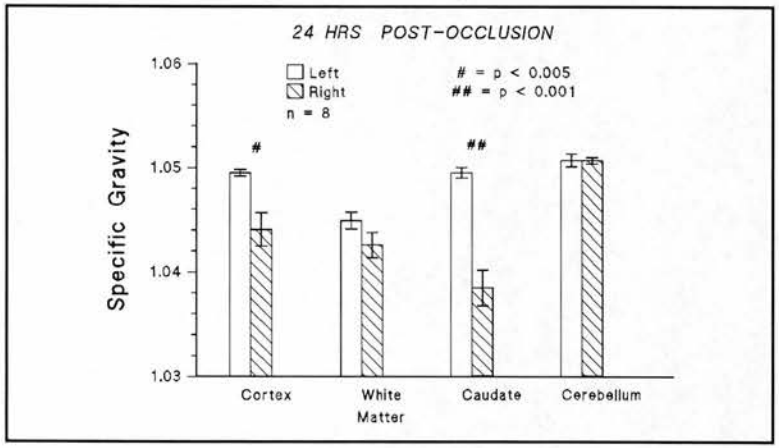


Figure 10d: Brain specific gravity at 72 hours post-occlusion.

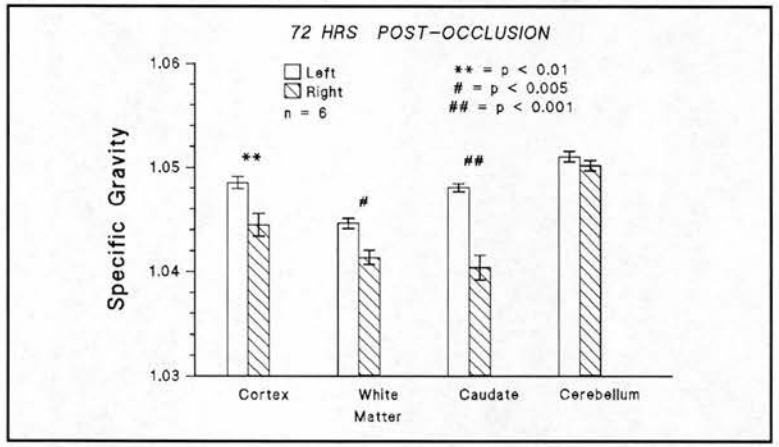


FIGURE 10 *Specific gravities in left (non-lesioned) and right (lesioned) hemispheres at 4 hours (normotensive and hypotensive groups), 24 and 72 hours after MCA occlusion. There is significantly greater oedema in the right hemisphere, especially in the caudate and less so in the cortex. In sham operated animals (not illustrated), there was no difference between hemispheres at 4 hours.*

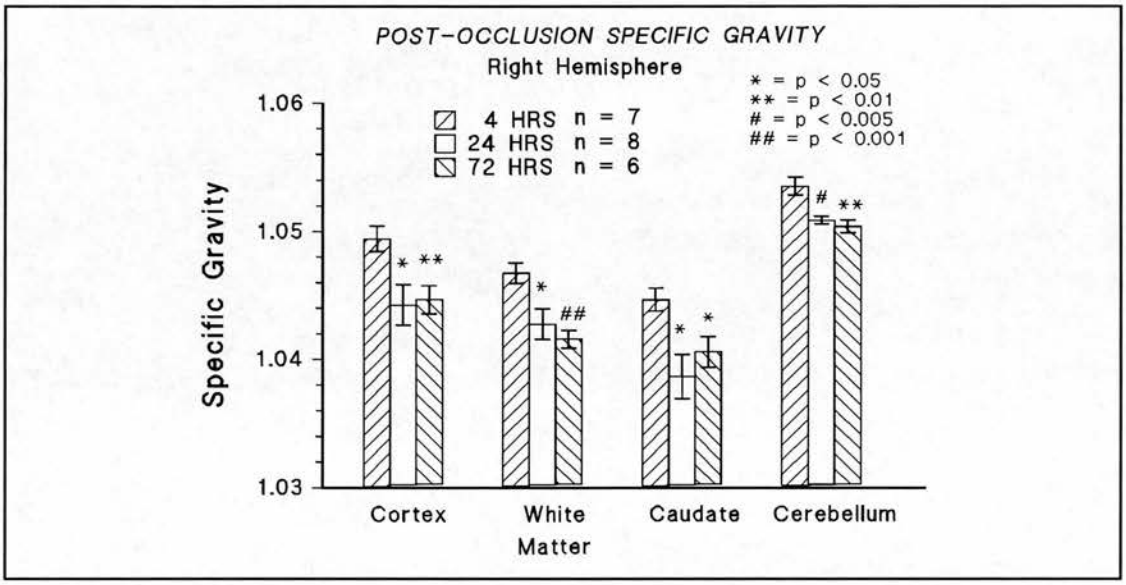


Figure 11a: Specific gravity in the right (lesioned) hemisphere at 4, 24 and 72 hours after MCA occlusion.

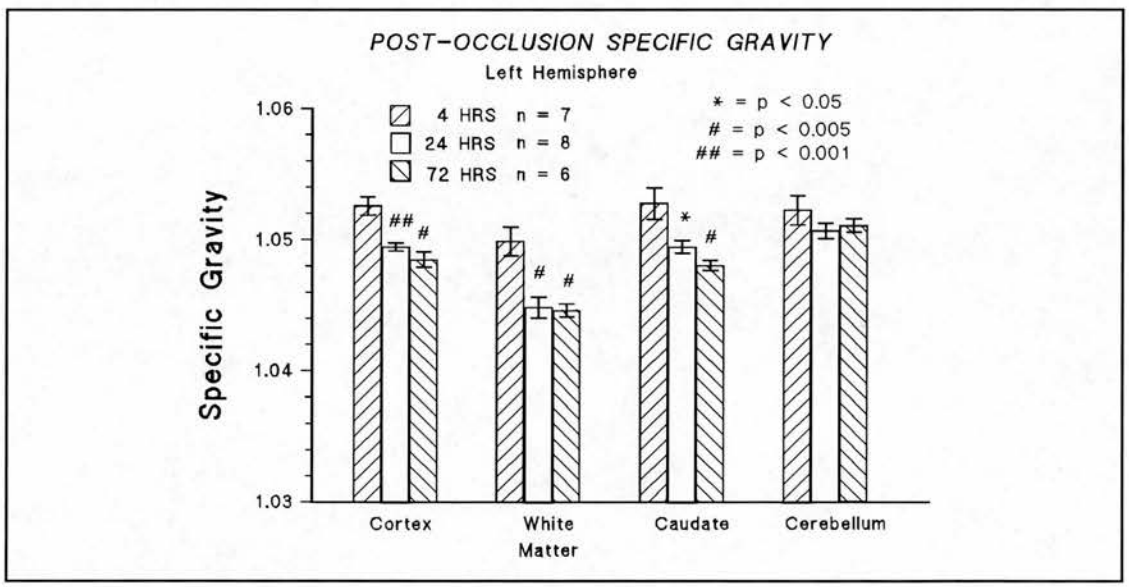


Figure 11b: Specific gravity in the left (non-lesioned) hemisphere at 4, 24 and 72 hours after right MCA occlusion.

FIGURE 11

The top figure would suggest that in this study the specific gravity is significantly less (i.e. oedema is significantly greater) in the lesioned hemisphere at 24 and 72 hours compared to 4 hours post-occlusion. This expected result is confounded by the lower figure, which shows that similar differences are found in the nonlesioned hemisphere, suggesting that it is not safe to compare the 24 and 72 hour groups with the 4 hour group. The possible reasons for this are discussed in the text. (p values are comparing 24 and 72 hour groups with the 4 hour group.)

± 0.0009, $p < 0.001$ and $p < 0.01$ respectively), indicating that in both groups MCA occlusion has caused greatest oedema formation within the caudate.

Comparing normotensive with hypotensive groups, the only significant difference was within the cortex (1.0493 ± 0.0011 v 1.0455 ± 0.0013, $p < 0.05$). Specific gravities in white matter and caudate were very similar in both groups. In the left (non-lesioned) hemisphere, no significant differences were noted between any two groups in any of the four areas, except in the white matter of normotensive and hypotensive groups (1.0499 ± 0.0011 v 1.0455 ± 0.0008, $p = 0.024$).

4.3.5. ***4 versus 24 versus 72 hours post-occlusion.***

A comparison of right (lesioned) hemisphere specific gravities between the 24 and 72 hour post-occlusion groups revealed no significant differences in any area. Comparing both these survival groups with the 4 hour post-occlusion group appeared to show that oedema formation was much greater at 24 and 72 hours than at 4 hours post-occlusion in cortex, white matter and caudate, with p values generally smaller ($p < 0.01$) in the 72 hour group (figure 11). However, it was noted that significant differences also occurred within the cerebellar samples. The standard errors in the all the cerebellar sample groups are small, suggesting reasonable homogeneity of testing within each group. Significant differences were also found when a comparison of *left* (non-ischaemic) hemisphere specific gravities was made between the 24/72 groups and the 4 hour group, suggesting that *it is not safe to compare non-survivor (4 hours) and survivor (24 and 72 hour) groups.*

4.4 CEREBRAL BLOOD FLOW [Groups 8, 9, & 10]

4.4.1. Cerebral blood flow estimations were carried out in the same experimental groups used for 4 hour specific gravity studies. Thus, recorded physiological variables are identical, and significant differences between groups are the same as paragraph 4.3.1. (above).

4.4.2. ***Absolute Cerebral Blood Flow: Right Hemisphere.*** **(table 16)**

There was no significant difference in baseline cerebral blood flows in all 3 groups,

although the baseline flows in group 10 (the hypotensive group) tended to be lower. Following the occlusion, hemispheric blood flows fell appropriately - this fall was more significant in the hypotensive group ($p < 0.05$). In the normotensive animals, blood flow began to recover in the lesioned hemisphere after 60 minutes especially in the right frontal electrode. Right frontal blood flows were consistently higher compared to right parietal blood flows. However, in the hypotensive group, although the blood flow recovered to some extent after reperfusion between 30 and 60 minutes, CBF always remained consistently lower and more linear over the 4 hour period (figure 12a, next page).

In the normotensive group, a total of 144 blood flows were recorded in the right (lesioned) hemisphere, of which the lowest blood flow recorded was 22.5 ml/100g/min (ie. only one blood flow measurement fell below 23 ml/100g/min - see discussion, para. 5.4.2). The overall mean hemispheric blood flow was 45.6 $\pm$14.0 ml/100g/min. Compare the hypotensive group, where the mean hemispheric blood flow fell to 35.2 $\pm$14.1 ml/100g/min, with 16 of 104 blood flow measurements falling below 23ml/100g/min. In this group, one blood flow recording fell below 15 ml/100g/min (11.8 ml/100g/min - the lowest recorded blood flow in this study).

4.4.3. ***Absolute Blood Flow: Left Hemisphere.*** **(table 16)**

In the normotensive group, blood flow fell slightly in the *non*-lesioned hemisphere after the occlusion on the other side, but rose above baseline values between 150 and 180 minutes. However, the *average* blood flow over the 4 hour experimental period was 62.3 ml/100g/min and 59.2 ml/100g/min in frontal and parietal areas respectively, which were very similar to baseline values. In the hypotensive group it was interesting to note that 10 minutes post-occlusion (during the hypotensive period) blood flows fell by 20-25% of baseline values, suggesting that in the non-lesioned hemisphere, autoregulation had not maintained CBF close to expected baseline values. After reperfusion, blood flow returned to baseline values and remained fairly consistent thereafter (figure 12b, next page).

Mean left hemispheric blood flows were comparable between the sham and normotensive groups (68.4 $\pm$17.1 and 60.5 $\pm$12.4 ml/100g/min). The induced fall in blood pressure after occlusion in the hypotensive group affected overall blood flow measurements in the non-lesioned hemisphere (mean = 51.6 $\pm$10.1 ml/100g/min), although this difference

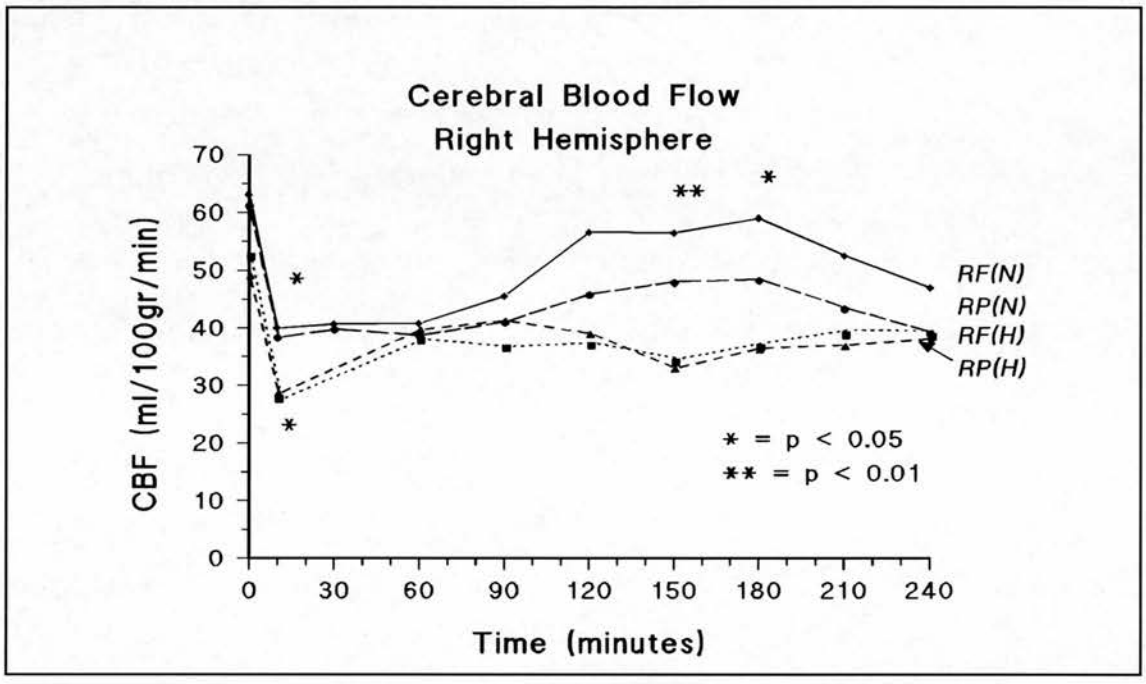


Figure 12a: Cerebral blood flows in the right (lesioned) hemisphere (absolute values).

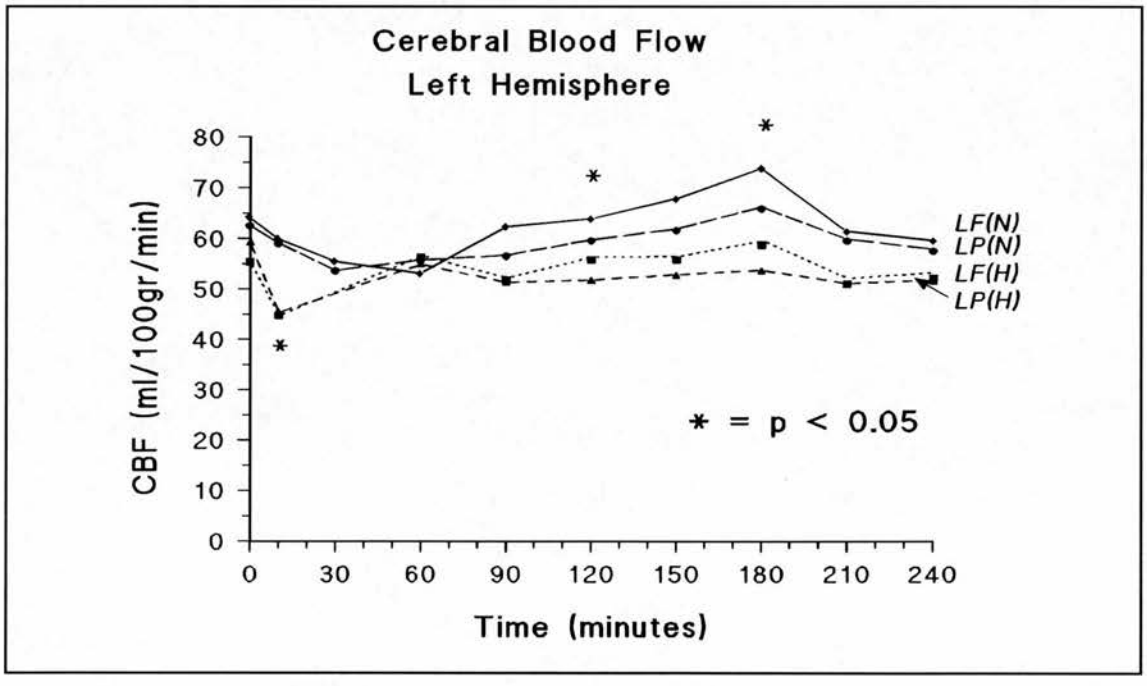


Figure 12b: Cerebral blood flow in the left (non-lesioned) hemisphere (absolute values).

FIGURE 12

Absolute cerebral blood flow graphs for both hemispheres. The lines represent each of the four electrodes: R,L = Right, Left; F,P = Frontal, Parietal; N,H = Normotensive, Hypotensive.

did not reach statistical significance.

4.4.4. ***Percentage Baseline Blood Flow (PBBF): Right Hemisphere.*** **(table 17)**

Percentage baseline blood flows (PBBF), where each CBF value is expressed as a percentage of the initial baseline value, were studied to compare trends and differences between the groups that would not be confounded by inter-experimental systems' or technical performance, giving rise to differences in absolute baseline blood flow measurements between animals. In the normotensive group, post-occlusion PBBF's averaged 74.1% and 70.2% in the frontal and parietal electrodes respectively with a mean hemispheric blood flow of 72.2%. Equivalent results in the hypotensive group were 72.4%, 67.8 % and 70.1%. Thus, although the mean percentage blood flows are lower in the hypotensive group, the period of hypotension has not affected the overall reduction in blood flow following the occlusion, when blood flow is measured as a proportion of the baseline value. In the normotensive group, once again blood flows improved after 90 minutes especially in the frontal area, diminishing again after 180 minutes. However, in the hypotensive group the values remained fairly linear after reperfusion (figure 13, next page).

4.4.5. Although PBBF values at 10 minutes post-occlusion were not significantly different between the groups, mean values were lower in the hypotensive animals (Frontal - 60.6% and 56.3%; Parietal - 63.2% and 53.6%), the difference being greater in the parietal area. Intriguingly, PBBF values were highest in the hypotensive group after reperfusion was completed at 60 minutes, and at this time post-occlusion were higher than in the normotensive group. This may a reflect a reperfusion hyperaemia not seen in normotensive animals.

4.4.6. ***Percentage Baseline Blood Flow (PBBF): Left Hemisphere.*** **(table 17)**

Left hemisphere PBBF values averaged 96.4% and 94.9% in the frontal and parietal electrodes of the normotensive animals, and similarly 87.9% and 97.9% in the hypotensive group. The reason for the significant difference between the average frontal PBBF's in the non-lesioned hemisphere is not entirely clear, but this has arisen in the hypotensive group because of the significant fall in blood flow during the period of hypotension 5-10 minutes

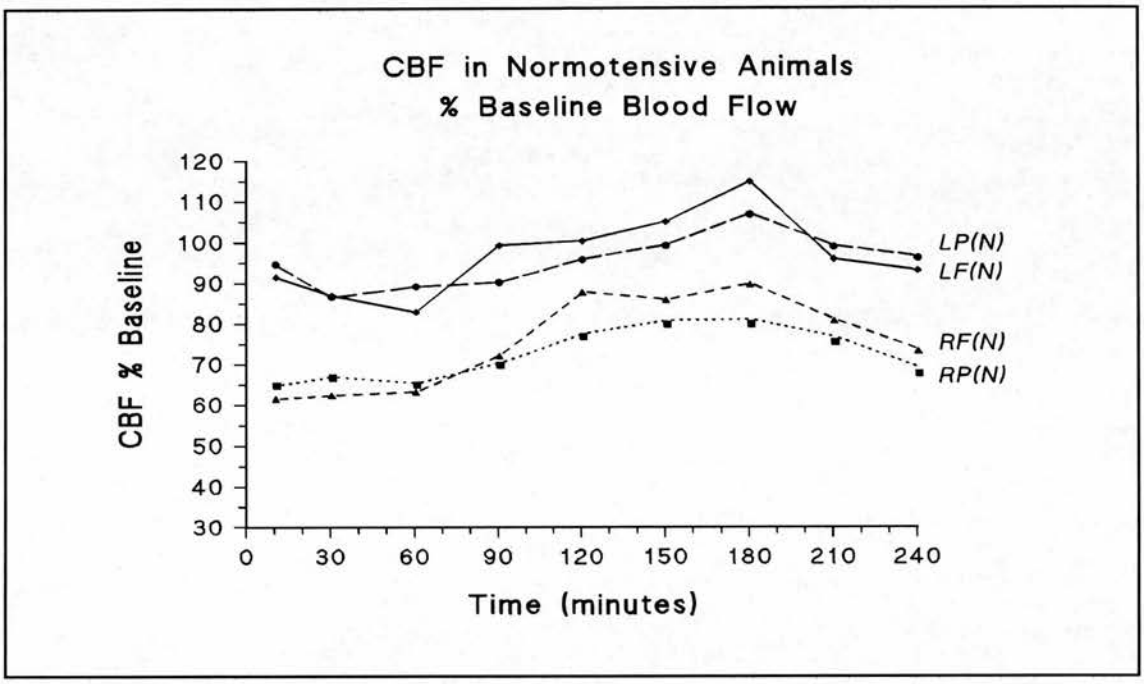


Figure 13a: Cerebral blood flows in Group 9 (normotensive) animals (% baseline values).

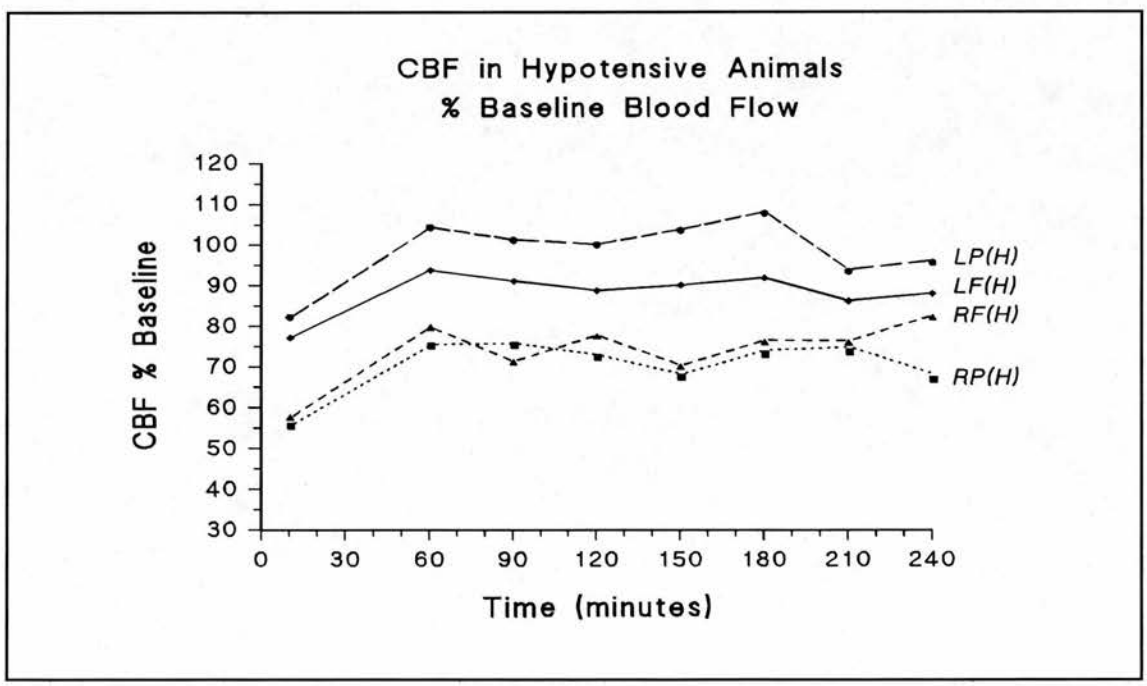


Figure 13b: Cerebral blood flows in Group 10 (hypotensive) animals (% baseline values).

FIGURE 13

Cerebral blood flow graphs with results converted to percentage baseline values. The lines represent each of the four electrodes: R,L = Right, Left; F,P = Frontal, Parietal; N,H = Normotensive, Hypotensive.

post-occlusion, in combination with lower blood flows occurring after 210 minutes when haemodynamic stability tends to deteriorate. Blood flows have fallen in the *left* (non-ischaemic) hemisphere in response to the acute fall in blood pressure after the *right* hemispheric infarction, and then recovered to baseline values after reperfusion. Once again, this suggests that under these conditions autoregulation has not been effective in maintaining blood flow in the *non*-ischaemic hemisphere (figure 13b).

4.4.7. ***Computer analysis versus Hand analysis.*** **(figures 14 & 15)**

Electronic on-line data analysis of hydrogen clearance using techniques of variable sophistication is not new (eg. Jones et al 1981; Nelson et al 1990). An integral part of this study involved the assessment of recently developed software which allowed the on-line acquisition of the hydrogen clearance data, and calculation/storage of CBF results using an Apple computer and a program written in Pascal (MacDonald et al 1993). A direct comparison of hand-calculated and computer-generated results has been made of all the cerebral blood flows estimated in this chapter, and the results have been graphically displayed in figures 14 & 15 (following pages). In both normotensive and hypotensive groups, the correlations were greater than 0.9. Despite the smaller number of data points in the hypotensive group, the correlation was better at 0.93. This arises from the greater number of lower flows in the latter group, and figure 15 illustrates that the correlation improves with blood flows below 50 ml/100g/min. This suggest that the system is more stable and performs better at lower flows. The best correlation is found with monoexponential curves ($r > 0.97$); it is likely that what errors do arise in this group will result from the hand analysis, where the drawing of "best-fit" lines is subject to some error. Providing that the methods of data generation and equipment performance are satisfactory, especially with regard to electrode construction and placement, computer-based systems for data acquisition render the tedious methods of hand analysis obsolete and are likely to be much more accurate.

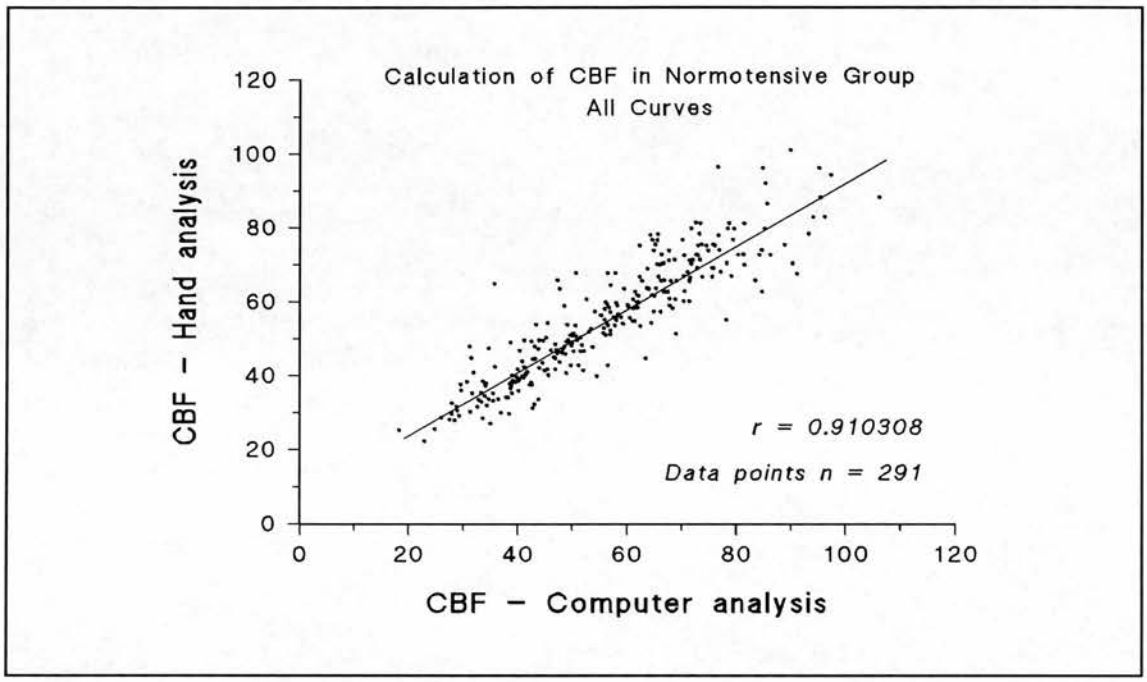


Figure 14a: Computer versus Hand analysis of H_2 clearance curves in Group 9 animals.

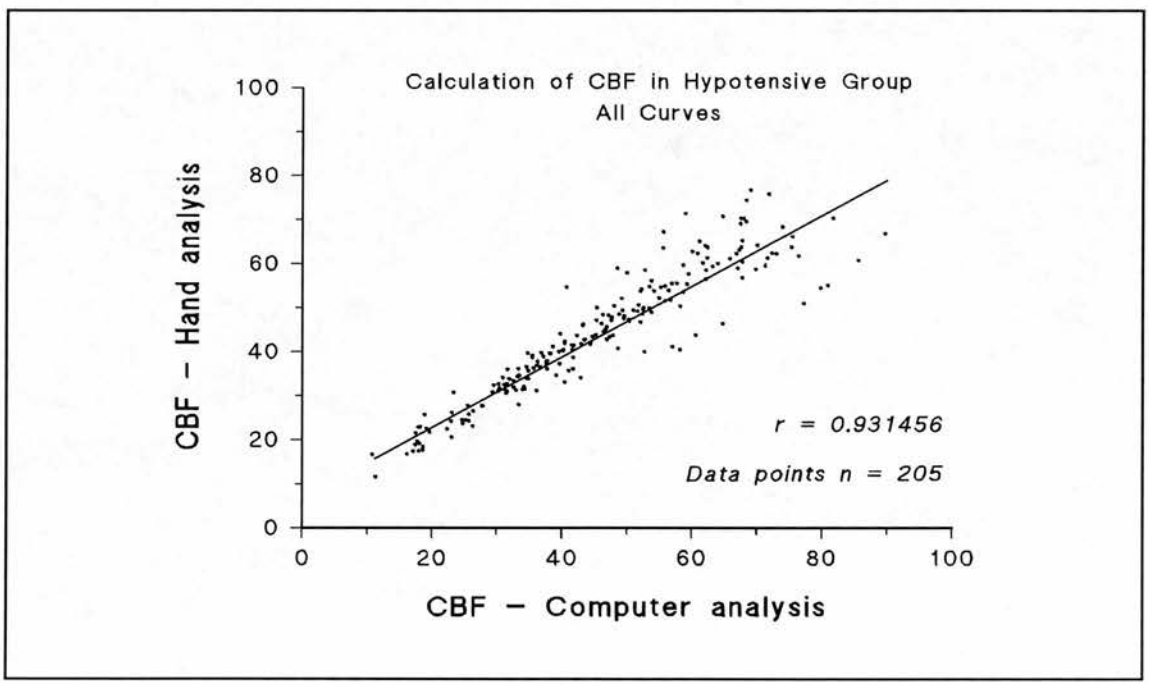
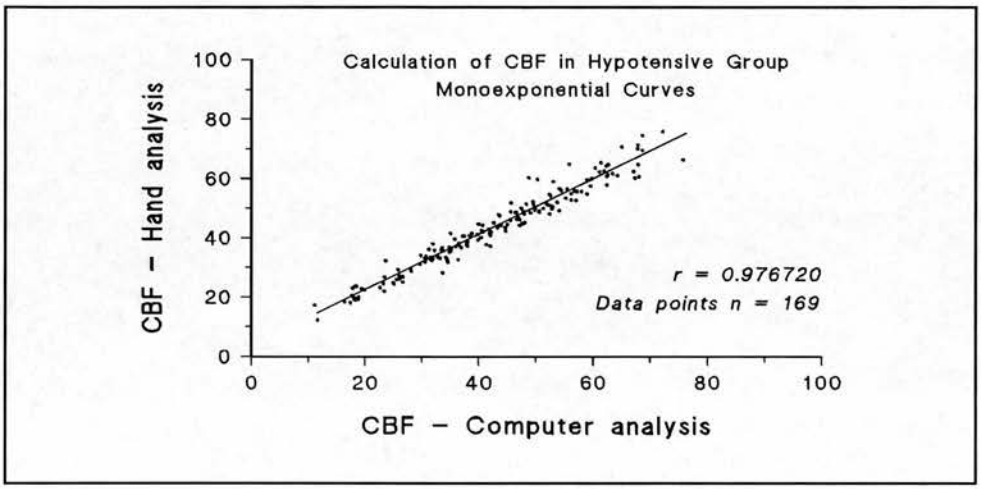
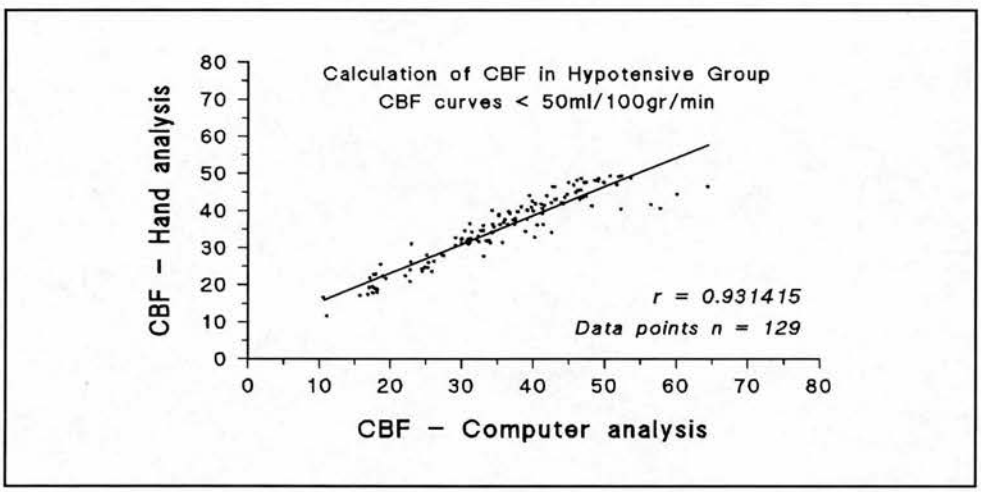


Figure 14b: Computer versus Hand analysis of H_2 clearance curves in Group 10 animals.

FIGURE 14

Computer versus Hand analysis of all the cerebral blood flow results in the normotensive (Group 9) and hypotensive (Group 10) animals (comparison of 496 results). Note that the correlations are greater than 0.9.





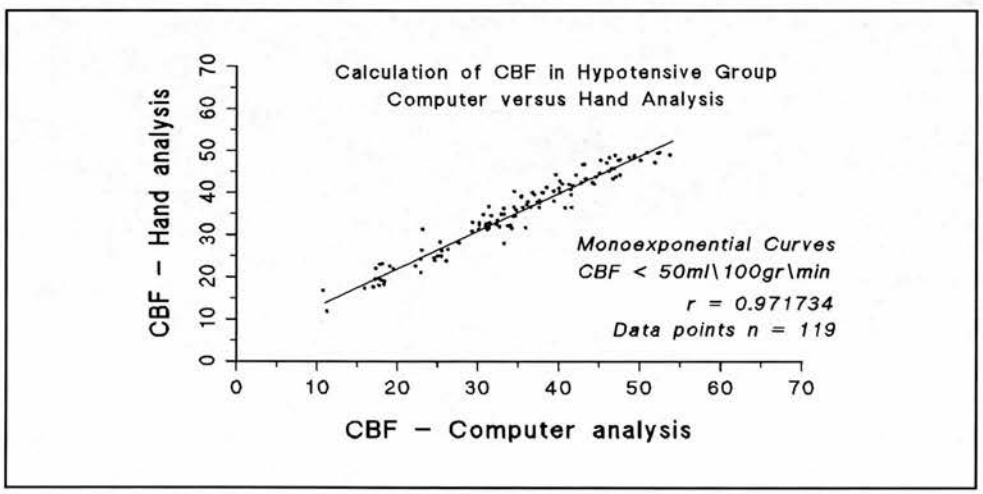


FIGURE 15

Computer versus Hand analysis of H_2 clearance curves in the hypotensive (Group 10) animals. Assessment of selected curves: Top = monoexponential curves; Middle = curves where CBF < 50ml/100g/min (hand analysis); Bottom = both parameters combined. Note that the best correlation is seen with monoexponential clearance curves.

4.5 IMMUNOSUPPRESSION [Groups 13 & 14]

4.5.1. The results of the peripheral blood counts and differentials are shown in table 18. There was no significant difference in any haematological parameter between the two groups prior to irradiation. Seven days post-irradiation, the neutrophil count was reduced in the irradiated group, but this reduction was not significant. Monocyte counts were unaffected. The lymphocyte count in the irradiated animals fell from a mean of 10.4 x 10^9/l to 2.0 x 10^9/l before surgery ($p < 0.001$). Platelet and total white counts were significantly reduced in the irradiated group ($p < 0.005$). At 24 hours following surgery, in the control group the platelet count was higher than the pre-operative value, in keeping with a normal response to surgery. In the irradiated group, however, platelet counts fell from a mean of 217 x 10^9/l to only 65.3 x 10^9/l, indicating a failure in these animals to mount a normal thrombocytosis.

4.5.2. Specific gravity measurements are shown in table 19. In both irradiated and non-irradiated (control) groups, comparisons between operated (right) and non-operated (left) hemispheres showed significant differences in the specific gravities of the white matter and caudate, but not the cerebellum. In the cortex, although specific gravity was less on the operated side in both groups (control - 1.0461 $\pm$0.0005 v 1.0497 $\pm$0.0008; irradiated - 1.0480 $\pm$0.0006 v 1.0507 $\pm$0.0014), the results were only statistically significant in the non-irradiated control group ($p < 0.01$), suggesting that irradiation had conferred some protection against the development of oedema in the lesioned hemisphere. Further, a significant reduction in oedema was demonstrated in the cortex of the lesioned hemisphere in irradiated animals compared with controls (1.0480 $\pm$0.0006 v 1.0461 $\pm$0.0005, $p < 0.05$).

4.5.3. These experiments (groups 13 and 14) offered the opportunity to compare specific gravity results measured at 24 hours after MCA occlusion with those recorded in group 11 (page 69 and table 14). Comparing the control non-irradiated animals in this series with those of group 11 (oedema measurements at 24 hours post-occlusion), there were no significant differences demonstrated. However, there was a significant reduction in oedema formation in the cortex of the right (lesioned) hemisphere in irradiated animals compared

with the lesioned cortex of group 11 animals (1.0480 $\pm$0.0006 v 1.0440 $\pm$0.0016, $p < 0.05$).

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Chapter 5

DISCUSSION & CONCLUSIONS

Critical discussion and overview of project results with concluding remarks

5.1 AUTOREGULATION AFTER MCA OCCLUSION

5.1.1. The results of the current studies reaffirm the fundamental idea of autoregulation of cerebral blood flow, but have only investigated autoregulation at the lower end of the pressure-flow curve. In sham operated animals, there is clearly a lower limit of mean systemic blood pressure at which the steady-state maintenance of blood flow fails (50-60 mmHg in this series: figure 8). Most of the original studies on autoregulation have been performed in primates (Strandgaard et al 1975; Symon et al 1976), cats (Waltz & Sundt 1968), dogs (Harper 1965, 1966; Mchedlishvili et al 1973) or even goats (Kindt et al 1967), and the paucity of recent studies in rats makes accurate comparisons with this study difficult. Dirnagl & Pulsinelli reported a lower limit of 80 mmHg in spontaneously hypertensive rats, but hypertension is known to shift the autoregulatory curve to the right so this experimental model may not be directly comparable. Despite the variety of animal models, the lower threshold is remarkably consistent in the 50-60 mmHg range, with an upper limit of 150-170 mmHg.

5.1.2. In contrast to the sham occlusion group, a less clear relationship between the fall in blood pressure and loss of autoregulation was noted in the MCA occlusion group. If anything, the onset of autoregulatory failure was slightly higher, between 60 and 70 mmHg, but this was more obvious in the *non*-ischaemic hemisphere, and the change to a passive flow relationship was less dramatic. In the ischaemic hemisphere, the cerebral blood flow appeared to deteriorate gradually in the right parietal area below 70 mmHg but autoregulation remained effective in the right frontal area until the MABP fell further to 60 mmHg. The significance of this is not entirely clear, but autoregulation has clearly been *globally* affected by the ischaemic event. Symon et al (1976) demonstrated in their primate studies that in the parasagittal area closest to the collateral supply there was some preservation of autoregulatory capacity, attributed to supply from the anterior cerebral artery. This would explain why autoregulation has been better maintained in the frontal rather than the parietal area, and supports the concept that the attenuation of autoregulation is dependent on the depth of ischaemia (Symon et al 1976; Dirnagl & Pulsinelli 1990).

5.1.3. The global effect of MCA occlusion on cerebral autoregulation is also demonstrated by analysing all the blood flow recordings using sequential linear regression analysis (Appendix 6, page 137). This presents the same data in a different way, demonstrating a clear change in the gradient of the regression slope at 60 mmHg in both hemispheres of sham operated animals. The equations for CBF derived from these lines are also similar, with constants approximating to 60 over the range of blood pressure 90 to 60 mmHg, in both hemispheres. In the MCA occlusion group, the change in gradient in the right (lesioned) hemisphere *appears* to occur at approximately 70 mmHg, with a more subtle fall in CBF occurring below this MABP. Interestingly, in the left non-lesioned hemisphere, there is a passive CBF-blood pressure response throughout, supporting the concept of a global dysautoregulatory effect arising from a unilateral ischaemic event. The author believes this is better demonstrated in the non-lesioned hemisphere because the MCA occlusion itself has significant effects on *local* blood flow that interfere with a passive pressure-flow relationship that might otherwise be demonstrated.

In other situations of cerebral ischaemia, impaired autoregulation with a passive CBF-arterial blood pressure response has been demonstrated in a clinical setting occurring at a cerebral perfusion pressure (CPP) of approximately 70 mmHg (Chan et al 1993). In head injured patients, sequential linear regression analysis of CPP versus pulsatility index (calculated from transcranial Doppler flow studies) and jugular venous oxygenation yielded breakpoint values of CPP of 70 mmHg and 68 mmHg respectively (Chan et al 1993). Thus, a linear correlation between CPP and either pulsatility index or jugular venous oxygenation was noted when CPP fell below 70 mmHg.

5.1.4. Other factors may explain the failure to demonstrate a clear change in CBF at a specific MABP in the occlusion group. The stabilisation of the blood pressure at each selected level, checking of arterial blood gases, and measurement of the hydrogen clearance takes about 30 minutes, so the estimation of blood flow at 50 and 40 mmHg is carried out between 2 and 3 hours after the occlusion. The combination of a fall in blood flow, hypotension, and associated tissue hypoxia will have generated progressive oedema formation over this time period. This will have a progressive effect on cortical perfusion adjacent to the electrodes. The dramatic fall of CBF noted in the sham operated group at 60

mmHg, when maximum vasodilation has occurred because of hypotensive hypoxia, will not be observed in the occlusion group where the heterogeneity of ischaemic injury has caused local differences in tissue acidosis and variable degrees of vasoparalysis. Thus, the flow studies confirm that a degree of dysautoregulation has occurred following MCA occlusion.

5.1.5. The effect of the anaesthetic on cerebral blood flow and autoregulation might be relevant. The quantitative effect of halothane in clinical concentrations still appears to be controversial. Many earlier studies have suggested it is a potent vasodilator, increasing CBF in normal brain (McDowall 1967; Keaney et al 1973; Anderson et al 1980). Another study has demonstrated a 17% *reduction* in CBF with 0.5% halothane, but confirmed an *increase* of about 25% at a concentration of 1% (Morita et al 1977). Its variability of effect may thus be concentration-dependent. If it also *increases* CBF in ischaemic brain (Smith et al 1973), it should not have a deleterious effect on regional blood flow in experimental ischaemia. Moreover, although halothane increases CBF, it has been shown to *decrease* cerebral metabolic rate and oxygen consumption ($CMRO_2$) both clinically and experimentally (McDowall 1967; Morita et al 1977), which may be beneficial in situations of ischaemia. Quantitatively, Morita et al (1977) quoted a 30% reduction in $CMRO_2$ with 0.5% halothane.

5.1.6. While concentrations of halothane in excess of 1% impair autoregulation (Okuda & Mcdowall 1975), Morita et al (1977) demonstrated intact autoregulation at concentrations of 0.5%, and McDowall (1967) reported that cortical blood flow was significantly *improved* by the administration of 0.5% halothane. The effect of halothane on blood pressure, especially in higher concentrations (eg. Osborne et al 1987), may be relevant in studies which suggest that halothane *reduces* CBF. Any significant concomitant fall in MABP induced by the halothane will adversely affect cerebral perfusion (Christensen et al 1965). Further, after cryogenic brain injury halothane exacerbates cerebral oedema but only in concentrations above 0.9% (Smith & Marque 1975). This may be related to its effects on brain pH and blood-brain barrier permeability (Anderson et al 1980). In this thesis, the effect of halothane on CBF and autoregulation has hopefully been minimised by maintaining inspired concentrations between 0.6 and 0.8%.

It is important not to underestimate the effects of the other volatile anaesthetics on

cerebral blood flow. Inhalation of N_2O is also associated with global and regional increases in CBF (Deutsch & Samra 1990). The significant effects of the anaesthetic on CBF and autoregulation need to be considered in any studies on cerebral ischaemia, and they require that the methods of anaesthesia are at least consistent.

5.1.7. The effect of reperfusion by transfusion in this study has important implications in any situation where the blood pressure has been restored after a period of hypotension. In the sham operated animals, reperfusion did not entirely restore the cortical blood flow to baseline levels, although the difference was only significant in the left frontal electrode. In the MCA occlusion group, the restoration of blood flow was almost complete in the left hemisphere electrodes. The blood flows remained depressed in the right hemisphere after reperfusion, but comparing them to the immediate post-occlusion blood flows (at 90 mmHg), there was no appreciable difference. Therefore, in this study, cortical reperfusion in the areas adjacent to the electrodes has not been *significantly* affected by the period of progressive hypotension, although in no area did the average blood flow on reperfusion ever attain *or exceed* baseline blood flow.

5.1.8. Comparable to this study, Waltz (1968) noted that after induced hypotension cortical blood flow after MCA occlusion in cats did not return to baseline levels when the blood pressure was restored. However, the failure of reperfusion in ischaemic regions analogous to the no-reflow phenomenon (Ames et al 1968) will also depend on the depth and duration of ischaemia. The reason why a failure in reperfusion *or* the presence of hyperaemia or "luxury perfusion" (*increased* CBF on reperfusion) has not been more clearly demonstrated in this study may be a function of the position of the electrodes i.e. clear of the MCA territory, and in an area where the collateral supply is good and relative ischaemia is minimal.

5.1.9. Fitch et al (1975) studied the effect of cervical sympathectomy on cerebral autoregulation in a baboon model, and concluded that haemorrhagic or cardiogenic shock is more detrimental to the brain's ability to regulate its own blood flow than drug-induced autonomic blockade. With cervical sympathectomy, CBF became pressure-dependent at a

lower MABP (40 mmHg) compared to animals subjected to oligaemic hypotension alone. Their findings support Harper's theory of two vascular resistances in series (intra- and extraparenchymal) (Harper et al 1972, Harper 1975). If hypotension leads to progressive hypoxic dilatation of the intraparenchymal vessels until they are fully dilated, then sympathetic vasoconstriction of the larger extraparenchymal vessels will impair cerebral perfusion further, so that a passive pressure-flow relationship becomes apparent. In this thesis, there are some stress-related indicators in MCA-occlusion groups subjected to hypotension eg. the lower serum glucose at 4 hours in Group 10 animals; the relatively lower pCO_2 with a larger standard error in Group 5 animals during the hypotensive period; and the progressive fall in pCO_2 with severe hypotension seen in the present study, related to a marked peripheral vasoconstriction. This "shock" response with related sympathicoadrenal stimulation will be detrimental to cerebral autoregulation, and will contribute to a further impairment in cerebral perfusion associated with moderate hypotension (Kovách et al 1975).

5.1.10. The profound effect of the arterial pCO_2 on cerebral blood flow and its autoregulation has already been alluded to (Sokoloff 1960; Reivich 1964; Lassen & Christensen 1976). In the current studies, the author has maintained pCO_2 with in tight limits, usually between 33 and 36 mmHg. However, with a progressive reduction in systemic blood pressure, the animal becomes peripherally vasoconstricted and arterial pCO_2 falls. Alterations in ventilation with reductions in stroke volume or rate can restore pCO_2 by induced hypoventilation, but this becomes increasingly more difficult as oligaemic shock progresses. The adjacent graph (figure 16) illustrates that pCO_2 has gradually fallen in the sham operated group, until MABP reaches 50 mmHg where it falls off more steeply. In the MCA occlusion group, pCO_2 has remained fairly linear until a MABP of 60 mmHg when there is a dramatic fall

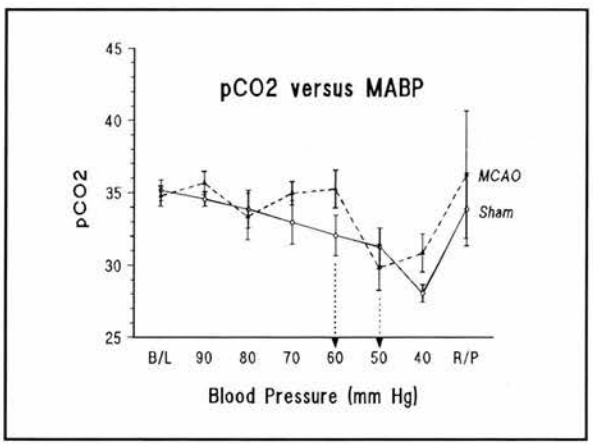

Figure 16
The relationship between mean arterial blood pressure and pCO_2.

in pCO_2. It is interesting to note that these levels tend to coincide with the equivalent levels of systemic blood pressure where cerebral autoregulation fails (figure 8). This suggests that the lower limit of cerebral autoregulation compares favourably with a level of mean blood pressure where the peripheral circulation is also significantly compromised. This implies a relationship between the autoregulatory threshold and the arterial pCO_2.

5.1.11. The intracerebral arteriole acts like a pCO_2 electrode: CO_2 diffuses freely through the endothelial membrane, while H^+ and HCO_3^- are less able to do so (Hutchison & Acheson 1975). The intravascular CO_2 and the extravascular HCO_3^- determine the pH around the endothelial cells, so that a change in the intracellular $[H^+]$ in the smooth muscle fibres is ultimately responsible for the control of cerebral vasomotor activity (Gotoh et al 1961; Pontén & Siesjö 1965; Hutchison & Acheson 1975; McHenry 1983). It is likely that as the systemic blood pressure falls and oligaemic shock develops, cerebral vasodilatation occurs due to profound tissue hypoxia and acidosis, especially in association with a focal ischaemic lesion. The point at which cerebral vasodilation is maximal due to tissue hypoxia will be equivalent to the lower limits of autoregulation in other tissues where relative hypoxia will promote anaerobic glycolysis, a reduction in CO_2 production and an associated fall in pCO_2. Despite the falling pCO_2, hypoxic/acidotic vasoparalysis of the intracerebral vessels prevents them from reacting to the low pCO_2, so that CBF becomes passively dependent on the systemic blood pressure (Harper & Glass 1965; Artru & Colley 1984). Thus, there is not necessarily a direct causal relationship between a failure of cerebral autoregulation and a falling arterial pCO_2; rather, both are occurring in parallel, and whereas other tissues can tolerate the acidosis generated by the failure of perfusion the brain is much more susceptible and ischaemic damage is made worse.

5.1.12. In summary, this study has reaffirmed that at the lower end of the pressure-flow curve cerebral autoregulation does exist, and that in the normal rat cortex autoregulation fails below a mean systemic blood pressure of 60 mmHg. Despite MCA occlusion, vascular reactivity within the parasaggital cortical area appears to be well maintained, especially within the frontal region. Nevertheless, in this model of focal ischaemia, autoregulation is disturbed globally, and that the normal relationship between MABP and cerebral perfusion

is affected even within the non-ischaemic hemisphere. Perhaps reflecting the position of the electrodes, reperfusion in this model did not demonstrate significant "no-reflow" or hyperaemic phenomena. This suggests that there is only a modest reduction in blood flow within the parasagittal cortex after MCA occlusion, perhaps related to good collateral circulation. However, it may be that a period of modest hypotension has subtle effects on the efficacy and integrity of the collateral circulation, sufficient to impair cerebral perfusion, exacerbate cerebral oedema and extend the area of infarction.

5.2 THE EFFECTS OF HYPOTENSION ON INFARCT SIZE

5.2.1. Although the simplicity of TTC perfusion in delineating infarcts is appealing, the technique has to be learned. Patchy perfusion is occasionally encountered, demonstrable in the non-ischaemic hemisphere, or in the sham animals. The reasons for this must relate to a poor saline washout, perhaps due to poor heparinisation and using an inadequate perfusing pressure. These problems were overcome by heparinising the animals 10 minutes before terminating the experiment (1000 units via the right femoral catheter), *and* an intra-atrial injection of 1000 units of heparin immediately after opening the chest. The perfusion fluids were pre-warmed to 37°C. Over-perfusion with heparinised saline should be avoided, as this has been associated with "hydropic cell" artifacts (Brierley et al 1973); 100 - 120 ml was usually sufficient to obtain a clear atrial effusate. Over-perfusion with TTC can produced uneven staining and less effective delineation of the infarcted/non-infarcted interface: 25-30 ml of a standard 2% solution per 300 gram rat was adequate. On one occasion, while the right (lesioned) hemisphere was well perfused, the whole of the left hemisphere failed to stain: the author felt that the position of the catheter in the ascending aorta had occluded the left common carotid artery, so it is important that only the ascending aorta is cannulated. A mean perfusion pressure of 150 mmHg, using a pulsatile method, was found to produce the best results.

5.2.2. The variability of infarct size in both groups, estimated by TTC perfusion and histology, is an important finding. MCA occlusion is advocated by its supporters as

producing reproducible results (Tamura et al 1981a, 1981b), yet there is wealth of evidence to the contrary (Robinson et al 1975; Hossmann & Schuier 1980; Crowell et al 1981; Coyle 1982; Bose et al 1984; Bederson et al 1986b; Duverger & MacKenzie 1988). As long ago as 1951, even Harvey & Rasmussen in their primate model of MCA occlusion stated that "the variation in the size of the infarcts was considerable"; one animal demonstrated a profound hemiparesis and at autopsy the MCA was completely divided, yet the brain appeared almost normal. They explained these differences on the basis of collateral circulation when only one branch of the circle of Willis is divided, commenting that the ultimate area of infarction is dependent on the *physiological* distribution of the particular vessel. There is no evidence since then that non-validates this concept. Despite these reservations, Bederson et al (1986b) concluded that infarcts of predictable size were obtained in the rat model of MCA occlusion providing the artery was coagulated all the way from its origin to the olfactory tract, ensuring that retrograde filling of the lenticulostriate arteries was prevented.

5.2.3. Previous studies have shown that the correlation between TTC perfusion or immersion and histology in the assessment of infarct size is good at 24 hours post-occlusion (Park et al 1988; Hatfield et al 1991). With *immersion* techniques, the relationship is less accurate before this time. Bedersen et al (1986a) found it "difficult to compare results for H & E and TTC-stained sections in rats sacrificed 6 hours or less after the onset of cerebral ischaemia". They explained this variation in sizes between the TTC staining and histological appearances (estimated on the *same* sections) as due to significant differences in areas of infarction in the basal ganglia; the correlation in this area was poor ($r = 0.65$). This is supported by the findings of Park et al (1988), who found at *4 hours* post-occlusion in rats, comparison between the two techniques in estimating the total infarct volume and infarction within the cortex demonstrated a close correlation ($r = 0.92$) but in the caudate nucleus the correlation was poor ($r = 0.66$). However, Taylor et al (1987) concluded that TTC *perfusion* reliably delineated cortical infarction from 6 to 168 hours after MCA occlusion in a rat model, with a regression value of 0.84 between TTC perfusion and histology.

5.2.4. In this study, TTC perfusion and Histology were performed in different experimental

groups, and therefore quantitative comparisons cannot be made. However, infarct volumes calculated by histological methods are much smaller than those in the TTC perfusion studies. Using identical experimental brain sections, Taylor et al (1987) and to a lesser extent Park et al (1988) and Bederson et al (1986a) noted that infarct size measured histologically was smaller than that delineated by TTC perfusion. This may support Harvey & Rasmussen's concept of differing anatomical and physiological perfusion defects. The concept that TTC is necessarily identifying *physiologically* inert or dead tissue associated with mitochondrial failure at 4 hours post-occlusion, rather than a simple *anatomical* perfusion deficit, must be regarded with some caution. For instance, Liszczak et al (1984) found that mitochondria did *not* rupture in conditions of no reflow at 6 hours after permanent MCA occlusion. Recent studies by Hatfield et al (1991) have made comparisons at various intervals following MCA occlusion between TTC immersion and perfusion staining techniques and conventional histology. These studies suggested that the pattern of staining delineated by TTC perfusion in the *acute* phase of ischaemia represents lack of delivery of the stain (anatomical perfusion deficit) rather than the true area of brain damage (pathological deficit). Thus, although TTC perfusion is perceived as a physiological technique, it may be simply demonstrating an anatomical perfusion deficit, which does not concur exactly with the ultimate physiological deficit made smaller by collateral circulation.

Further, Cole et al (1990) compared infarct sizes by TTC perfusion in 2 groups of rats 3 hours after MCA occlusion. In the second group, a period of 2 hours reperfusion was allowed before the animals were killed. The area of injury was significantly less in the reperfusion group, and they concluded that the histochemical abnormality revealed by TTC is "reversible in some circumstances and does not necessarily represent inevitable infarction". The author supports their conclusion that in situations of acute ischaemia (less than 6 hours), TTC is an indicator of cerebral injury but is not a definitive marker of irretrievable infarction.

5.2.5. In this thesis, other technical reasons might explain why there are substantial size differences between the TTC perfusion and Histology groups. Formalin fixation causes tissue shrinkage. Taylor et al (1987) commented that the size differences between their TTC perfused and histologically-fixed sections was partly explained by the preferential

dehydration of oedematous tissue by fixation. Although both groups in this study involve identical perfusion fixation, the TTC perfusion animals were sectioned and photographed in groups of two or three within several days of brain removal, whereas the specimens for histological analysis were left for several *months* before sectioning, staining and analysis were carried out. It is known that cerebral vessels shrink to about one half of their *in vivo* calibre after fixation (McDonald & Potter 1951), and in view of the rich blood supply to the brain this must also have an effect on post-fixation size. However, the author has not tested this hypothesis by carrying out studies to estimate the rate of shrinkage after specified periods of fixation.

The methods of data extraction and analysis differ between the two studies: the first uses summation of areas on 9 equivalent 1.5 mm sections, the second uses integration from 8 stereotactic planes with different interplanar distances, using appropriate computer software. The standard charts used in the latter study are derived from Konig & Klippel's stereotactic atlas of the rat brain, which was based on observations from a 150 g female rat. The brains of male rats are 10% larger, and many of the rats used in the current studies were substantially larger than 150 g; this will have some effect on quantification data especially where swelling of the affected areas further exaggerates the differences (Graham, 1991, personal communication). A comparative study of the *area* differences between sections taken from rats of different sizes (eg. 150g and 300g) has not been carried out, but the reader is referred to the graph in appendix 4 which illustrates that there is a correlation between brain *weight* and body weight in the series of 15 rats used for volumetric validation.

5.2.6. Compared to other studies, the infarct volumes assessed by TTC perfusion appeared inappropriately large (range = 150.5 - 259.7 mm^3) eg. Graham et al (1985) and Osborne et al (1987) quote ranges of 84 - 120 mm^3 in Sprague-Dawley rats, and Duverger & MacKenzie (1988) quoted a mean volume of 62.2 mm^3 with a coefficient of variation of 49%. However, these sizes compare favourably with the *histological* volumes in this study, and in Wistar rats, Brint et al (1988) quote a range of 107 to 209 mm^3 and Jacewicz et al (1990) a range of 87.3 to 209.0 mm^3 in their studies. Unfortunately, many of the recent studies using TTC have assessed infarct sizes either as cross-sectional areas on individual sections (Lye et al 1987; Bedersen et al 1986b; Cole et al 1990), or by measuring

hemispheric infarct *surface* areas (Chen et al 1986), so direct comparisons with this study are difficult. The quantification methods used in this thesis have been validated by assessing the hemispheric volume of the normal rat brain without using image analysis (Appendix 4, q.v.). The mean hemispheric weight was measured in 15 control animals, and the average brain density derived using specific gravity measurements in 7 of these controls. The hemispheric volume was calculated using the formula $Volume = {}^{Mass} / {}_{Specific\ Gravity}$. This method gave a mean volume of 714 mm^3, which compares favourably with the left (non-lesioned) hemisphere in normotensive and hypotensive groups (725.9 $\pm$14.5, and 753.2 $\pm$27.6 mm^3 respectively).

5.2.7. An increase in hemispheric size after arterial occlusion is recognised in the current studies and may be due to oedema formation. The lesioned hemisphere has increased in size by about 5% (figure 17). However, cerebral ischaemia will cause cerebral vasodilation in the area around the infarct where circulatory arrest is incomplete, and even at 4 hours post-occlusion some of this swelling may be due to vascular dilatation and hyperaemia (i.e. an increase in cerebral blood volume). This effect might also be greater in the hypotensive group, where the depth of ischaemia is greater due to the fall in blood pressure. Despite reperfusion after the hypotensive insult, the vascular responses may not be entirely reversible ("no-reflow" phenomenon). Thus, the small increase in the hemispheric size in the hypotensive group compared to the normotensive group could be explained by increases in oedema and cerebral blood volume within the cortex. The *cortical* area as a percentage of the hemisphere is

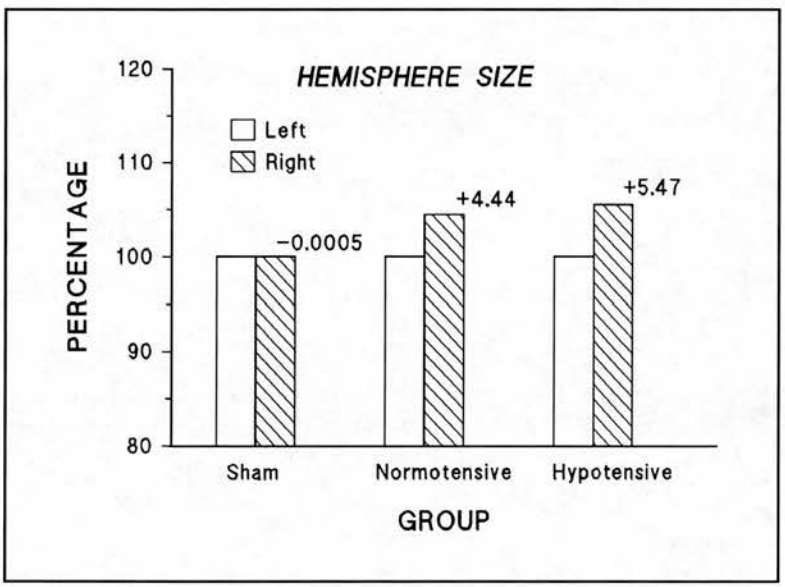

Figure 17
The effect of right MCA occlusion on hemisphere size. The left side is taken to be 100% and the right (lesioned) hemisphere compared with it.

greater in the hypotensive group (55.0 ±3.9% and 52.5 ±3.2%) but these differences did not reach statistical significance. Sundt & Michenfelder (1972) also noted that the ischaemic hemisphere in their model of MCA occlusion in squirrel monkeys was visually larger after 3 hours, and estimated the water content to be 0.5-2% greater. In a middle cerebral artery occlusion model in cats, Fenske et al (1975) noted that the lesioned hemisphere was 8.3 ±1.4% heavier at 24 hours. Specific gravity measurements were significantly lower in cortex and caudate of the lesioned hemisphere when compared to the non-lesioned hemisphere, more in support of oedema formation rather than increased cerebral blood volume as the cause of increased hemispheric size 4 hours after MCA occlusion.

5.3 THE EFFECTS OF HYPOTENSION ON CEREBRAL OEDEMA

5.3.1. Hatashita & Hoff (1986a, 1986b, 1988) have studied the complex interaction between tissue pressure, tissue resistance to flow of oedema fluid, and tissue compliance in the generation of cerebral oedema. Based on their "biomechanical" concepts, they conclude that soon after the development of cerebral ischaemia, early oedema formation is driven by the hydrostatic pressure gradient occurring across the capillary. Although the spread of fluid is initially limited by brain tissue hydraulic resistance, as the ischaemic injury progresses the accumulation of oedema fluid leads to a dramatic increase in tissue compliance and reduction in tissue resistance to flow. Thus, the migration of fluid is driven down the pressure gradient that develops between oedematous tissue and the CSF. In their model, they have even calculated this pressure gradient between oedematous tissue and ventricular fluid to be 5.3 mmHg.

5.3.2. In support of the theory that the "driving force for fluid accumulation in vasogenic brain oedema is the transmural pressure gradient in the capillary bed" (Hatashita & Hoff 1988), acute hypertension is known to dramatically increase cerebral oedema and even cause focal oedema in normal brain (Fenske et al 1975; Katzman et al 1977; Hatashita et al 1988; Kuroiwa et al 1990). Hypotension might therefore be expected to *reduce* oedema formation. However, the opposite effect is observed, because the reduction in the hydrostatic pressure

gradient is off-set by greater ischaemic cell injury caused by the reduced flow eg. the degradation of structural cellular elements and release of osmotically active compounds (Katzman et al 1977; O'Brien 1979). *Intracellular* fluid accumulation is perhaps more relevant than *interstitial* oedema formation in low-flow situations early after an ischaemic insult. In support of this, Hossmann (1976) has demonstrated in a cat model of focal ischaemia that a significant reduction in the extracellular space occurs (18.9 to 8.5 volume %) despite only small changes in net water content of the brain (75.9% to 76.1%). This re-emphasises the initial importance of cytotoxic oedema rather than vasogenic oedema, and represents compartmental fluid shifts rather than increases in total tissue water content.

5.3.3. There are other factors that suggest that the early cytotoxic effects of ischaemia are equally important to hydrostatic pressure gradients in the generation of oedema. In the first place, although the accumulation of intracellular water is accompanied by an increase in the concentration of sodium (coupled with a decrease in the concentration of potassium), there is a disassociation between the respective rises in intracellular water and Na^+ observed up to 6 hours after experimental MCA occlusion with a disproportionate rise in water observed (Gotoh et al 1985; Hatashita & Hoff 1990). The marked increase in brain intracellular osmolality 1 hour after global ischaemia in a cat model cannot be explained only on the basis of changes in the concentration of major solutes such as Na^+, K^+, glucose, lactate or pyruvate (Katzman et al 1977). Ischaemic cell damage must liberate a number of other osmotically active compounds that accelerate intracellular fluid accumulation. Significant increases in intracellular osmolality can be demonstrated up to 6 hours after MCA occlusion, and this correlates well with the period of cytotoxic oedema before vasogenic oedema is established (Hatashita et al 1988).

5.3.4. In the hypotensive group used in this study, after *permanent* focal ischaemia, the 30 minute period of hypotension followed by normalisation of the blood pressure might simulate a model of *reversible* ischaemia in some areas within the penumbra of the infarct. Ito et al (1979) have shown that restoration of blood flow after 1 hour of ischaemia (following common carotid occlusion in Mongolian gerbils) leads to resolution of oedema, suggesting that glial membrane Na^+/K^+ ion exchange function is not permanently damaged after

ischaemia of this duration. It might be expected therefore that if the additional ischaemia induced by the period of hypotension is reversible, then little difference in oedema formation would be expected between the two groups. The author believes that the difference seen in the cortex between the two groups arises because collateral circulation is in fact *irreversibly* affected by the reduction in blood flow, in keeping with a "no-reflow" phenomenon. This concept is supported by the blood flow studies, and also explains why little difference was noted in oedema formation within the caudate nucleus where collateral circulation is poor.

5.3.5. After proximal MCA occlusion, the absence of good collateral circulation in the lateral caudate nucleus will lead to greater ischaemia in this area. As oedema formation is known to be related to the depth of ischaemia, this absence of good collateral circulation may explain the relative differences in oedema formation in the cortex compared to the subcortical structures. Thus, even in the normotensive group, oedema formation within the penumbra of the caudate infarct is substantial, and in the hypotensive group a short period of hypotension will have little additional effect. Further, while significant oedema is known to develop within the core of the MCA territory by 1 hour, Hatashita & Hoff (1986a) found that significant increases in oedema formation only occurred at the periphery of the MCA territory at 6 hours, and that the increase in water content in this area was significantly less than that found in the core. It may be too early at 4 hours post-occlusion to expect more significant differences in tissue specific gravity, especially at the level of hypotension used in this study. However, the greater reduction in tissue specific gravity within the caudate nucleus compared to the cortex agrees with the findings of Hatashita & Hoff.

5.3.6. In the present study, survival experiments were carried out to ascertain if cerebral oedema was clearly established by 24 hours. This had implications for studies relating to the effects of immunosuppression on cerebral oedema, as morbidity and mortality increases significantly in immunosuppressed rats after 48 hours following a major ischaemic insult. Even in the present study, mortality in the 72-hour survival group was 20%. It was also hoped that some comparisons could be made between oedema formation at 24 and 72 hours, and in non-survivor experiments at 4 hours (although such a comparison is not crucial to the aims of this thesis). However, the significant differences arising in specific gravities of the

cerebellar and non-lesioned (left) hemispheric samples between normotensive non-survivor and survivor groups cast doubts on the legitimacy of such comparisons.

5.3.7. There are significant methodological differences between survivor and non-survivor groups which might influence comparability of results. Firstly, the method of anaesthesia is different, and halothane is thought to exert a protective effect on post-ischaemic brain oedema, at least in a reperfusion model (Cahn et al 1990). Secondly, specific gravity measurements were made by the author himself after non-survivor experiments, but the help of an experienced technician (S. Cook) was essential in the survivor experiments where the rapid measurement of specific gravities in several consecutive animals was required. Finally, haematocrits are significantly higher in the non-survivor animals perhaps suggesting that their hydration status is different (see adjacent table). Nevertheless, the author suggests (but has not confirmed experimentally) that progressive oedema in the survivor animals causing right to left hemispheric shifts, raised ICP, and the concept of diaschisis (secondary "remote" ischaemia) might account for the fall in specific gravities seen in the left hemisphere after 24 and 72 hours. Other studies have also demonstrated the formation of oedema in distant non-ischaemic areas of the brain in situations of severe ischaemia and major infarction (O'Brien et al 1974a; Katzman et al 1977). Whatever reasons explain these differences between non-survivor and survivor groups, it is best to avoid making any direct comparisons.

GROUP	HAEMATOCRIT Mean ± SE
9 - 4 Hours	*37.2 ± 0.9*
11 - 24 Hours	*44.6 ± 1.3*
12 - 72 Hours	*44.2 ± 1.1*
p Values *9 v 11 < 0.001* *9 v 12 < 0.001* *11 v 12 N/S*	

5.3.8. The survivor groups are however methodologically identical, apart from the experimental time-course. In this study, oedema formation was similar at 24 hours compared to 72 hours post-occlusion, confirming that cerebral oedema is well established by 24 hours after focal ischaemia. Although some studies have shown oedema to progress up to 72 hours, the increases in tissue water content after 24 hours appear to be comparatively small

(O'Brien et al 1974a, 1974b; Hatashita & Hoff 1990). Recent studies have demonstrated that after MCA occlusion in male Sprague-Dawley and Wistar rats, oedema was maximal at 24 hours, and in fact marginally less by 48 hours (Hatashita et al 1988, 1990). It seems legitimate therefore to use a 24-hour post-ischaemia model of cerebral oedema to study haemodynamic or therapeutic effects.

5.3.9. In summary, this study suggests that even at 4 hours after MCA occlusion, a modest short-term reduction in systemic blood pressure will cause more oedema within the cortex. While significant reductions in MABP have dramatic affects on cerebral perfusion, it might have been expected that a post-occlusion reduction of 25-30% would have been tolerated, with no measurable effect on the formation of oedema as early as 4 hours. The finding that oedema is significantly worse in the cortex emphasises the importance that after any ischaemic insult to the brain, *any* degree of hypotension should be avoided. The prevention of a minor fall in blood pressure after stroke will be even more relevant in hypertensive patients where the autoregulatory curve is shifted to the right and a "normal" systemic blood pressure leads to relative hypoperfusion.

5.4 THE EFFECT OF HYPOTENSION ON CEREBRAL BLOOD FLOW & THE IMPORTANCE OF COLLATERAL CIRCULATION

5.4.1. Middle cerebral artery occlusion is known to cause substantial decreases in local CBF (Symon et al 1974b; Heiss et al 1976; Symon et al 1979; Jones et al 1981). However, the extent of tissue damage due to profound ischaemia will depend to a large extent on the collateral blood flow and its ability to maintain cellular viability (even if it is unable to restore normal neuronal function). The concept of an ischaemic penumbra, where cell function is lost but potential viability maintained, and its physiological relationship to the collateral supply is important in terms of improving clinical outcome by haemodynamic or therapeutic manipulation. The existence of an ischaemic penumbra has been demonstrated experimentally (Symon et al 1974b; Astrup et al 1981; Jones et al 1981) and clinically (Olsen et al 1983), and significant therapeutic improvements in the outcome following stroke

might be possible if the perfusion characteristics of this area adjacent to an infarct are better understood. Olsen et al (1983) demonstrated that in stroke patients blood flow in collaterally perfused brain including the ischaemic penumbra was improved by increasing MABP. Therefore, a fall in MABP is likely to impair perfusion in this area, and this study was designed to confirm this hypothesis.

5.4.2. As cerebral blood flow diminishes, three important physiological thresholds have been identified that correlate with significant changes in neuronal function and integrity. In the range of 23-15 ml/100g/min, the so-called ischaemic "penlucida" (Drummond et al 1989), energy supply cannot quite meet normal physiological demands. Although the first signs of electrophysiological dysfunction are apparent and the neurons are functional impaired, they can maintain their structural viability indefinitely (Morawetz et al 1978). Below 15-18 ml/100g/min, the ischaemic "penumbra", neurons are non-functional and their viability is only temporary depending on the duration and depth of ischaemia. The onset of this stage (CBF = 15 ml/100g/min) is heralded by the abolition of cortical somatosensory evoked potentials (Astrup et al 1981; Branston et al 1984). At 6 ml/100g/min, substantial release of intracellular K^+ occurs, associated with membrane failure and rapid cell death (Astrup et al 1981; Crockard et al 1987; Drummond et al 1989). Jones et al (1981) described similar thresholds as "functional" (below 23 ml/100g/min), when reversible paralysis occurred, and "structural" (below 17 ml/100g/min) when irreversible anatomical damage was demonstrable. However, the development of morphological damage is time dependent and is a function of the severity and duration of local reduction of blood flow rather than necessarily occurring at a particular flow value (Jones et al 1981; Heiss 1983). It will also depend on the selective vulnerability to ischaemia of the particular neurons under study.

5.4.3. In the current study, average blood flows were well above these critical levels. However, the lowest recorded post-occlusion blood flow in the hypotensive group was 11.8 ml/100g/min, with 15% of right hemispheric recorded blood flows falling within the 15-23 ml/100g/min range. In the normotensive group, only one recorded blood flow fell below 23 ml/100g/min, indicating in general terms the detrimental effect of a modest reduction in

blood pressure on cortical blood flow. The higher non-ischaemic blood flows recorded in this study reflect the position of the cortical electrodes in relation to the infarct, and may confirm that normal blood flow per unit tissue is higher in the rat brain related to cellular density and higher metabolic requirements (Tamura et al 1981b). It is important to appreciate that the aim of this study was to investigate the flow characteristics within the cortex adjacent to, but not within, the infarct area. The author believes that the effects of hypotension on the *collateral* circulation is particularly relevant in understanding the mechanism whereby minor reductions in systemic blood pressure affect outcome in cerebral ischaemia.

5.4.4. With regard to hydrogen clearance, it is worth commenting that more biexponential curves occurred in the non-ischaemic hemispheres and sham operated animals, where blood flows were generally higher, than in the ischaemic (lesioned) hemisphere following the occlusion where the blood flows were reduced. Symon et al (1974b) found that under conditions of normal perfusion, 56% of clearance curves were monoexponential, and that after MCA occlusion in their baboon model electrodes which originally recorded biexponentially reverted to a monoexponential clearance, similar to the author's own experience. Jones et al (1981) also noted that before the MCA occlusion in their primate model 30% of the hydrogen clearance curves were biexponential, whereas almost all the washout curves after the occlusion were monoexponential. They commented that the reason for this was uncertain, but this author believes that in non-ischaemic tissue where blood flow and vascular integrity is preserved, diffusion of the hydrogen between different compartments is likely to be more of a problem because local perfusion gradients are intact and blood flows normal (or high). In situations of relative ischaemia, the equilibration of blood flow and loss or reversal of local perfusion gradients might discourage inter-compartmental diffusion.

5.4.5. Another methodological problem common to the measurement of cerebral blood flow using hydrogen clearance is appreciable variability between basal blood flow recordings from one animal to other, or between electrodes in the same hemisphere. This is also reported by Symon et al (1974b) who relate this to local differences in capillary blood flow.

It is not related to electrode performance, but to true inhomogeneity of blood flow within the microenvironment of the electrode. Such irregularities are evened out by the use of larger electrodes, but given the size of a rat brain (compared to a primate brain) relative to the size of the electrode larger electrodes would not be appropriate in the current model. If this variability in blood flow measurement is combined with the variability of infarct size (and presumably size and configuration of the penumbra) as already demonstrated, then absolute precision in electrode placement seems unnecessary. In defence of the methodology, the point of the study is to identify trends in cerebral perfusion changes and differences in flow characteristics that arise as a result of the period of hypotension. Tests of significance that predict differences in small groups of animals despite large standard errors are clearly important. The author has also converted the absolute CBF values to percentage baseline values to help remove inter-animal variability. However, inter-hemispheric and group differences can still be clearly identified.

5.4.6. A final comment on hydrogen clearance relates to the problem of very slow flows. This was also identified by Symon et al (1974b), who found that in areas of dense ischaemia the electrodes saturated slowly and continued to saturate after the H_2 inhalation was discontinued. They explained this on the delayed flow and delivery of hydrogen to the ischaemic area via anterior and posterior cerebral collaterals. To establish a measurable flow in these areas, they waited several minutes until a monoexponential slope eventually developed. In similar situations, this is exactly what the author has done in the present study.

5.4.7. The induction of a large infarct in the right hemisphere also apparently affected blood flow in the *left*, non-lesioned hemisphere. In the normotensive group, there was a slight fall in blood flow up to 60 minutes, which was not observed in the sham operated group. Although this reduction in blood flow was not statistically significant, it suggests that infarction in one hemisphere has to some extent a *global* affect on CBF. After 60 minutes, perfusion in left hemisphere begins to increase up to 180 minutes where it exceeds baseline flow (figure 12b, page 74). This is not paralleled by any significant change in mean arterial blood pressure, and may reflect a relative hyperaemia even in the non-ischaemic hemisphere

(diaschisis). Although other experimental studies have not demonstrated significant changes in the blood flow of the contralateral hemisphere (Yamaguchi et al 1971; Heiss et al 1976; Hossmann & Schuier 1980), acute cerebral ischaemia has been shown to affected the water content of the opposite hemisphere (O'Brien et al 1974a) and remote effects on blood flow have been described in man (Meyer et al 1970).

5.4.8. It has been demonstrated that autoregulation of cerebral blood flow is effective down to a mean blood pressure of 50-60 mmHg. Theoretically, therefore, reducing blood pressure by 25-30% to 70 mmHg might be better tolerated than expected. However, it is well recognized that in ischaemic tissue, autoregulation is attenuated because of a metabolically-induced vasoparalysis (Shima et al 1983). In the current context it is important to appreciate that even minor changes in blood flow within the ischaemic penumbra can have highly significant effects on the extent of neuronal damage, because cell viability is already severely compromised. If oxygen availability is the critical factor, then hypotension will cause further tissue hypoxia. Controlled hypotension combined with hypoxia causes electrical failure in the rat brain at a much higher flow than in normoxic animals (78 mmHg v. 46 mmHg) (Astrup et al 1981). Presumably in focal ischaemia, a reduction in MABP to 70 mmHg can cause similar dysfunction in areas which are relatively hypoxic.

5.4.9. In the hypotensive group, the fall in MABP after the occlusion *significantly* affected blood flow in the left hemisphere. Thus, with an acute fall in blood pressure, autoregulation has *not* been effective in maintaining blood flow in the non-ischaemic hemisphere. In this group, blood flow also rises marginally in the left hemisphere up to the watershed of 180 minutes post-occlusion, but this trend is much less obvious than in the normotensive animals. It may be the period of hypotension has had some affect on vascular calibre and cerebrovascular resistance even in the non-ischaemic hemisphere. These subtle changes in the haemodynamic profile of the non-ischaemic hemisphere are more apparent in the frontal region rather than the parietal region. The author believes that this reflects differences in collateral supply within the boundary zones between anterior/middle cerebral artery and middle/posterior cerebral artery territories.

5.4.10. Neurogenic mechanisms may have only minor effects on the control of CBF (para. 1.3.8.), but in circumstances of hypovolaemic hypotension, because increased sympathetic activity with sympathetic vasoconstriction of cerebral vessels is known to shift the autoregulatory curve to the right, the lower limit of CBF autoregulation is increased (Lassen & Christensen 1976). Thus, in haemorrhagic hypotension, brain ischaemia develops at a higher mean systemic blood pressure. The effect of sympathetic vasoconstriction might explain why in the face of an acute fall in blood pressure, blood flow is reduced even within the non-ischaemic hemisphere as demonstrated in this study. Lassen & Christensen (1976) suggest that, as it is the larger arteries and arterioles that are under sympathetic control, this mechanism may be important in regulating cerebral blood volume. In the context of a large infarct in one hemisphere, a global reduction in cerebrovascular blood volume may be physiological important in reducing ICP.

5.4.11. The differences in the perfusion characteristics of the two groups following MCA occlusion is perhaps the most significant finding in the study. Blood flow in the cortex in normotensive animals recovered significantly after 90 minutes, especially in the frontal area (figure 12a, page 74). This suggests that perfusion improved after the initial ischaemic event, related to improvements in collateral circulation. A similar pattern was not seen in the hypotensive animals (figure 12b, page 74), suggesting that the hypotensive period after the occlusion has impaired the ability of the collateral circulation to restore pre-occlusion perfusion. This difference is also reflected in the PBBF curves (figures 13a and 13b, page 76), where after restoration of normal blood pressure at 30-60 minutes in the hypotensive group the blood flow remains fairly linear, but in the normotensive group the blood flows recovered from 63% to 83% at 180 minutes post-occlusion.

5.4.12. Heubner's pial anastomosis forms a highly branched network of small vessels (40-100 μ diameter) on the surface of the cerebral cortex, which forms the main collateral blood supply to ischaemic tissue after MCA occlusion (Vander Eecken & Adams 1953). The importance of collateral supply in experimental cerebral infarction has been established angiographically (Crowell et al 1971). Shima et al (1983) monitored pial artery pressure (PAP) in a cat model of MCA occlusion, and found that PAP fell dramatically after the

occlusion to 15% of the control value. However, it recovered to some degree indicating what they termed as "delayed collateralization of the ischaemic territory". In 1968, Waltz demonstrated that in ischaemic cerebral cortex after MCA occlusion in cats, autoregulation was lost and induced hypotension caused severe ischaemic changes in the superficial cortical microvasculature. He also found that the cortical blood flow did not return to former levels when the blood pressure was increased again. These findings parallel the author's own results, and suggest that the irreversible effects of a period of hypotension on the leptomeningeal collateral circulation might explain why cortical blood flow fails to improve after MCA occlusion in the hypotensive group unlike the normotensive group. Moreover, the relatively greater partial recovery of cortical blood flow recorded by the frontal electrode compared to the parietal electrode in the normotensive animals probably reflects differences in anterior and posterior collateral circulations. Interestingly, Tamura et al (1981b) noted a substantial reduction in the blood flow within the *occipital* area in the same experimental model, which was unusual based on anatomy of the blood supply (Yamori et al 1976).

5.4.13. Symon et al (1979) demonstrated that the reduction in CBF after MCA occlusion in the baboon was least in the parasagittal area corresponding to the region supplied by anterior cerebral collaterals, which compares favourably to the area where cortical electrodes were implanted in this study. In the same model, they demonstrated that CO_2 reactivity in the superficial circulation was remarkably intact (Symon et al 1974a). Shima et al (1983) also demonstrated that although vascular reactivity was lost in the intracortical vessels after MCA occlusion (failed autoregulation), the extracortical vessels, in particular Heubner's pial anastomosis, remained reactive to both changes in blood pressure and CO_2. Their results suggest that the collateral vessels react to such changes in a similar fashion to vessels in normal brain. If such vascular reactivity exists in adjacent cortical areas, then the effect of reducing systemic blood pressure might be further dilatation of these vessels in response to a reduced perfusion. This fall in vascular resistance would potentially divert blood away from the ischaemic penumbra where vasoparalysis renders the local circulation incapable of favourably altering its vascular resistance. Even a minor reduction in the afferent blood pressure would therefore significantly compromise blood flow within the ischaemic penumbra. This effect has also been suggested by Symon et al (1974a).

5.4.14. The importance of collateral circulation in maintaining cerebral perfusion is well supported by many studies. Over 30 years ago, Meyer and Denny-Brown (1957) using a primate model of permanent MCA occlusion concluded that the severity and duration of cerebral ischaemia depended on the effective contribution of the collateral circulation. After division of the MCA, a fall in blood flow within the arterial stump was matched by a rapid increase in flow within the distal stump that must have been derived entirely from the collateral circulation. Distal anastomotic vessels were seen to return to normal calibre within 10 seconds of dividing the proximal MCA, and reversal of flow was noted from anterior cerebral to middle cerebral territory. Further, they demonstrated that when blood pressure was maintained at 100 mmHg, small fluctuations in oxygen tension and a delayed increase in collateral blood flow were noted over the cerebral cortex of the MCA territory when the artery was occluded for 6 minutes. However, if arterial occlusion was combined with systemic hypotension (bp = 85 mmHg), then the collateral circulation failed to increase and severe cortical hypoxia was noted. In another study, fluorescein angiography and perfusion with carbon black indicated considerable collateral blood supply to affected cortex, enough to maintain normal distribution of water and electrolytes, after proximal MCA occlusion in dogs subjected to haemorrhagic hypotension for 1 hour (bp = 50 mmHg) (Schibata 1974). Clinically, in patients with unilateral ICA occlusion, infarcts are larger where the collateral circulation is poor as assessed by angiography (Takagi et al 1981).

5.4.15. In conclusion, collateral circulation to cortical areas adjacent to the ischaemic area is important in limiting the extent of ischaemic damage after cerebral infarction. The ischaemic penumbra will depend on it to maintain or improve tissue perfusion, severely compromised by loss of blood supply via the occluded parent artery. This collateral supply appears to be more effective within anterior circulation rather than posterior circulation boundary zones, as suggested by this study. A period of modest hypotension after MCA occlusion affects regional CBF and may lead to further neuronal injury by compromising blood flow within the ischaemic penumbra. This probably occurs because vascular dilatation in cortex adjacent to the penumbra diverts blood from vasoparalysed areas, and stasis occurring within these areas of low flow is not wholly reversible on restoration of the blood pressure.

5.5 THE EFFECTS OF IMMUNOSUPPRESSION ON THE DEVELOPMENT OF CEREBRAL OEDEMA

5.5.1 There is growing evidence that the development of cerebral oedema is secondarily mediated to some extent by white cells or platelets (Obrenovitch & Hallenbeck 1985; Hallenbeck et al 1986; Kochanek & Hallenbeck 1992). Cerebral infarcts are known to be infiltrated by leucocytes (Pozzilli et al 1985) and lymphocytes (Barcikowska-Litwin et al 1987). Platelets also accumulate in regions of low blood flow (Obrenovitch & Hallenbeck 1985), and peripheral platelet function is altered after cerebral ischaemic insults (D'Andrea et al 1988; Joseph et al 1989). In animal models of intracerebral haematoma, the depth of ischaemia and extent of oedema is greater when the haemorrhage is simulated by the injection of blood rather than an inert substance, suggesting that in addition to the mass effect of the haematoma the cellular blood components contribute to the development of oedema (Jenkins et al 1990). Moreover, immunological reactions are also being implicated in the development of cerebral vasospasm after subarachnoid haemorrhage (Østergaard et al 1987; Harada et al 1990).

5.5.2. It is still not clear what aspect of blood cell rheology is most important in the pathogenesis of cerebral ischaemia or generation of cerebral oedema, but polymorphonuclear leucocytes (PMNL) are currently receiving the most serious attention. In an excellent review, Kochanek & Hallenbeck (1992) describe many studies that demonstrate a relationship between circulating leucocytes and the pathogenesis of cerebral ischaemia and stroke. They postulate a number of mechanisms that may explain this relationship. Cerebral blood flow may be reduced by small vessel occlusion or vasoconstriction mediated by vasoactive substances released from activated leucocytes. Blood brain barrier (BBB) permeability and neuronal cell damage could be exacerbated by hydrolytic enzyme release or the production of oxygen radicals. Leucocytes may be directly involved in activating or initiating small vessel thrombosis.

5.5.3. PMNL's are known to increase vascular permeability in other inflammatory processes, but conflicting evidence exists on the effect of activated leucocytes within the

cerebral circulation on BBB permeability (Kochanek & Hallenbeck 1992). For example, one study has suggested that activated leucocytes increase BBB permeability (Faustmann et al 1985), while another has shown that PMNL's can move across the BBB without its disruption and where the endothelial tight junctions remain preserved (Dorovini-Zis et al 1992). Grøgaard et al (1989) studied the effects of delayed hypoperfusion in rats depleted of polymorphs by antineutrophil serum using reversible bilateral carotid occlusion and hypotension to 50 mmHg for 15 minutes. They noted a significant improvement in the post-ischaemic local CBF in animals depleted of peripheral PMNL's, and suggested that PMNL's initiate endothelial damage and subsequent swelling. The precise effect of activated leucocytes on brain parenchyma is also not known. Giulian et al (1990) however have demonstrated that human immunodeficiency-virus infected human macrophages and microglia produce neurotoxic factors that kill neuronal cells in culture.

5.5.4. While some compelling evidence is emerging that PMNL's are implicated in the development of cerebral oedema, the data remains conflicting. Some of the most impressive effects of therapy aimed at impairing leucocyte accumulation and function have been observed in spinal cord ischaemia models (Clark et al 1991; Lindsberg et al 1991). Clark et al (1991) demonstrated that treatment with a leucocyte adhesion antibody in a rabbit model of spinal cord ischaemia led to a significant reduction in neurological dysfunction. In a similar model, Giulian & Robertson (1990) demonstrated that treatment which inhibited mononuclear phagocyte accumulation and function improved neurological function 3 days after ischaemia. However, against this has to be considered a study by Schürer et al (1990) which suggested that neutropaenia *enhanced* oedema formation in a rat model of cerebral ischaemia. They noted that oedema formation and hemispheric swelling was *greater* in rats subjected to trauma after treatment with antineutrophil serum, despite the hypothesis that white blood cells produce proteases and toxic oxygen radicals that damage the plasma membranes of the vascular endothelium and open the BBB.

5.5.5. This latter study might lend support to the concept that it is not PMNL's that are playing the primary immunological role in the pathogenesis of cerebral ischaemia - perhaps lymphocytes require greater consideration. In a study of 74 patients dying from cerebral

infarcts, Barcikowska-Litwin et al (1987) demonstrated lymphocytic infiltration of the infarct in 23% of cases, not dependent on the age or duration of survival of the patient. After MCA occlusion in cats, Takeshima et al (1992) demonstrated *no* improvement in recovery of CBF, or CSEP amplitude, and *no* reduction in infarct volume measured by TTC staining, despite treatment with antileucocyte adhesion antibody. Recent studies have shown that vinblastine-induced neutropenia in rats failed to alter significantly oedema formation at 4 hours after either an experimental intracerebral haematoma (Modha et al 1988) or middle cerebral artery occlusion (Alvarez et al, *personal communication*). Moreover, in the current study, there was a significant reduction in the lymphocyte count following irradiation, paralleled by a much less marked reduction in the neutrophil count, and little effect on the monocyte count. These findings suggest that lymphocytes rather than neutrophils or monocytes may be more important as cellular mediators in the generation of cerebral oedema.

5.5.6. Platelet function is an alternative area for study. In a model of air embolism-induced cerebral ischaemia in dogs, Obrenovitch & Hallenbeck (1985) demonstrated that the distribution of platelets paralleled areas where delayed impairment of microvascular perfusion had occurred. They concluded that the reduction in microvascular perfusion that occurs in damaged brain was associated with platelet accumulation. They added that it was difficult to identify cause and effect i.e. was platelet accumulation leading to impaired perfusion, or was ischaemic damage to vascular endothelium causing platelet aggregation? One criticism of this model is that air embolism is a unique form of ischaemic insult that also produces a primary endothelial insult and PMNL involvement at the blood-bubble interface (Kochanek & Hallenbeck 1992). In this thesis, although platelet counts preoperatively were significantly reduced compared to controls, a mean count of 217.0 x 10^9/l would not suggest a serious thrombocytopenia. However, the failure of these animals to mount a normal thrombocytosis following surgery confirms that platelet production was severely affected. Moreover, in irradiated animals the platelet count itself does not necessarily equate with normal platelet function (Coggle 1971).

5.5.7. Recent studies have suggested that vasoactive substances released from platelets, such as thromboxane, ADP, Ca^{++}, and 5-hydroxytryptamine (Holmsen 1987; De Clerk et

al 1985) can result in vascular and neuronal damage. After subarachnoid haemorrhage, platelet-derived vasoconstictors (eg. serotonin, thromboxane A_2, and platelet derived growth factor [PDGF]) are implicated in initiating cerebral vasospasm (Harada et al 1990). Platelet activating factors eg. arachnidonic acid, have caused significant neuronal injury when injected directly into the carotid artery of rats, not associated with occlusive platelet thrombi (Furlow & Bass 1975). Joseph and others have concluded that "platelet secretion may be of separate importance to mechanical occlusion of blood vessels by platelet aggregates in the pathogenesis of cerebral infarction". Further investigation by selective depletion of platelets may prove decisive in determining whether or not they are a significant factor in the development of cerebral oedema.

5.5.8. In conclusion, the significant reduction in cortical oedema following middle cerebral artery occlusion in irradiated, immunosuppressed rats suggests that cerebral oedema may be mediated by an immune mechanism. It is not clear via which peripheral blood component this effect is mediated, but this study suggests that lymphocytes and/or platelets are the most likely candidates. This question might be answered if methods that selectively deplete specific peripheral blood components could be developed in experimental models of cerebral ischaemia, perhaps using other forms of immunosuppression. If a specific cell type can be clearly identified as being more responsible for the generation of cerebral oedema, the therapeutic implications could be substantial.

5.6 CONCLUDING REMARKS

5.6.1. After proximal MCA occlusion in the rat, it has been shown that cerebral autoregulation has been impaired but not lost within the parasagittal area of the cortex supplied by the middle cerebral artery. However, within this area of relative ischaemia, cerebral blood flow is compromised after the occlusion, and is even more compromised by a period of hypotension after the ischaemic event. With hypotension, perfusion fails to recover within this penumbra, and this is reflected in greater cortical oedema at 4 hours post-occlusion, and larger areas of histologically-proven infarction. TTC perfusion deficits

are also made greater by a period of hypotension. Where ischaemia is far more severe (close to the core of the infarct) and collateral circulation is poor (caudate nucleus), oedema formation is much greater and less affected by a change in systemic blood pressure. This is also reflected in changes in infarct configuration that occur with hypotension, where medial and inferior cortical areas of ischaemic damage are made larger, while effects on the size and configuration of sub-cortical (caudate) areas are less apparent. Blood flow within the frontal area is generally better than the parietal area, reflecting better anterior cerebral collateral circulation. This collateral circulation is impaired by a fall in blood pressure after MCA occlusion, as frontal blood flow becomes less effective and more equal to parietal blood flow (see figure 12a), and frontal blood flow seems less able to recover when normal blood pressure is re-established.

5.6.2. In a rat model of permanent arterial occlusion, the author has shown that even a 25-30% reduction in mean arterial pressure for a relatively short time (30 minutes) has significant deleterious effects on the extent of cerebral ischaemia and presumably neuronal injury. In 1968, Waltz concluded: "There is little doubt that hypotension has a deleterious effect on ischemic cerebral cortex.....therefore, in patients with transient ischemic attacks, progressive strokes or recently completed strokes, every effort should be made to maintain systemic blood pressure at a level which is normal for that patient". The author concurs with that conclusion, and would also council against *any* falls in blood pressure in *any other* situation of cerebral ischaemia. The use of induced hypotension during aneurysmal surgery has rightly lost favour, and in the light of the present experimental findings ought to be condemned. There is growing evidence that "secondary insults", including periods of hypotension, occur unrecognised in many head-injured patients, and such events may adversely affect outcome (Andrews et al 1990; Miller 1992). The author emphasises that the maintenance of normal systemic arterial pressure in any situation of cerebral ischaemia is of paramount importance, and rightly deserves greater vigilance from all who are involved in the care of such patients.

5.6.3. The role of white cells in the generation of cerebral oedema still requires a great deal of study, as many important questions remain unanswered. The author thus concludes this

aspect of his study on a cautionary note. Despite the current rapid growth in experimental studies looking at the effects of white blood cell function in cerebral ischaemia, no clinical studies have been undertaken to ascertain the effect of modifying PMNL or lymphocyte function after cerebral ischaemic events. Even a transient and reversible effect on suppressing immune function might be potentially hazardous. Such a strategy might substantially increase the risk of infection, or even disturb the normal regenerative processes within the CNS that are essential to recovery. Complex mechanisms that lead to the elaboration of nerve growth factors and stimulation of neovascularisation depend on the normal activity of leucocytes (Kochanek & Hallenbeck 1992). Thus, any approach at suppressing white cell function will have to be considered very cautiously. With regard to the role of platelets, if specific platelet depletion or functional modification becomes clinically possible, it is difficult to imagine that any benefits in terms of reducing neuronal injury will be outweighed by the obvious clinical disadvantages.

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TABLES

Numerical list of tables as referenced in the text

PHYSIOLOGICAL VARIABLES 1 - *Autoregulation*

		GROUP 1	*GROUP 2*
Haematocrit	*B/L*	36.6 ±0.6	38.6 ±0.6 *
	2nd	34.5 ±1.1	35.8 ±1.9
	3rd	24.0 ±2.3	28.2 ±2.4
Glucose	*B/L*	160 0 ±4.8	154.8 ±2.3
	2nd	148.8 ±10.1	131.2 ±4.7
	3rd	118.0 ±25.4	118.4 ±12.6
Full Blood Counts	*Hb g/dl*	12.4 ±0.5	13.7 ±0.4
	WBC x 10^9/l	7.3 ±1.2	8.1 ±0.6
	Plat x 10^9/l	789 ±51	708 ±42
	Hct % [Derived]	32.3 ±1.5	35.2 ±1.6
	Hct % [Actual]	36.3 ±0.6	38.6 ±0.6

TABLE 1

Haematocrits, Serum glucose results and Full Blood Counts in the autoregulation experimental groups (1 & 2). Key: ***B/L*** *= Baseline;* ***2nd*** *= prior to MCA occlusion;* ***3rd*** *= before termination of experiment;* ***Hb*** *= Haemoglobin;* ***WBC*** *= White blood count;* ***Plat*** *= Platelets;* ***Hct*** *= Haematocrit;* ***Derived*** *= returned with the full blood count from the haematology department;* ***Actual*** *= obtained within the laboratory using centrifuge capillary samples. Data is Mean ± SE. * = $p < 0.05$, comparing group 1 with group 2.*

PHYSIOLOGICAL VARIABLES 2 - *Infarct Size: TTC Perfusion*

		GROUP 3	*GROUP 4*	*GROUP 5*
Haematocrit	*B/L*	39.3 ±1.1	38.6 ±0.5	39.2 ±0.9
	2nd	38.3 ±1.5	37.8 ±0.8	36.5 ±0.7
	3rd	34.8 ±2.9	33.6 ±1.3	27.1 ±1.6 #
Glucose	*B/L*	165.2 ±7.9	165.8 ±7.7	156.0 ±3.3
	2nd	156.8 ±10.7	163.4 ±6.1	145.9 ±7.5
	3rd	127.8 ±9.5	121.9 ±6.7	122.2 ±7.0
Full Blood Counts	*Hb g/dl*	12.3 ±0.9	13.0 ±0.2	13.3 ±0.3
	WBC x 10^9/l	9.8 ±1.0	8.5 ±0.7	8.3 ±0.6
	Plat x 10^9/l	929 ±67	760 ±46	806 ±28
	Hct % [Derived]	33.2 ±1.0	33.8 ±0.6	33.9 ±1.0
	Hct % [Actual]	39.3 ±1.1	38.6 ±0.5	39.2 ±0.9

TABLE 2

Haematocrits, Serum glucose results and Full Blood Counts in the TTC perfusion experimental groups (3,4 & 5). Key: ***B/L*** *= Baseline;* ***2nd*** *= prior to MCA occlusion;* ***3rd*** *= before termination of experiment;* ***Hb*** *= Haemoglobin;* ***WBC*** *= White blood count;* ***Plat*** *= Platelets;* ***Hct*** *= Haematocrit;* ***Derived*** *= returned with the full blood count from the haematology department;* ***Actual*** *= obtained within the laboratory using centrifuge capillary samples. Data is Mean ± SE. # = $p < 0.005$, comparing group 5 with group 4.*

PHYSIOLOGICAL VARIABLES 3 - *Infarct Size: Histology*

		GROUP 6	*GROUP 7*
Haematocrit	*B/L*	41.8 ±0.9	41.0 ±0.9
	2nd	43.7 ±1.1	42.0 ±1.2
	3rd	42.0 ±0.9	39.8 ±1.2
	4th	41.4 ±0.7	40.2 ±0.2
Glucose	*B/L*	184.6 ±11.4	164.8 ±7.8
	2nd	194.4 ±25.0	153.8 ±13.8
	3rd	146.6 ±9.9	119.2 ±12.8
	4th	113.6 ±9.3	105.0 ±2.5
Full Blood Counts	*Hb g/dl*	13.8 ±0.6	14.6 ±0.5
	WBC x 10 ^{9}/l	9.8 ±2.1	21.1 ±2.0
	Plat x 10 ^{9}/l	602 ±42	594 ±98
	Hct % [Derived]	35.2 ±1.7	38.4 ±1.5
	Hct % [Actual]	41.8 ±0.9	41.0 ±0.9

TABLE 3

Haematocrits, Serum glucose results and Full Blood Counts in the histology experimental groups (6 & 7). Key: ***B/L*** *= Baseline;* ***2nd*** *= prior to MCA occlusion;* ***3rd*** *= 2 hours post-occlusion;* ***4th*** *= before termination of experiment;* ***Hb*** *= Haemoglobin;* ***WBC*** *= White blood count;* ***Plat*** *= Platelets;* ***Hct*** *= Haematocrit;* ***Derived*** *= returned with the full blood count from the haematology department;* ***Actual*** *= obtained within the laboratory using centrifuge capillary samples. Data is Mean ± SE.*

PHYSIOLOGICAL VARIABLES 4 - *Cerebral Blood Flow/Oedema*

		GROUP 8	*GROUP 9*	*GROUP 10*
Haematocrit	*B/L*	40.2 ±0.9	40.9 ±0.6	42.4 ±0.7
	2nd	40.5 ±1.9	40.3 ±0.5	41.9 ±0.6
	3rd	37.0 ±2.0	37.2 ±0.9	39.6 ±0.6
Glucose	*B/L*	162.2 ±7.6	163.1 ±6.3	156.7 ±7.4
	2nd	131.7 ±7.3	131.7 ±2.3	146.0 ±6.5
	3rd	117.8 ±10.5	117.8 ±5.9	98.6 ±4.3 #
Full Blood Counts	*Hb g/dl*	13.7 ±0.5	13.3 ±0.2	13.5 ±0.3
	WBC x 10^9/l	9.7 ±1.0	7.1 ±0.9	8.5 ±0.4
	Plat x 10^9/l	732 ±55	805 ±69	742 ±40
	Hct % [Derived]	34.8 ±0.8	34.7 ±0.8	34.4 ±0.8
	Hct % [Actual]	40.2 ±0.9	40.9 ±0.6	42.4 ±0.7

TABLE 4

Haematocrits, Serum glucose results and Full Blood Counts in the cerebral blood flow and specific gravity experimental groups (8,9 & 10). Key: ***B/L*** *= Baseline;* ***2nd*** *= prior to MCA occlusion;* ***3rd*** *= before termination of experiment;* ***Hb*** *= Haemoglobin;* ***WBC*** *= White blood count;* ***Plat*** *= Platelets;* ***Hct*** *= Haematocrit;* ***Derived*** *= returned with the full blood count from the haematology department;* ***Actual*** *= obtained within the laboratory using centrifuge capillary samples. Data is Mean ± SE. # = $p < 0.05$, comparing group 10 with group 9.*

PHYSIOLOGICAL VARIABLES 5 - *Autoregulation*

PARA.	GROUP	B/L	PRE-OCC.	BLOOD PRESSURE (mmHg)						
				90	80	70	60	50	40	R/P
pH	1	7.44 ±0.01	7.44 ±0.02	7.43 ±0.01	7.40 ±0.02	7.40 ±0.01	7.38 ±0.02	7.36 ±0.02	7.29 ±0.03	7.12 ±0.03
	2	7.44 ±0.02	7.45 ±0.01	7.42 ±0.01	7.42 ±0.01	7.39 ±0.01	7.38 ±0.02	7.37 ±0.02	7.32 ±0.03	7.24 ±0.04
pO_2	1	159.9 ±5.2	147.6 ±3.3	153.4 ±8.0	145.8 ±8.3	163.2 ±11.2	162.4 ±11.2	168.3 ±13.1	162.1 ±14.1	162.9 ±9.0
	2	144.7 ±8.9	173.9 ±6.0**	148.7 ±8.5	162.8 ±8.9	163.7 ±8.8	158.3 ±6.2	163.0 ±4.7	166.2 ±10.1	132.1 ±9.7
pCO_2	1	35.0 ±1.3	35.2 ±0.7	34.6 ±0.5	33.9 ±1.3	33.0 ±1.5	32.1 ±1.4	31.3 ±1.3	28.1 ±0.6	33.9 ±2.5
	2	34.5 ±1.3	34.8 ±0.7	35.7 ±0.8	33.4 ±1.6	35.0 ±0.8	35.3 ±1.3	29.9 ±1.6	30.9 ±1.3	36.3 ±4.4
MABP (mmHg)	1	100.2 ±7.1	104.5 ±4.2	89.8 ±0.6	80.4 ±0.8	70.2 ±0.6	59.6 ±0.5	50.2 ±0.6	40.2 ±0.8	88.0 ±7.0
	2	100.6 ±5.0	106.0 ±7.1	90.4 ±0.2	80.4 ±0.5	69.4 ±0.7	59.6 ±0.4	50.2 ±0.6	41.0 ±1.1	89.0 ±0.6
TEMP (°C)	1	36.9 ±0.04	37.0 ±0.04	36.8 ±0.06	36.9 ±0.05	36.9 ±0.04	36.8 ±0.04	37.0 ±0.07	36.7 ±0.09	36.8 ±0.11
	2	37.1 ±0.11	37.0 ±0.04	37.0 ±0.02*	36.9 ±0.04	37.0 ±0.02	36.9 ±0.04	37.0 ±0.02	36.8 ±0.10	36.9 ±0.07

* = $p < 0.05$; ** = $p < 0.01$ *(cf. Sham operated and MCA occlusion groups i.e. groups 1 and 2).*

TABLE 5

Physiological variables in autoregulatory study groups, where the post-occlusion blood flow is reduced in increments of 10mmHg, and cerebral blood flow measured at each level of blood pressure. ***Para.*** *= physiological parameter;* ***B/L*** *= baseline values;* ***Pre-occ.*** *= prior to MCA/Sham occlusion;* ***Temp*** *= core temperature. Data = means ±SE.*

PHYSIOLOGICAL VARIABLES 6 - *TTC Perfusion*

PARA.	GROUP	B/L	PRE-OCC	TIME Post-occlusion (minutes)					
				10	30	60	120	180	240
pH	3	7.44 ±0.04	7.41 ±0.03	-	7.42 ±0.03	7.41 ±0.03	7.36 ±0.04	7.32 ±0.05	7.38 ±0.02
	4	7.42 ±0.03	7.43 ±0.02	-	7.41 ±0.02	7.40 ±0.02	7.40 ±0.02	7.39 ±0.02	7.39 ±0.04
	5	7.43 ±0.01	7.43 ±0.01	7.41 ±0.02	-	7.38 ±0.02	7.39 ±0.03	7.40 ±0.02	7.41 ±0.02
pO_2	3	161.5 ±6.2	155.8 ±10.0	-	153.9 ±8.9	155.9 ±8.1	148.6 ±4.6	141.6 ±7.8	140.3 ±9.2
	4	154.8 ±7.3	159.4 ±3.4	-	148.2 ±4.9	138.7 ±4.6	144.7 ±4.6	137.1 ±4.2	146.9 ±3.6
	5	155.1 ±7.4	160.1 ±5.9	164.2 ±4.1	-	156.8 ±4.6*	159.1 ±5.3	148.5 ±5.8	138.7 ±4.7
pCO_2	3	35.9 ±1.5	35.7 ±0.9	-	33.6 ±0.9	33.4 ±0.9	34.8 ±1.1	33.4 ±0.9	34.9 ±0.5
	4	34.1 ±0.7	34.3 ±0.6	-	35.3 ±0.8	33.6 ±0.6	34.6 ±0.8	34.7 ±0.6	35.1 ±0.8
	5	35.5 ±0.9	36.3 ±0.7	33.7 ±1.2	-	33.9 ±1.1	34.2 ±0.8	35.7 ±0.6	35.2 ±0.8
MABP (mmHg)	3	103.5 ±10.4	112.3 ±3.3	-	113.8 ±6.8	121.5 ±6.8	110.3 ±7.2	104.3 ±6.1	97.2 ±5.1
	4	107.4 ±5.6	99.3 ±3.9	-	106.3 ±4.3	110.3 ±2.5	107.3 ±3.9	103.8 ±3.1	105.9 ±7.3
	5	115.7 ±4.1	110.5 ±7.9	69.9 ±0.5	-	101.4 ±3.3*	102.0 ±4.4	99.4 ±5.2	99.7 ±4.7
TEMP (°C)	3	37.0 ±0.16	36.9 ±0.15	-	36.9 ±0.25	37.1 ±0.09	37.0 ±0.07	37.1 ±0.11	37.1 ±0.25
	4	37.1 ±0.02	37.0 ±0.08	-	37.1 ±0.03	37.1 ±0.08	36.9 ±0.09	37.0 ±0.04	37.1 ±0.05
	5	37.1 ±0.07	36.9 ±0.08	37.1 ±0.07	-	37.1 ±0.08	37.1 ±0.09	36.9 ±0.08	37.1 ±0.04

* = $p < 0.05$ *(cf. normotensive and hypotensive groups i.e. groups 4 and 5).*

TABLE 6

Physiological variables in the TTC perfusion groups: 3 = Sham operated; 4 = Normotensive; 5 = Hypotensive (BP reduced to 70mm Hg for 30 minutes post-occlusion). ***Para.*** *= physiological parameter;* ***B/L*** *= baseline;* ***Pre-occ.*** *= prior to middle cerebral artery occlusion;* ***Temp*** *= core temperature. Data = means ±SE.*

PHYSIOLOGICAL VARIABLES 7 - *Histology*

PARA.	GROUP	B/L	PRE-OCC.	TIME post-occlusion (minutes)						
				10	60	120	180	240	300	360
pH	6	7.46 ±0.02	7.46 ±0.01	-	7.42 ±0.01	7.39 ±0.02	7.39 ±0.02	7.38 ±0.02	7.35 ±0.01	7.34 ±0.02
	7	7.42 ±0.03	7.43 ±0.02	7.39 ±0.02	7.38 ±0.02	7.43 ±0.02	7.38 ±0.03	7.38 ±0.02	7.38 ±0.01	7.37 ±0.01
pO_2	6	162.7 ±3.3	155.9 ±14.2	-	157.5 ±5.2	139.9 ±6.5	141.7 ±8.1	139.8 ±4.6	141.8 ±4.5	144.4 ±4.1
	7	124.7 ±20.9	130.3 ±9.2	140.2 ±15.0	148.8 ±8.1	124.7 ±7.4	133.7 ±9.7	139.2 ±10.4	134.0 ±9.3	129.8 ±9.3
pCO_2	6	35.6 ±0.4	34.2 ±0.7	-	35.4 ±1.0	34.7 ±1.5	34.8 ±0.8	34.2 ±0.5	34.8 ±0.4	35.6 ±0.9
	7	35.0 ±1.1	36.1 ±1.0	34.7 ±0.7	36.0 ±0.8	33.6 ±0.6	34.1 ±0.7	33.8 ±0.7	34.6 ±1.2	34.2 ±0.5
MABP (mmHg)	6	113.2 ±4.9	109.6 ±6.1	-	113.2 ±4.2	110.0 ±6.1	106.8 ±3.0	105.6 ±3.7	102.0 ±6.6	96.0 ±7.9
	7	100.2 ±7.9	95.4 ±5.0	70.0 ±0.8	99.0 ±4.3*	92.0 ±6.1	93.2 ±3.5*	91.4 ±2.3*	92.2 ±1.1	92.7 ±7.5
TEMP (°C)	6	37.2 ±0.11	36.9 ±0.07	-	37.0 ±0.03	37.0 ±0.0	37.1 ±0.08	37.0 ±0.02	37.0 ±0.0	37.0 ±0.0
	7	37.3 ±0.10	36.9 ±0.04	37.1 ±0.06	37.1 ±0.06	37.0 ±0.04	36.9 ±0.03*	37.0 ±0.02	37.0 ±0.02	37.0 ±0.0

* = $p < 0.05$ *(cf. normotensive and hypotensive groups i.e. groups 6 and 7).*

TABLE 7

Physiological variables in animals perfusion-fixed at 6 hours post-occlusion for histological examination. ***Para.*** *= physiological parameter;* ***B/L*** *= baseline values;* ***Pre-occ.*** *= values prior to middle cerebral artery occlusion;* ***Temp*** *= core temperature. Data = means ±SE.*

PHYSIOLOGICAL VARIABLES 8 - *Cerebral Blood Flow/Oedema*

PARA.	GROUP	B/L	PRE-OCC.	TIME Post-occlusion (minutes)					
				10	30	60	120	180	240
pH	8	7.47 ±0.01	7.46 ±0.01	-	7.45 ±0.01	7.42 ±0.01	7.41 ±0.02	7.40 ±0.02	7.39 ±0.01
	9	7.46 ±0.01	7.46 ±0.01	-	7.46 ±0.01	7.44 ±0.01	7.42 ±0.01	7.39 ±0.02	7.39 ±0.02
	10	7.42 ±0.01#	7.42 ±0.01#	-	-	7.35 ±0.02##	7.38 ± 0.01#	7.36 ±0.01	7.35 ±0.02
pO_2	8	150.5 ±10.7	149.1 ±10.2	-	142.1 ±7.4	137.9 ±7.4	135.9 ±4.2	137.5 ±6.4	137.8 ±8.9
	9	149.7 ±9.0	153.7 ±7.6	-	152.0 ±7.4	150.1 ±7.8	156.8 ±9.6	157.2 ±7.6	160.5 ±11.6
	10	136.6 ±8.3	123.6 ±5.5**	-	-	113.3 ±4.6**	115.8 ±7.7**	120.7 ±11.5*	113.5 ±8.2**
pCO_2	8	33.8 ±0.8	33.8 ±0.8	-	34.3 ±0.8	35.2 ±0.5	35.9 ±0.6	34.4 ±1.0	35.2 ±0.3
	9	34.8 ±0.7	34.2 ±0.8	-	33.0 ±0.2	34.0 ±0.8	34.3 ±0.3	34.4 ±0.9	33.5 ±0.5
	10	34.8 ±0.6	34.1 ±0.3	-	-	34.1 ±0.7	33.9 ±0.7	34.5 ±0.4	35.5 ±0.7*
MABP (mmHg)	8	109.5 ±4.9	100.5 ±6.2	-	105.7 ±3.6	104.5 ±3.6	97.0 ±4.6	97.3 ±6.1	93.0 ±8.0
	9	105.1 ±4.5	97.3 ±4.8	-	101.4 ±3.6	100.4 ±3.4	97.4 ±4.0	97.3 ±2.4	96.8 ±4.1
	10	100.4 ±3.7	99.7 ±2.7	69.8 ±1.3	-	100.4 ±2.9	91.3 ±1.3	91.6 ±1.7	89.8 ±1.3
TEMP (°C)	8	37.0 ±0.03	37.0 ±0.03	-	37.0 ±0.05	36.9 ±0.05	37.0 ±0.07	36.9 ±0.04	37.0 ±0.07
	9	36.8 ±0.04	36.9 ±0.04	-	36.9 ±0.05	36.9 ±0.04	36.8 ±0.05	36.9 ±0.05	36.8 ±0.05
	10	37.0 ±0.04*	37.0 ±0.01	-	-	37.1 ±0.05*	37.0 ±0.05**	37.0 ±0.03**	37.1 ±0.05**

* = $p < 0.05$; ** = $p < 0.01$; # = $p < 0.005$; ## = $p < 0.001$ (cf. Normotensive and Hypotensive groups i.e. 9 and 10).

TABLE 8

Physiological variables in the cerebral blood flow/specific gravity groups: 8 = Sham operated; 9 = Normotensive; 10 = Hypotensive (BP reduced to 70 mmHg for 30 minutes post-occlusion). ***Para.*** *= physiological parameter;* ***B/L*** *= baseline;* ***Pre-occ.*** *= prior to middle cerebral artery occlusion;* ***Temp*** *= core temperature. Data = means ±SE.*

SYRINGE DRIVER 1 - *Infusion Rate*

Absolute Pressure Difference (Target - Measured Pressure) (mm Hg)	Pump Rate (ml/min)	VDU Report of Pump Activity
0	0	*"stopped"*
< 5	0.29	*"slow"*
5 - 10	0.65	*"medium"*
> 10	2.65	*"quick"*

TABLE 9

Delivery rates adopted by the syringe driver, calculated using a 5 ml Plastipak syringe.

SYRINGE DRIVER 2 - *Blood Pressure Control*

Rat no.	1	2	3	4	5	Average
Mean Pressure ± SE (mmHg) *	*69.6 ±0.59*	*70.2 ±0.35*	*69.8 ±0.34*	*68.8 ±0.36*	*70.4 ±0.55*	*69.8 ±0.43*
Average Deflection (mmHg) #	*-1.4 +1.03*	*-0.56 +0.8*	*-0.8 +0.53*	*-1.4 +0.23*	*-0.9 +0.73*	*-1.21 +0.95*

**** Calculated using 30 data points, one from each minute, while the pump is operational.***

 # The deflection of each data point above or below the "best fit" line drawn through the polygraph tracing of the mean blood pressure.

TABLE 10

Blood pressure control in 5 rats where the target pressure was set at 70 mmHg and maintained at that level for 30 minutes post-occlusion.

VOLUMETRIC ANALYSIS 1 - *TTC Perfusion*

GROUP		Sham	Normotensive	Hypotensive
SIZE mm³	**Right Hemisphere**	*790.7 ±26.4*	*757.7 ±14.0*	*794.3 ±28.3*
	Left Hemisphere	*791.1 ±25.8*	*725.9 ±14.5*	*753.2 ±27.6*
	Lesion (Infarct)	—	*190.2 ±8.8*	*230.7 ±10.1***
	Right Caudate	—	*361.1 ±12.9*	*355.7 ±10.8*
	Caudate Lesion	—	*35.6 ±2.5*	*47.0 ±4.4**
DIFFERENCES mm³	**Right v. Left Hemisphere**	*1.7 ±4.3*	*31.7 ±6.9*	*41.1 ±6.9*
	Right v. Left Caudate	—	*38.1 ±5.9*	*22.2 ±4.3*
PERCENTAGE RATIOS %	***Left to Right Hemisphere***	*100 ±0.6*	*95.8 ±0.9*	*94.8 ±0.8*
	Lesion to Right Hemisphere	—	*25.1 ±1.1*	*29.2 ±1.6**
OTHER RATIOS	**Right to Left Caudate**	—	*1.12 ±0.02*	*1.07 ±0.01**
	Right Caudate to Right Hemisphere	—	*0.47 ±0.01*	*0.45 ±0.01*
	Cortical Lesion to Cortex	—	*0.39 ±0.02*	*0.42 ±0.03*
	Caudate Lesion to Caudate	—	*0.10 ±0.01*	*0.13 ±0.01**
	Caudate Lesion to Lesion	—	*0.19 ±0.01*	*0.20 ±0.01*

** = $p < 0.05$; ** = $p < 0.01$ (cf. hypotensive with normotensive groups).*

TABLE 11

Volumetric analysis following TTC perfusion, sectioning and photography. Areas were estimated on 9 slices using a Kontron MOP-Videoplan image analyzer, summated and multiplied by the slice thickness (1.49 mm) to calculate the volume. Headings in italics are of primary interest. (See also appendix 4).

VOLUMETRIC ANALYSIS 2 - *Histology*

GROUP		NORMOTENSIVE	HYPOTENSIVE
Whole Volumns (mm^3)	**Hemisphere**	*738.5 ±139.5*	*997.7 ±86.7*
	Cortex	*435.9 ±122.1*	*649.0 ±79.2*
	Caudate	*306.1 ±17.8*	*313.2 ±9.8*
Infarct Volume (mm^3)	**Hemisphere**	*57.6 ±7.5*	*84.9 ±7.6 **
	Cortex	*33.9 ±7.1*	*55.8 ±6.9*
	Caudate	*22.2 ±1.5*	*24.0 ±0.7*
Infarct Volume (% whole)	**Hemisphere**	*13.3 ±1.7*	*19.6 ±1.8 **
	Cortex	*17.1 ±3.6*	*28.2 ±3.5*
	Caudate	*65.3 ±4.3*	*70.5 ±2.1*

TABLE 12

Volumetric analysis on 10 perfusion fixed brains, prepared, sliced and stained with H & E (haematoxylin and eosin). Volumes were calculated by summation of areas from 8 representative stereotactic sections, estimated using a Quantimet 970 image analysis system (Osborne et al 1987). The methods of volumetric analysis are therefore different from those used in the TTC perfusion experimental groups, so direct comparisons cannot be made between the two methods. Note that infarct volumes were significantly greater in the hypotensive group (= $p < 0.05$).*

CEREBRAL OEDEMA 1 - *Full Blood Counts*

GROUP		9	11	12
TIME Post-Occ.		**4 Hours**	**24 Hours**	**72 Hours**
Hb g/dl		*13.3 ±0.2*	*14.0 ±0.9*	*13.5 ±1.0*
WBC x 10^9/l		*7.1 ±0.9*	*8.2 ±0.9*	*9.3 ±0.6*
Plat x 10^9/l		*805 ±69*	*850 ±75*	*858 ±104*
Hct %	**Derived**	*[35 ±1] #*	*34 ±2*	*34 ±2*
	Actual	*37 ±1 **	*44 ±2*	*43 ±2*

* = $p < 0.01$, comparing group 9 with group 11 or 12.

TABLE 13

Full blood count estimations in the three groups of animals studied to compare brain specific gravities at different times after middle cerebral artery occlusion. ***Hb*** *= Haemoglobin;* ***WBC*** *= total white cell count;* ***Plat*** *= Platelet count;* ***Hct*** *= Haematocrit. The terms "derived" and "actual" are explained in the legend to table 1. The bracketed result (#) is a baseline, pre-lesion haematocrit (as group 9, table 4), and cannot be directly compared to the results in groups 11 and 12, which were taken just prior to the termination of the animal and removal of the brain. The "actual" result however in group 9 was estimated after 3 hours post-occlusion, and will reflect the fluid status of these animals prior to brain removal at 4 hours.*

CEREBRAL OEDEMA 2 - *Specific Gravity*

GROUP	8 [n = 6]	9 [n = 7]	10 [n = 7]	11 [n = 8]	12 [n = 6]
MCO	- (Sham)	+	+	+	+
POST-OCC. BP	Normotensive	Normotensive	Hypotensive	Normotensive	Normotensive
TIME POST-OCCLUSION	4 Hours	4 Hours	4 Hours	24 Hours	72 Hours
RIGHT HEMISPHERE					
Cortex	*1.0525 ±0.0013*	*1.0493 ±0.0011*	*1.0455 ±0.0013* *	*1.0440 ±0.0016*	*1.0444 ±0.0011*
White Matter	*1.0497 ±0.0011*	*1.0466 ±0.0009*	*1.0477 ±0.0006*	*1.0425 ±0.0012*	*1.0413 ±0.0007*
Caudate	*1.0509 ±0.0009*	*1.0445 ±0.0010*	*1.0448 ±0.0017*	*1.0384 ±0.0017*	*1.0403 ±0.0012*
Cerebellum	*1.0514 ±0.0014*	*1.0532 ±0.0008*	*1.0526 ±0.0018*	*1.0506 ±0.0003*	*1.0501 ±0.0005*
LEFT HEMISPHERE					
Cortex	*1.0511 ±0.0013*	*1.0526 ±0.0007*	*1.0508 ±0.0008*	*1.0495 ±0.0003*	*1.0485 ±0.0006*
White Matter	*1.0484 ±0.0009*	*1.0499 ±0.0011*	*1.0465 ±0.0008*	*1.0449 ±0.0008*	*1.0446 ±0.0005*
Caudate	*1.0512 ±0.0009*	*1.0528 ±0.0012*	*1.0507 ±0.0010*	*1.0495 ±0.0005*	*1.0480 ±0.0004*
Cerebellum	*1.0517 ±0.0012*	*1.0523 ±0.0011*	*1.0530 ±0.0009*	*1.0507 ±0.0006*	*1.0510 ±0.0005*

* = $p < 0.05$ *(comparing cortical SG of lesioned hemisphere at 4 hours in normotensive and hypotensive groups).*

TABLE 14 *Specific Gravity measurements. Data = Mean ±SE.*

CEREBRAL BLOOD FLOW 1 - *Autoregulation*

GROUP	SIDE	E/T	MEAN BLOOD PRESSURE							
			B/L	90	80	70	60	50	40	R/P
Sham (1) n = 5	Right	Frontal	86.4 ±21.6	87.5 ±16.8	87.8 ±11.9	91.4 ±13.1	85.6 ±17.8	56.1 ±15.2	46.2 ±14.6	80.7 ±0.7
		Parietal	59.3 ±8.9	59.7 ±6.6	74.9 ±13.5	70.4 ±8.6	72.4 ±13.3	49.0 ±10.2	40.0 ±6.7	53.6 ±7.4
	Left	Frontal	77.3 ±19.0	86.8 ±20.8	76.2 ±6.0	91.3 ±13.4	79.7 ±8.1	50.1 ±10.1	44.2 ±10.6	52.3 ±1.0
		Parietal	73.2 ±14.4	65.5 ±7.5	71.6 ±6.2	65.7 ±11.4	66.5 ±10.3	51.4 ±10.7	40.0 ±4.7	62.0 ±0.9
MCA Occlusion (2) n = 5	Right	Frontal	82.1 ±13.9	41.1 ±8.5	42.9 ±6.5 *	44.2 ±5.3 *	47.8 ±5.4	42.2 ±7.6	34.2 ±8.9	38.6 ±4.5 **
		Parietal	71.8 ±9.4	36.7 ±3.6 *	36.8 ±3.5 *	38.3 ±2.8 **	34.9 ±5.8 *	27.4 ±4.2	25.5 ±2.1 *	36.3 ±2.5
	Left	Frontal	71.4 ±7.1	55.4 ±6.2	64.3 ±6.3	62.8 ±8.9	50.2 ±7.7 *	43.7 ±7.8	50.4 ±4.4	70.3 ±8.5
		Parietal	76.3 ±14.1	77.1 ±11.4	82.6 ±8.7	82.1 ±8.6	79.7 ±11.1	52.9 ±9.1	44.2 ±9.5	67.2 ±8.4

*p values: * = $p < 0.05$; ** = $p < 0.01$ (comparing Sham with MCA occlusion group).*

TABLE 15

*Cerebral Blood Flow estimations in autoregulatory experimental groups 1 and 2, measured at 10 mmHg MABP increments using the computer-driven syringe driver (described in chapter 2, para. 2.4). **E/T** = Electrode; **B/L** = Baseline (prior to MCA occlusion); **R/P** = Reperfusion. Data is mean CBF in ml/100g/min ±SE.*

CEREBRAL BLOOD FLOW 2 - *Absolute Values*

E/T	GROUP	B\L	TIME Post-occlusion (minutes)								
			10	30	60	90	120	150	180	210	240
Right Frontal	8 - Sham	66.2 ±6.6	67.2 ±9.0	72.5 ±9.1	63.0 ±11.0	65.6 ±7.1	58.3 ±6.8	67.8 ±11.1	65.2 ±6.8	69.7 ±9.6	70.3 ±16.5
	9 - Normotensive	63.1 ±4.7	40.0 ±2.4	40.7 ±2.8	40.8 ±1.8	45.6 ±5.3	56.6 ±4.5	56.5 ±6.4	59.0 ±7.9	52.5 ±7.8	47.0 ±7.1
	10 - Hypotensive	48.4 ±5.7	28.0 ±4.4*	-	39.0 ±6.4	40.5 ±6.3	38.2 ±7.7	32.1 ±2.5**	35.5 ±3.8*	36.0 ±5.9	37.0 ±7.3
Right Parietal	8 - Sham	61.5 ±7.9	63.4 ±5.4	71.2 ±7.1	65.1 ±10.5	62.4 ±4.8	72.4 ±7.9	64.3 ±5.5	64.4 ±7.6	55.8 ±3.2	70.8 ±13.3
	9 - Normotensive	61.0 ±3.6	38.2 ±3.5	39.6 ±4.7	38.5 ±11.3	40.8 ±5.9	45.6 ±3.4	47.7 ±5.6	48.1 ±6.3	43.1 ±4.4	38.8 ±4.4
	10 - Hypotensive	51.2 ±5.8	26.5 ±2.5*	-	36.7 ±5.2	35.3 ±6.5	35.8 ±6.2	32.9 ±4.3	35.5 ±4.6	37.6 ±8.2	37.3 ±10.5
Left Frontal	8 - Sham	62.3 ±3.6	68.9 ±6.5	72.6 ±5.3	67.0 ±6.1	69.5 ±6.8	64.7 ±6.0	70.8 ±8.6	70.8 ±10.6	64.5 ±8.7	71.1 ±12.6
	9 - Normotensive	64.4 ±2.8	60.1 ±6.0	55.7 ±2.8	53.3 ±4.1	62.5 ±5.5	64.1 ±2.7	68.0 ±6.4	74.0 ±6.2	61.6 ±5.1	59.8 ±4.8
	10 - Hypotensive	58.7 ±3.8	44.9 ±3.6	-	54.4 ±3.3	50.8 ±4.0	51.2 ±3.5*	52.2 ±5.1	53.0 ±4.2*	50.3 ±5.3	50.9 ±7.7
Left Parietal	8 - Sham	65.1 ±4.6	70.6 ±7.0	71.0 ±6.9	65.8 ±7.0	71.2 ±7.0	65.8 ±7.2	66.8 ±9.8	73.5 ±10.9	62.7 ±14.4	62.2 ±12.3
	9 - Normotensive	62.6 ±3.2	59.1 ±4.4	53.7 ±3.1	55.7 ±4.7	56.6 ±6.0	59.6 ±3.7	61.7 ±4.0	66.0 ±4.2	59.6 ±3.2	57.6 ±5.0
	10 - Hypotensive	54.4 ±3.6	43.8 ±2.3*	-	55.4 ±2.8	50.9 ±2.2	52.8 ±2.2	54.9 ±4.2	57.8 ±4.9	50.1 ±4.5	51.2 ±7.0

* = $p < 0.05$; ** = $p < 0.01$ (cf. normotensive with hypotensive groups)

TABLE 16

*Absolute cerebral blood flow values in all three groups. **E/T** = Electrode; **B/L** = Baseline (pre-occlusion) values. The normotensive (group 9) and hypotensive (group 10) results are shown graphically in figure 12, page 74 (q.v.). Data expressed is ml/100g/min, means ± SE.*

CEREBRAL BLOOD FLOW 3 - *Percentage Baseline Values*

E/T	GROUP	TIME POST-OCCLUSION (Minutes)								
		10	30	60	90	120	150	180	210	240
Right Frontal	8 - Sham	113.0 ±8.1	108.7 ±6.3	106.8 ±11.0	99.8 ±7.2	104.4 ±6.7	111.3 ±9.0	102.1 ±8.4	117.4 ±8.3	99.8 ±5.7
	9 - Normotensive	60.6 ±4.2	61.4 ±3.6	62.2 ±4.3	71.1 ±8.0	86.8 ±8.4	84.8 ±8.4	88.6 ±10.7	79.6 ±9.6	72.1 ±9.6
	10 - Hypotensive	56.3 ±3.8	-	78.3 ±5.1*	69.8 ±8.7	76.1 ±7.6	68.6 ±4.7	74.6 ±3.5	74.4 ±17.3	80.6 ±15.6
Right Parietal	8 - Sham	105.3 ±6.0	117.7 ±6.1	104.4 ±4.6	105.3 ±10.6	118.8 ±3.6	107.1 ±6.4	111.8 ±6.0	113.0 ±1.3	141.4 ±21.4
	9 - Normotensive	63.2 ±6.1	65.2 ±6.7	63.4 ±6.3	68.2 ±8.8	75.2 ±5.5	78.2 ±7.7	78.3 ±8.2	73.8 ±7.6	66.0 ±6.2
	10 - Hypotensive	53.6 ±5.5	-	73.1 ±8.6	73.2 ±12.7	70.2 ±10.0	65.3 ±7.5	70.9 ±8.7	71.4 ±9.9	64.7 ±11.4
Left Frontal	8 - Sham	110.6 ±8.0	116.5 ±5.8	106.7 ±4.5	110.7 ±5.6	114.1 ±9.7	112.1 ±8.5	112.1 ±11.0	107.6 ±9.7	117.5 ±14.6
	9 - Normotensive	91.3 ±9.9	86.8 ±4.4	82.6 ±5.3	99.0 ±7.4	100.1 ±4.5	104.8 ±7.3	114.6 ±8.1	95.6 ±5.5	92.8 ±4.6
	10 - Hypotensive	76.6 ±3.6	-	93.3 ±3.8	90.7 ±6.0	88.3 ±6.0	89.6 ±7.5	91.4 ±7.4	85.7 ±6.5	87.5 ±8.7
Left Parietal	8 - Sham	108.0 ±6.1	108.9 ±7.5	100.9 ±8.2	108.9 ±7.3	109.8 ±7.9	101.3 ±8.3	114.8 ±10.0	98.7 ±13.9	106.6 ±14.1
	9 - Normotensive	94.3 ±5.4	86.3 ±4.3	88.9 ±6.4	88.9 ±8.5	95.5 ±5.0	98.9 ±5.5	106.5 ±8.1	98.6 ±5.4	95.9 ±9.1
	10 - Hypotensive	81.6 ±4.6	-	103.7 ±7.4	100.6 ±8.9	99.5 ±8.6	103.0 ±10.2	107.2 ±8.6	92.6 ±8.0	95.1 ±8.7

* = $p < 0.05$ *(cf. normotensive and hypotensive groups).*

TABLE 17

Cerebral blood flow estimations, expressed as a percentage of baseline (pre-occlusion) values. The normotensive (group 9) and hypotensive (group 10) results are displayed graphically in figure 13, page 76 (q.v.). Data is mean percentages ±SE.

IRRADIATION 1 - *Full Blood Counts*

	PRETREATMENT		PREOPERATIVE		POSTOPERATIVE	
GROUP	14	13	14	13	14	13
White Blood Count x 10^9/l	*11.9 ± 1.1*	*9.2 ± 0.8*	*2.4 ## ± 0.4*	*9.4 ± 1.0*	*2.1 # ± 0.4*	*6.4 ± 1.2*
Haemoglobin g/dl	*13.0 ± 0.5*	*12.4 ± 0.4*	*10.9 ± 0.9*	*12.3 ± 0.7*	*10.8 * ± 0.8*	*13.4 ± 0.3*
Haematocrit %	*33.5 ± 1.0*	*33.0 ± 1.2*	*27.9 ± 2.2*	*32.6 ± 1.5*	*27.9 * ± 2.1*	*35.0 ± 1.2*
Platelets x 10^9/l	*882 ± 30*	*893 ± 50*	*217 # ± 25*	*639 ± 134*	*65 ## ± 6*	*939 ± 45*
Neutrophil x 10^9/l	*1.2 ± 0.3*	*1.1 ± 0.5*	*0.8 ± 0.2*	*1.3 ± 0.2*	*0.9 * ± 0.2*	*2.5 ± 0.5*
Lymphocytes x 10^9/l	*10.4 ± 2.8*	*7.8 ± 0.5*	*2.0 ## ± 0.2*	*7.9 ± 0.8*	*1.2 * ± 0.4*	*3.7 ± 0.7*
Monocytes x 10^9/l	*0.3 ± 0.03*	*0.3 ± 0.06*	*0.3 ± 0.11*	*0.2 ± 0.03*	*0.1 ± 0.01*	*0.3 ± 0.09*

*p values : * = < 0.05 ; ** = < 0.01 ; # = < 0.005 ; ## = < 0.001 (comparing group 13 with group 14).*

TABLE 18

A comparison of the peripheral blood counts between irradiated animals (Group 14) and normal controls (Group 13). Mean values ± SE.

IRRADIATION 2 - *Specific Gravity*

GROUP	Group 13 - Control *[n = 7]*		Group 14 - Irradiated *[n = 8]*	
SIDE	**Right**	**Left**	**Right**	**Left**
Cortex	*1.0461 ± 0.0005* **	*1.0497 ± 0.0008*	*1.0480 ± 0.0006*	*1.0507 ± 0.0014*
White Matter	*1.0426 ± 0.0010* *	*1.0456 ± 0.0005*	*1.0424 ± 0.0007* **	*1.0459 ± 0.0009*
Caudate	*1.0393 ±0.0011* ##	*1.0476 ± 0.0005*	*1.0403 ±0.0008* ##	*1.0483 ± 0.0006*
Cerebellum	*1.0504 ± 0.0006*	*1.0522 ± 0.0018*	*1.0512 ± 0.0008*	*1.0517 ± 0.0010*

*p values : * = < 0.05; ** = < 0.01; ## = < 0.001 (comparing right to left hemisphere).*

TABLE 19

Mean specific gravities ± SE, after right middle cerebral artery occlusion in non-irradiated, control (Group 13) and irradiated (Group 14) animals. Comparing cortical oedema in the lesioned hemisphere between the two groups, there was a significant improvement ($p < 0.05$).

PHYSIOLOGICAL VARIABLES 9 - *Survivors*

TIME (minutes)		0	15	30	45
STAGE		Set-up	Diss.	Cran.	Post-occ.
pH		*7.32 ±0.01*	*7.36 ±0.01*	*7.35 ±0.02*	*7.36 ±0.01*
pCO_2 [mmHg]		*55.8 ±0.6*	*48.9 ±2.4*	*45.8 ±1.7*	*46.9 ±1.3*
pO_2 [mmHg]		*199.3 ±18.8*	*166.6 ±24.3*	*163.3 ±26.4*	*215.7 ±22.7*
Sat. [%]	ABG	*99.2 ±0.1*	*99.1 ±0.2*	*99.1 ±0.2*	*99.4 ±0.1*
	* Pulse Oximeter	*98.7 ±0.6*	*99.2 ±0.2*	*98.0 ±0.6*	*99.3 ±0.3*
MABP [mmHg]		*84.2 ±2.4*	*89.5 ±2.9*	*85.7 ±1.4*	*88.7 ±3.5*

* *Novametrix pulse oximeter on rear paw.*

TABLE 20

The effect of intraperitoneal anaesthesia and nitrous oxide/oxygen (400 : 600 ml/min) by mask on physiological variables in a group of 4 rats (weight range 472 - 529 grams). Means ± SE. ***Set-up*** *= after insertion of arterial line;* ***Diss.*** *= during subtemporal dissection;* ***Cran.*** *= after craniectomy;* ***Post-occ.*** *= after middle cerebral artery occlusion;* ***Sat.*** *= arterial oxygen saturation;* ***ABG*** *= arterial blood gas.*

APPENDICES

Additional comments on hydrogen clearance, volumetric analysis and data collection

APPENDIX 1 - *H_2 Clearance Equations*

Several assumptions are implicit in the calculation of cerebral blood flow from the clearance of a circulating tracer. Many of these theoretical assumptions are discussed in chapter 3. The following mathematical outline assumes that once hydrogen gas is introduced into the arterial circulation, it distributes itself between brain and blood according to its partition coefficient. This distribution is related to blood flow by the Fick Principle :

$$dQ \; / \; dt \; = \; F \; (C_a - C_v) \qquad (1)$$

Amount of tracer taken up by the brain (Q) per unit time (t) is equal to the difference between the arterial (C_a) and venous (C_v) concentrations multiplied by the blood flow (F), where arterial flow is assumed to equal venous flow.

The hydrogen gas is assumed to equilibrate instantaneously with a brain volume V and partition coefficient λ :

$$dC \; / \; dt \; = \; - F \; (C_b - \lambda C_a) \; / \; \lambda V \qquad (2)$$

$$\Rightarrow \quad C_b \; = \; C_{b0} \; (e^{-kt}), \; k \; = \; F \; / \; \lambda V \qquad (3)$$

assuming the brain is saturated with hydrogen and the arterial concentration is zero, C_b is the concentration of hydrogen in the brain at time t, C_{b0} is the brain hydrogen concentration at the beginning of desaturation, and k equals blood flow per brain volume divided by the partition coefficient.

The partition coefficient (λ) for hydrogen is almost 1.0, so the exponential slope (k) approximates blood flow. Solving for k :

$$k \; = \; - \log_n \; (C_b \; / \; C_{b0}) \; / \; t \qquad (4)$$

The time required for the hydrogen concentration to fall to half of its original value ($t_{½}$) will depend roughly on the blood flow. Thus :

$$k \; = \; - \log_n \; (1/2) \; / \; t_{½} \; = \; 0.693 \; / \; t_{½} \qquad (5)$$

Therefore :

$$CBF \; = \; 0.693 \; \times \; 100 \text{ (grams)} \; \times \; 60 \text{ (seconds)} \; / \; t_{½} \; = \; 4158 \; / \; t_{½} \qquad (6)$$

 # after Young, 1980.

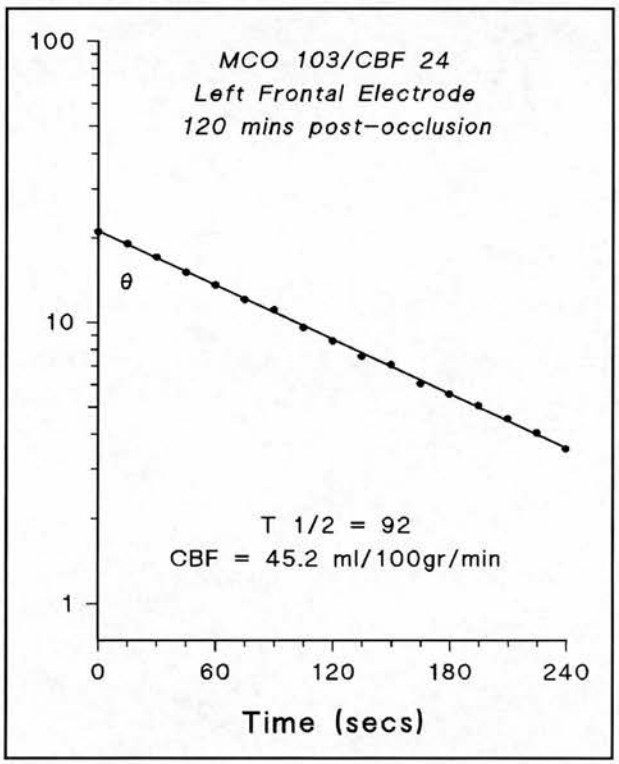
APPENDIX 2 - *Cerebral blood flow.*

This table gives the blood flow in ml/100g/min, calculated from the $t_{½}$, or time taken for the H_2 concentration to fall to half its original value (CBF = 0.693 x 100g x 60 sec / $t_{½}$).

$T_{½}$	CBF	$T_{½}$	CBF	$T_{½}$	CBF	$T_{½}$	CBF	$T_{½}$	CBF
25	166.3	80	52.0	135	30.8	190	21.9	245	17.0
26	159.9	81	51.3	136	30.6	191	21.8	246	16.9
27	154.0	82	50.7	137	30.4	192	21.7	247	16.8
28	148.5	83	50.1	138	30.1	193	21.5	248	16.8
29	143.5	84	49.5	139	29.9	194	21.4	249	16.7
30	138.6	85	48.9	140	29.7	195	21.3	250	16.6
31	134.2	86	48.3	141	29.5	196	21.2	251	16.6
32	129.9	87	47.8	142	29.3	197	21.1	252	16.5
33	126.0	88	47.2	143	29.1	198	21.0	253	16.4
34	122.3	89	46.7	144	28.9	199	20.9	254	16.4
35	118.8	90	46.2	145	28.7	200	20.8	255	16.3
36	115.5	91	45.7	146	28.4	201	20.7	256	16.2
37	112.4	92	45.2	147	28.3	202	20.6	257	16.2
38	109.4	93	44.7	148	28.1	203	20.5	258	16.1
39	106.6	94	44.2	149	27.9	204	20.4	259	16.0
40	103.9	95	43.8	150	27.7	205	20.3	260	16.0
41	101.4	96	43.3	151	27.5	206	20.2	261	15.9
44	99.0	97	42.9	152	27.4	207	20.1	262	15.9
43	96.7	98	42.3	153	27.2	208	20.0	263	15.8
44	94.5	99	42.0	154	27.0	209	19.9	264	15.7
45	92.4	100	41.6	155	26.8	210	19.8	265	15.7
46	90.4	101	41.2	156	26.7	211	19.7	266	15.6
47	88.5	102	40.8	157	26.5	212	19.6	267	15.6
48	86.6	103	40.4	158	26.3	213	19.5	268	15.5
49	84.9	104	40.0	159	26.2	214	19.4	269	15.5
50	83.2	105	39.6	160	26.0	215	19.3	270	15.4
51	81.5	106	39.2	161	25.8	216	19.2	271	15.3
52	80.0	107	38.9	162	25.7	217	19.2	272	15.3
53	78.5	108	38.5	163	25.5	218	19.1	273	15.2
54	77.0	109	38.1	164	25.4	219	19.0	274	15.2
55	75.6	110	37.8	165	25.2	220	18.9	275	15.1
56	74.2	111	37.5	166	25.0	221	18.8	276	15.1
57	72.9	112	37.1	167	24.9	222	18.7	277	15.0
58	71.7	113	36.8	168	24.7	223	18.6	278	15.0
59	70.5	114	36.5	169	24.6	224	18.6	279	14.9
60	69.3	115	36.2	170	24.5	225	18.5	280	14.8
61	68.2	116	35.8	171	24.3	226	18.4	285	14.6
62	67.1	117	35.5	172	24.2	227	18.3	290	14.3
63	66.0	118	35.2	173	24.0	228	18.2	295	14.1
64	65.0	119	34.9	174	23.9	229	18.2	300	13.9
65	64.0	120	34.6	175	23.8	230	18.1	305	13.6
66	63.0	121	34.4	176	23.6	231	18.0	310	13.4
67	62.1	122	34.1	177	23.5	232	17.9	315	13.2
68	61.1	123	33.8	178	23.4	233	17.8	320	13.0
69	60.3	124	33.5	179	23.2	234	17.8	325	12.8
70	59.4	125	33.3	180	23.1	235	17.7	330	12.6
71	58.6	126	33.0	181	23.0	236	17.6	335	12.4
72	57.7	127	32.7	182	22.8	237	17.5	340	12.2
73	57.0	128	32.5	183	22.7	238	17.5	345	12.0
74	56.2	129	32.2	184	22.6	239	17.4	350	11.9
75	55.4	130	32.0	185	22.5	240	17.3	355	11.7
76	54.7	131	31.7	186	22.4	241	17.3	360	11.5
77	54.0	132	31.5	187	22.2	242	17.2	365	11.4
78	53.3	133	31.3	188	22.1	243	17.1	370	11.2
79	52.6	134	31.0	189	22.0	244	17.0	375	11.0

APPENDIX 3

The Practice of Plotting Data Points from Hydrogen Clearance Curves & Calculating Cerebral Blood Flow by Hand Analysis.



The adjoining graph is constructed from actual data points taken from the hydrogen clearance curve generated from the left frontal electrode at 120 minutes post-MCA occlusion during experiment MCO 103/CBF 24. Data points are taken from the curve at each 15 second interval, and plotted on log-graph paper. After discontinuing the hydrogen, the first minute of the clearance curve is ignored, so that the tissue extraction of hydrogen is in a "steady-state" prior to analysis. In this particular example, the graph is almost linear, indicating that the fall in tissue hydrogen concentration has described a monoexponential curve. This is generally seen with good electrode placement and function (within the cortex only, and minimal trauma during placement). A linear regression (best-fit line) gives an excellent correlation of 0.999, with a hand calculated CBF of 45.2 ml/100g/min. The computer generated result could not better this, giving the same result! Clearly, this is one of the best generated hydrogen clearance curves: the overall correlation between hand and computed analyses of 36 curves in this particular experiment was 0.824.

The author has experimented with two other methods of calculating the cerebral blood flow from the desaturation curve. The first utilises the same mathematics as described in appendix 1, but employs the use of a scientific calculator to aid the calculation. 9 data points are selected from the curve (ie. at each 30 second interval over 4 minutes), and the logarithm of each value is entered into the statistics register, converting the exponential curve into a straight line. A program is created and stored in the calculator, so that a single key-press calculates the intercept with the y-axis (the logarithmic value at time zero), determines the antilog, halves this value, evaluates the resultant corresponding x-value (the $t_{½}$ value), and thus calculates the CBF. This saves a great deal of time

if access to on-line computer data analysis is not available - it is the plotting of the data on to logarithmic graph paper and construction of hand-drawn best-fit lines that is so time consuming. It is also likely to be more accurate and free from "analyst variability", but only if the curves are monoexponential.

The second alternative method is more innovative. The CBF is proportional to the slope of the line plotted on logarithmic graph paper i.e. the steeper the slope, the faster the blood flow. Thus, the blood flow will depend on the angle the line makes with the y-axis - shown as θ in the above figure:

$$\tan \theta = x / y \qquad (1)$$

$$\Rightarrow x = \tan \theta \times y \qquad (2)$$

On standard logarithmic graph paper, half the y-value is a constant irrespective of where the line intercepts the y-axis, and measures 25 mm. The x value, or $t_{½}$, will depend on the scale adopted by the researcher, but in the author's case this was 30 seconds/cm, or 3 seconds/mm. Thus :

$$x \times 3 = \tan \theta \times y \times 3 \qquad (3)$$

$$\Rightarrow t_{½} = \tan \theta \times 75 \qquad (4)$$

$$\Rightarrow CBF = (0.693 \times 100 \times 60) / (\tan \theta \times 75) \qquad (5)$$

$$\Rightarrow CBF = 55.44 / \tan \theta \qquad (6)$$

Cerebral blood flow is inversely related to the angle θ, and can be calculated directly if the angle between the line and the y-axis is measured. In the example illustrated on the previous page, θ equalled 51°. Tan θ = 1.235 ⇒ CBF = 44.9 ml/100g/min. This method still involves plotting the points and drawing the lines for a hand analysis, but if it is incorporated into a different program on a scientific calculator, the savings in time without loss of accuracy is obvious.

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APPENDIX 4 - *Volumetric Validation*

Infarct volumes in chapter 4 ranged from 150 to 260 mm^3, which was larger than those volumes reported by several other studies (see Ginsberg & Busto 1989). A study was conducted to assess the volume of a normal rat brain, to confirm that the methods used in this thesis for volumetric analysis gave results for hemispheric volumes equivalent to those calculated by a different method. 15 male Wistar rats (weight range 321 - 489 g; mean weight 418 ± 13.9 g) were sacrificed. After induction of anaesthesia with nitrous oxide/oxygen (70:30) and halothane 3-4%, blood was taken for a serum glucose estimation, and the rats killed by intracardiac pentobarbitol (***Expiral***). The brains were rapidly removed, whole brain and bi-hemispheric (cerebellum removed) weights were measured, and specific gravity measurements carried out on a random sample of seven animals.

PARAMETER		Range	Mean ±SE
Glucose mg% mmol/l		*131 - 194 mg%* *7.3 - 10.8 mmol/l*	*153.7 ±4.6 mg%* *8.5 ±0.26 mmol/l*
Weights g	***Whole Brain***	*1.837 - 2.069 g*	*1.952 ±0.015 g*
	Bi-hemisphere	*1.414 - 1.566 g*	*1.499 ±0.010 g*
Specific Gravity (Right + Left Hemisphere / 2)	***Cortex***	*1.0481 - 1.0509*	*1.0497 ±0.0002*
	White Matter	*1.0433 - 1.0460*	*1.0452 ±0.0002*
	Caudate	*1.0465 - 1.0492*	*1.0487 ±0.0002*
	Cerebellum	*1.0488 - 1.0526*	*1.0508 ±0.0002*

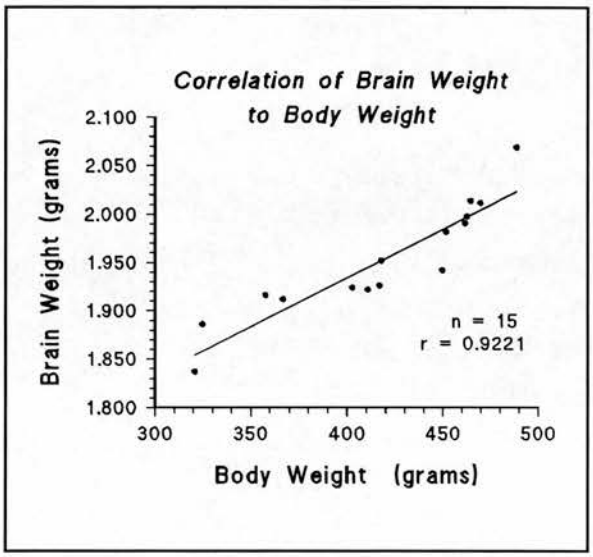
The average hemispheric specific gravity depends on contributions from cortex, white matter and caudate (sub-cortical) areas. Image analysis of brain sections of the sham operated animals confirmed that the relative contributions of these areas to the whole hemisphere were 52%, 6% and 42% respectively. Thus, the average normal hemispheric specific gravity will equal (1.0497 × 0.52) + (1.0451 × 0.06) + (1.0487 × 0.42), or 1.0490. Specific gravity of the brain or its relative density is the ratio of the weight of a unit volume of brain to the weight of an equivalent volume of water. Thus :

$$SG_{Hemisphere} = M_{Hemisphere} / V$$

$$\Rightarrow \quad V = M_{Hemisphere} / SG_{Hemisphere}$$

$$= 749\ mg / 1.0490$$

$$= 714\ mm^3$$



Correlation of Brain weight to Body weight in this series of 15 rats.

The left (non-lesioned) hemisphere in the normotensive and hypotensive groups in chapter 4 measured 726 mm^3 and 753 mm^3 respectively, which compares favourably with the current estimated hemispheric volume of 714 mm^3 (+1.7% and +5.5% respectively). The adjacent graph has been included to illustrate the fairly close correlation between brain weight (and presumably brain volume) and body weight in this experimental series.

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APPENDIX 5 - *Calibration Data for Gravimetric Columns.*

The bromobenzene/kerosene gravimetric columns used in this thesis were all generated according to the method originally described by Shigeno in 1982. The organic solvents are layered in rectangular glass columns that measured 2 × 15 × 25 cm. The column homogeneity was checked by dropping potassium sulphate calibration standards at 1 cm intervals across the width of several randomly selected columns. This confirmed that the layering was remarkably consistent across the width of the column, except within last centimetre of each side. Here, the fluid density tended to be marginally greater, presumably due to a surface effect adjacent to the glass. This area was therefore avoided in tissue flotation estimations.

It had occurred to the author that early evaporation from the surface of the fluid may affect the linearity of the upper area of the column. Although it had been customary to carry out a 7-point calibration, the specific gravity results in these experiments only once fell below 1.035 (1.0329). Therefore, it was considered appropriate to exclude to first 2 calibration points (1.03 and 1.035), and do a 5-point calibration on the remainder of the column. As shown below, 5-point calibration tended to give better correlations, confirming that the upper part of the column did adversely affect the overall regression. However, the difference this made to the actual calculated tissue specific gravities was often negligible, or very small (± 0.0000 - 0.0008). The column regressions are shown below (range 0.9935 - 0.9998).

CALIBRATION GROUP	*7-point*	*5-point*	*7-point*	*5-point*
8 4 Hours Sham *n* = *6*	*0.9990*	*0.9996*	*0.9984*	*0.9993*
	0.9956	*0.9987*	*0.9947*	*0.9993*
	0.9957	*0.9990*	*0.9987*	*0.9989*
9 4 Hours Normotensive *n* = *7*	*0.9962*	*0.9959*†	*0.9969*	*0.9987*
	0.9964	*0.9972*	*0.9909*	*0.9967*
	0.9973	*0.9935*†	*0.9923*	*0.9995*
	0.9977	*0.9984*		
10 4 Hours Hypotensive *n* = *7*	*0.9943*	*0.9995*	*0.9986*	*0.9994*
	0.9998	*0.9995*†	*0.9983*	*0.9985*
	0.9950	*0.9947*†	*0.9969*	*0.9962*†
	0.9972	*0.9994*		
11 24 Hours *n* = *8*	*0.9922*	*0.9998*	*0.9941*	*0.9997*
	0.9969	*0.9998*	*0.9955*	*0.9996*
12 72 Hours *n* = *6*	*0.9928*	*0.9996*	*0.9957*	*0.9983*
	0.9919	*0.9986*		
Post-irradiation + Controls *n* = *15*	*0.9936*	*0.9996*	*0.9975*	*0.9993*
	0.9995	*0.9995*	*0.9970*	*0.9988*

† *7-point better than 5-point calibration.*

APPENDIX 6 - *Regression Analysis of CBF in Autoregulation Study Groups*

The same data presented in figure 8 (page 63) was analysed by plotting all the cerebral blood flows obtained in each experimental group at mean blood pressures of 40 to 90 mmHg, and calculating sequential linear regressions to estimate break-points in the CBF when plotted against MABP. The slope equations above 60 mmHg for CBF in the sham operated animals were: right hemisphere - y = 1.2x + 60.3; left hemisphere - y = 1.3x + 62.7. As expected, the constants are similarly close to 60 in both hemispheres. In the MCA occlusion animals, the slope of the regression line changed at 70 mmHg in the lesioned hemisphere, but this change is less marked than in the sham operated group. In the non-lesioned hemisphere, there appears to be no obvious demonstrable break-point, suggesting a passive pressure-flow relationship. [CBF = ml/100g/min; Blood pressure = mmHg.]

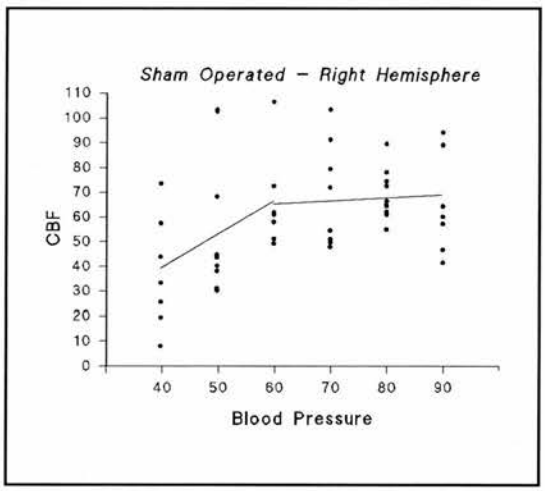

Figure 1. *Regression analysis of all CBF recordings in the right hemisphere of sham operated animals (group 1).*

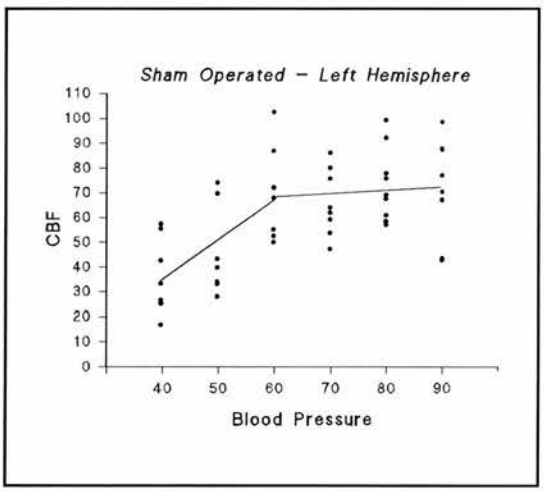

Figure 2. *Regression analysis of all CBF recordings in the left hemisphere of sham operated animals (group1).*

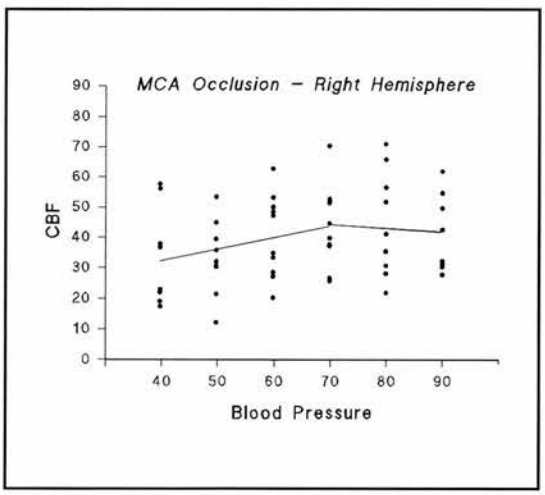

Figure 3. *Regression analysis of all CBF recordings in the right hemisphere of MCA occlusion animals (group 2).*

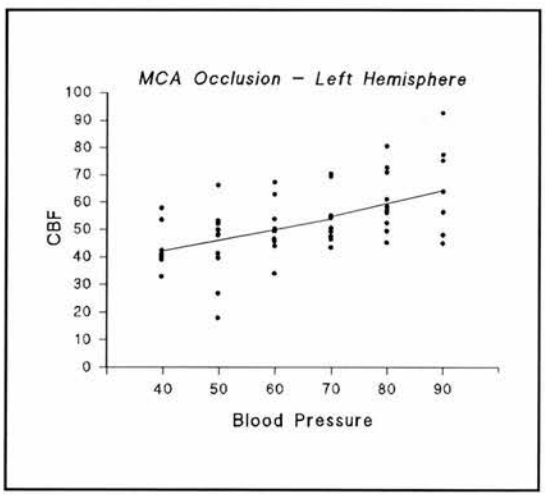

Figure 4. *Regression analysis of all CBF recordings in the left hemisphere in MCA occlusion animals (group 2).*

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Presentations & Publications

List of presentations to learned societies and current publications relevant to this thesis

PRESENTATIONS

1. *Controlled hypovolaemic hypotension in the rat and its effect on infarct size following middle cerebral artery occlusion.* North American Research Society of Neurological Surgeons, Boston, Massachusetts, USA; June 1990.

2. *The effects of immunosuppression by irradiation on cerebral oedema following middle cerebral artery occlusion in the rat.* Neurosurgical Satellite meeting of the Surgical Research Society, St. Bartholomew's Hospital, London; January 1991.

3. *The effect of hypotension on infarct size, cerebral oedema, and cerebral blood flow after experimental middle cerebral artery occlusion.* Society of British Neurological Surgeons, Autumn meeting, London; September 1991.

PUBLICATIONS

1. Chambers IR, **Strachan RD**, Kane PJ, Cooke A, Mendelow AD.
Closed-loop blood pressure control for cerebral perfusion studies.
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2. Mendelow AD, Kane P, **Strachan R**, Chambers I, Modha P, Cook S.
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3. **Strachan RD**, Kane PJ, Cook S, Chambers IR, Clayton CB, Mendelow AD.
Immunosuppression by whole-body irradiation irradiation and its effect on oedema in experimental cerebral ischaemia.
Acta Neurologica Scandinavica 1992; 86: 256-259.

4. MacDonald AG, Kane PJ, **Strachan RD**, Chambers IR, Mendelow AD.
Cerebral blood flow measurement using the hydrogen clearance technique: a microcomputer-based system for data collection and analysis.
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