

**THE CONSEQUENCES OF REPERFUSION ON
CEREBRAL ISCHAEMIC DAMAGE**

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 Summary **GAIL GARTSHORE, B.Sc. (Hons.), MRPharmS**

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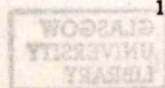
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**A thesis submitted for the Degree of Doctor of Philosophy
 to the Faculty of Medicine, University of Glasgow**

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**Wellcome Surgical Institute & Hugh Fraser Neuroscience Laboratories,
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Preface

Stroke is the most common life threatening neurological disease and is the third leading cause of death in the United States after heart disease and cancer. However stroke is considered to be more disabling than lethal and is a catastrophic event for both the patient and his relatives. It is not unusual for a previously normal intelligent person to be rendered aphasic, paralysed and incontinent. Much interest has focused on the therapy of stroke but it should be considered that prevention is far superior to a cure. Indeed prevention of stroke by the treatment of modifiable atherogenic host and environmental risk factors is likely to be the most effective means of reducing morbidity and mortality in this fatal condition. Factors such as antihypertensive therapy, cessation of cigarette smoking, reduction in alcohol consumption and control of serum cholesterol, constitute realistic measures which could prevent the occurrence of atherothrombotic stroke. However despite avid attempts to prevent stroke, in reality it is still a common pathological event and a major health problem. As such pre-clinical research into the pathophysiology of stroke, utilising both *in vitro* and *in vivo* techniques has been accelerated over the past few decades. It is accepted that physiologically regulated reproducible animal models must be utilized to gain insight into the very complex pathology of cerebral ischaemia and to investigate potential therapeutic strategies.

However in an era of aggressive animal rights activism it has been proposed that advances in technology capable of deriving morphological, physiological and even metabolic data from patients should permit animal testing and experimentation into cerebral ischaemia to be scaled down. Nevertheless, there are many fundamental issues that cannot be addressed in humans and therapeutic strategies must be investigated at a basic level for both efficacy and toxicity before moving to the clinical arena. The advantages of animal models of cerebral ischaemia are numerous and have been discussed in detail in section 1.6 of this thesis. This thesis also discusses the complex and divergent biochemical events proposed to be involved in the ischaemic cascade and attempts to emphasize that the glutamate theory of ischaemic cell death although important, does not contain all the answers. A vast array of experimentation has been performed within the scientific community to reach the present level of knowledge but in my opinion the most exciting advances in ischaemic research came with the revelation that the majority of human embolic strokes in general involve some degree of vessel recanalization and spontaneous reperfusion. This heralded the beginning of research into post-ischaemic reperfusion. Subsequent reports have implicated that the restoration of cerebral blood flow following an ischaemic event may have a dark side whereby delayed or late reperfusion is paradoxically detrimental to the final outcome.

The aim of this PhD thesis was to investigate the consequences of reperfusion on cerebral ischaemic damage. Ultimately, this required the use of an appropriate animal model of transient focal cerebral ischaemia and the endothelin reperfusion model fulfilled this role.

It incorporates a period of ischaemia within middle cerebral artery (MCA) territory followed by a gradual reperfusion. Of particular interest to me was the profile of cerebral blood flow (CBF) changes following endothelin-1 application to the MCA. There is much discussion in this thesis on the regulation and control of CBF with particular reference to endogenous mediators of cerebrovascular homeostasis. A discussion on the numerous techniques available for both clinical and pre-clinical assessment of CBF highlighted the importance of autoradiographic procedures in pre-clinical research involving animal experimentation. As such a novel double label autoradiography technique was developed within our laboratory. The practical aspects and utility of the procedure were subsequently investigated by myself and the outcome reported in this thesis.

The work presented in this thesis also intended to address certain fundamental issues pertinent to the characterisation of post-ischaemic reperfusion. Studies were designed and information obtained on the consequences of reperfusion on brain swelling and oedema formation, microvascular permeability and at the cellular level, on neurotransmitter transduction systems and second messengers. Of fundamental importance to the pathology of reperfusion injury is the contribution that free radical damage makes to the overall cascade. The role free radicals play in this process is discussed in detail and the results of an investigation into the neuroprotective effects of the glutathione peroxidase mimic ebselen reported. Thus the content of this PhD thesis is of interest from both a mechanistic and therapeutic angle. The ultimate aim of pre-clinical research is to propel effective and safe therapeutic strategies into the clinic. However to focus entirely on this angle of ischaemic research because it is presently in vogue would be naive. Of equal importance is research aimed at characterising fully the complex mechanisms contributing to the pathology of both cerebral ischaemia and reperfusion injury. We fully appreciate the enormity of this task and meet the challenge with enthusiasm. I propose that the content of this PhD thesis contributes to the vast array of information currently available and paves the way for future research into the exciting field of post ischaemic reperfusion and reperfusion injury.

Declaration

This thesis comprises my own original work and has not been presented previously as a thesis in any form. Certain experiments were carried out in collaboration with other researcher. Mr. P. Bannan, an experienced neurosurgeon, carried out the permanent middle cerebral artery (MCA) occlusion in the cat (section 3.1). The permanent MCA occlusion carried out in the rat (section 3.2) was performed by Dr. M. McAuley and the transient MCA occlusion induced by ET-1 application to the MCA reported in sections 3.3 (study 1) and 3.5 was performed by Dr. D. Dawson. In each case all additional experimental procedures excluding MCA occlusion and all analysis of results were carried out by myself. In all other studies reported in thesis MCA occlusion was carried out by myself.

Summary

The aim of this PhD thesis was to investigate the consequences of reperfusion on cerebral ischaemic damage using a new model of transient focal cerebral ischaemia developed at the Wellcome Surgical Institute. The model involves the abluminal application of the potent vasoconstrictor peptide endothelin-1 (ET-1) to the exposed MCA of the anaesthetised rat. Previous studies using the hydrogen clearance CBF technique and quantitative autoradiographic and histopathological procedures, revealed that ET-1 application to the MCA produced a profound ischaemia followed by a gradual, spontaneous reperfusion resulting in a reproducible lesion (within the cortex and caudate nucleus) by 4h post insult. The object of this thesis was to further characterise the pathological mechanisms associated with this model of transient cerebral ischaemia by investigating such parameters as CBF, brain tissue swelling, microvascular permeability, free radical damage and neurotransmitter transduction systems and second messengers.

Characterisation of CBF profile

A novel double label CBF autoradiography technique was utilised to investigate the CBF profile following ET-1 induced transient cerebral ischaemia. The CBF tracers ^{99m}Tc -hexamethylpropyleneamine oxime (HMPAO) and ^{14}C -iodoantipyrine (IAP) were used to provide a topographic profile of CBF at two distinct time points in the same animal. Previous work demonstrated that ^{99m}Tc -HMPAO is inherently unstable in the vial after reconstitution, and a 30min shelf life was therefore recommended. It was demonstrated in this thesis that the addition of cobalt chloride hexahydrate to the reconstituted vial can extend the *in vitro* shelf life to at least 4h post reconstitution. The addition of the stabiliser does not alter the ability of ^{99m}Tc -HMPAO to image CBF and does not have any influence on the absolute CBF in the cat.

In this cat study and in a separate rat study, ^{99m}Tc -HMPAO was shown to underestimate absolute CBF (assessed with ^{14}C -IAP) in high flow areas of the brain due to back diffusion from brain to blood. This well known phenomenon attributed to the complex *in vivo* kinetics of ^{99m}Tc -HMPAO was previously demonstrated by comparison with other CBF tracers. The complex *in vivo* kinetics of ^{99m}Tc -HMPAO may also be responsible for the apparent discrepancy noted between normalised ^{14}C -IAP and ^{99m}Tc -HMPAO in low and normal flow areas when administered 2min apart. Indeed ^{99m}Tc -HMPAO may overestimate flow in these areas but the possibility of ^{14}C -IAP underestimating flow should also be considered. This study highlighted the importance of the double label CBF autoradiography technique for providing topographic profiles of CBF and emphasises that the discrepancy between tracers should not pose a restriction to the use of the technique providing it is considered when interpreting results.

Subsequent studies utilised the double label autoradiography technique to investigate the topographic profile of reperfusion into ischaemic tissue following ET-1 induced reversible MCA occlusion. Blood flow changes were assessed with ^{99m}Tc -HMPAO during ischaemia (5min) and ^{14}C -IAP during reperfusion (initially at 2h and then at 30min, 1h, 2h and 4h post insult in a subsequent study). Following a significant ischaemic insult, reperfusion was relatively homogeneous within MCA territory but incomplete by the 4h time point. A strong positive correlation exists between the severity of ischaemia and subsequent reperfusion with evidence for differential reperfusion in the cortex and caudate nucleus and increased collateral supply from the anterior cerebral artery.

Brain tissue swelling

Consequent with the CBF changes during ET-1 induced ischaemia and reperfusion, there was a substantial ipsilateral hemispheric swelling. An exponential relationship exists between CBF in the parietal cortex during ischaemia and the increase in the ipsilateral hemispheric volume. During the first 4h post ET-1 application, swelling was maximal at the 1-2h time points and appeared to resolve as the quality of reperfusion improved.

Microvascular permeability

A quantitative autoradiographic technique utilising the small neutral, amino acid tracer ^{14}C -aminoisobutyric acid (AIB) revealed no significant changes in the microvascular permeability 1 and 2h post ET-1 application to the MCA, despite significant ipsilateral hemispheric swelling at these time points. The acute changes in brain swelling induced by ET-1 application to the MCA, are most likely a consequence of a disturbed ion homeostasis following energy failure and ion pump failure. Studies designed to investigate the consequences of oedema formation at later time points (e.g. 24h) following ET-1 application may reveal changes in microvascular permeability.

Free radical damage

Free radical formation may play a prominent role in the pathological events associated with post-ischaemic reperfusion. A previous study demonstrated that the Glutathione peroxidase mimic, Ebselen, designed to limit free radical mediated damage, is neuroprotective when administered 40min prior to ET-1 application to the MCA. The final study of this thesis revealed that Ebselen administered 15min after ET-1 application to the MCA does not reduce the volume of ischaemic damage and may in fact be detrimental to tissue survival. Dosing ebselen at various time points post ET-1 application may produce more promising results. Interestingly, Ebselen did not significantly influence the CBF profile in the parietal cortex during the first hour after administration.

Second messengers and neurotransmitter binding

The consequences of ET-1 induced ischaemia and reperfusion on brain injury at a cellular level were investigated by quantitative ligand binding autoradiography. The blood flow profile consistent with the ET-1 model of transient focal cerebral ischaemia was associated with a significant reduction in forskolin binding (a marker of the adenylate cyclase second messenger system) throughout MCA territory (e.g. by 25% in the parietal cortex, 11% in the caudate nucleus). The most marked losses in forskolin binding were in areas where ischaemia was severe and reperfusion poor. However, the same changes in CBF had no significant effect on D1 dopamine receptor binding (e.g. < 2% reduction in caudate nucleus). Thus, ligand binding characteristics are significantly affected as early as 2h post insult, with evidence of differential sensitivity for forskolin and D1 dopamine binding. Comparison with the results of a study investigating the consequences of maintained ischaemia on these systems revealed reperfusion-related salvage of dopamine and forskolin binding in the caudate nucleus but possible exacerbation of forskolin binding loss in the cortex.

Part 1 - Introduction

1.1 Cerebrovascular disease in man - an overview

Stroke - *"the rapid development of clinical signs of a focal (or global) disturbance of cerebral function with symptoms lasting 24 hours or longer or which leads to death with no apparent cause other than of vascular origin"*

World Health Organisation 1988

Acute cerebrovascular disease (synonymous with "cerebrovascular accident" or "stroke") is a major public health problem in most parts of the world. Despite modern advances in the management strategies of cerebrovascular disease, stroke is still a major cause of mortality and disability. About half of all individuals who survive a stroke have significant persisting neurological impairment and physical disability. In individual cases the immediate outlook for life depends on several factors, such as age of the patient and the type, size and anatomical site of the cerebrovascular lesion. The type of lesion influences not only the ultimate outcome but also the length of the survival period. It was established over three decades ago that more than half of the patients who succumb to cerebral haemorrhage die within 2 days of the onset of the symptoms and about 80% within 2 weeks. In contrast less than one third of deaths due to cerebral infarction occur within a week of stroke (Brown & Glassenberg 1973). In addition it is apparent that a hemispheric infarct, however large, only becomes fatal if a secondary oedematous swelling of the brain occurs causing transtentorial herniation and death. It appears, therefore, that an accurate clinical diagnosis of the type and severity of stroke is fundamental to the management of the patient and ultimately to the overall prognosis. Before the various subtypes of ischaemic stroke are discussed, it is important to consider the vascular anatomy of the brain in order to fully appreciate the devastation of stroke to such a complex organ.

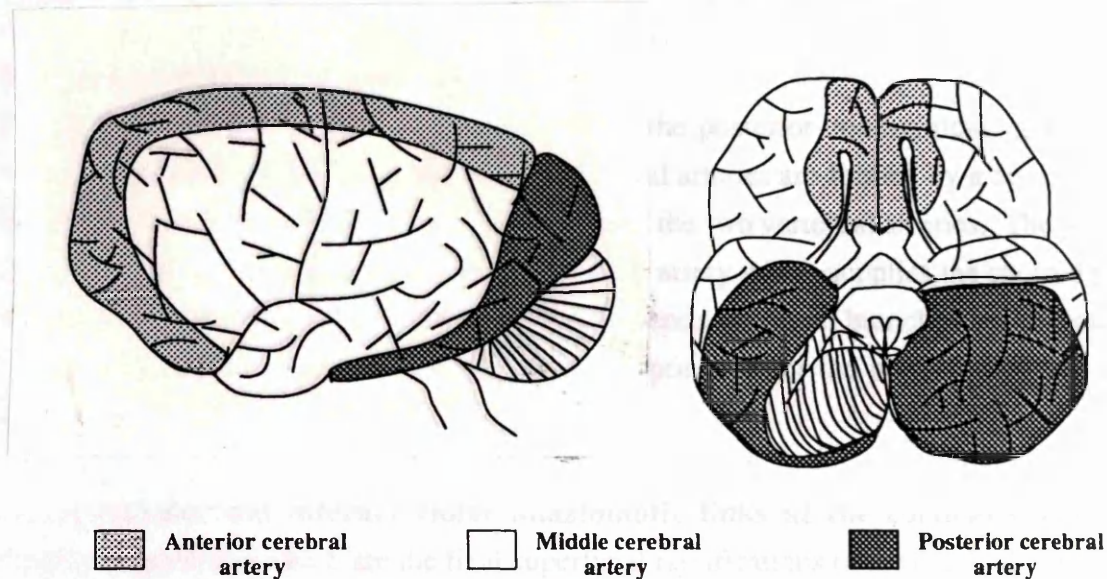
1.1.1 Vascular anatomy of the human brain

The general anatomical distribution in many lower species is similar to that in man and therefore many aspects of the following discussion may be pertinent to both rat and cat vascular anatomy (section 1.6).

The brain is instrumental in that it is supplied by four major arteries that eventually coalesce to form a structure known as the circle of Willis. These primary vessels are the two carotid arteries and the two vertebral arteries (which unite intracranially to form the basilar artery). If one of these major arteries became occluded over a prolonged period, the arrangement is such that the brain would still receive an adequate perfusion under normal circumstances. In

man the carotid arteries each contribute approximately 40% of the total perfusion of the brain. They divide into four major branches : the anterior cerebral, the middle cerebral, the anterior choroidal and the posterior communicating arteries. Figure 1 illustrates the areas of the brain supplied by the anterior cerebral, middle cerebral and posterior cerebral arteries. Although this represents the situation in the majority of cases it is not absolute and there may be some degree of variation especially in experimental animals.

Figure 1 - Arterial distribution of brain perfusion



Anterior cerebral artery

Various orbital, middle and posterior branches of the anterior cerebral artery supply the frontal and parietal lobes. The anterior cerebral artery is also responsible for supplying the corpus callosum. The medial striate artery of Heubner (a large branch of the anterior cerebral artery) is frequently responsible for the perfusion of part of the inferior portion of the caudate nucleus and the most rostral portion of the putamen, in addition to the anterior limb of the internal capsule. It is known that the medial striate artery forms an anastomotic connection with the lenticulostriate arteries of the basal ganglia.

Middle cerebral arteries

The middle cerebral arteries are the major branches of the internal carotid arteries and supply the majority of the lateral aspects of the cerebral hemispheres. The lateral striate and lenticulostriate branches of the middle cerebral arteries are responsible for the perfusion of the putamen, globus pallidus and the internal capsule.

Anterior choroidal artery

Branches of the anterior choroidal artery supply not only the choroid plexus of the lateral ventricle but also cerebral structures as diverse as the optic tract, hippocampus, tract of the caudate nucleus, pyriform cortex, amygdala, part of the globus pallidus, the posterior limb

of the internal capsule and particularly the middle one third of the cerebral peduncle and the lateral portion of the geniculate body.

Posterior communicating arteries

The bridge between the carotid and vertebrobasilar systems is formed by the posterior communicating arteries. A limited number of brain structures are supplied by the medial branches of these arteries including the genu of the corpus callosum, part of the posterior rim of the internal capsule, the rostral thalamus and the walls of the third ventricle.

The posterior cerebral arteries

The posterior cerebral arteries anastomose with the posterior communicating arteries to complete the circle of Willis. The posterior cerebral arteries are formed by a division of the basilar artery which itself was formed by fusion of the two vertebral arteries. The posterior cerebral arteries give rise to the posterior choroidal artery which supplies the choroid plexus of the third and lateral ventricles and in addition sends off direct branches to the thalamus. The posterior cerebral arteries are themselves responsible for the perfusion of part of the hippocampus and the thalamus.

Pial arterioles and interarteriolar anastomotic links at the cortical surface

Small pial arterioles which are the final superficial ramifications of the cerebral arteries, run over the surface of the brain and then enter the brain substance at various points. The mode of entry of these pial arterioles has been a point of great debate (Dahl 1973, Jones 1970, Krahn 1982). More than a century ago Heubner (1874) reported that there were extensive anastomotic links between arterioles and venules in the cortical surface which was in contrast to the minimal interarterial connections noted within the cortex. It was thought that the numerous anastomotic connections between the anterior, middle and posterior cerebral arteries on the cortical surface provided a system for redistributing blood supply. This may be analogous to that provided by the circle of Willis at the base of the brain but the capacity of the cortical anastomoses in maintaining adequate perfusion is substantially less than of the circle of Willis. This is highlighted by the fact that occlusion of an artery distal to the cortical interarteriolar anastomoses leads to severe reductions in cerebral blood flow whereas occlusion of an artery proximal to the circle of Willis minimally alters the basal level of flow.

Collateral circulations : arterial boundary zones

The collateral channels that exist between major cerebral arteries are of marked clinical importance because it is in these regions - which have been designated arterial boundary zones - that ischaemic brain damage may occur following an ischaemic attack. Indeed severe hypotension typically encountered following cardiac arrest in man leads to ischaemic brain damage which is frequently restricted to these arterial boundary zones. The boundary zones, which have also been termed border or watershed zones, appear to be localised at the territory limits of the major cerebral arteries (Brierly & Graham 1984). The most frequently

affected region in the neocortex is in the depth of the parietooccipital sulci, which is at the limits of the fields of irrigation of the anterior, middle and posterior cerebral arteries.

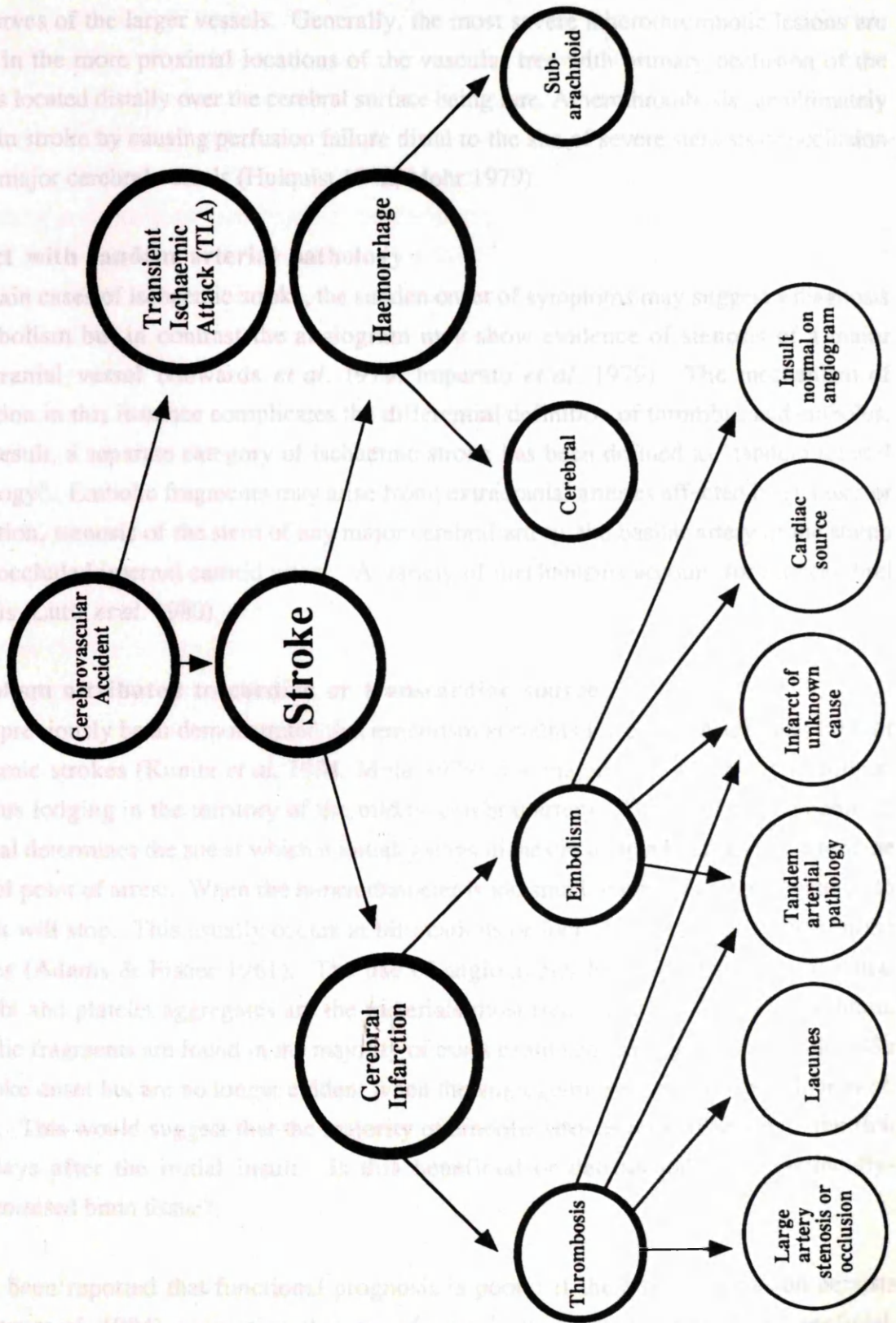
Microvascular organisation and capillary density

One of the major features of the cortical microcirculation is that arterioles penetrate the cortex at right angles to the surface and give rise to capillaries at all laminae. This vertical organisation of arterioles results in the production of minute boundary zones between penetrating arterioles at the distal end of the capillaries. The columnar pattern of local cerebral blood flow and glucose metabolism seen in the cerebral cortex during hypoxia or ischaemia probably reflects the orientation of these penetrating arterioles. The distribution of capillaries in the CNS is heterogeneous but structurally organised. It has been shown that the capillary density correlates with the number of synapses within a particular region (Dunning & Wolff 1937). In addition it has been suggested that capillary density provides an anatomic indicator of oxidative metabolism. This theory was further supported by the findings that in general the brain areas that have a higher basal glucose use and blood flow such as the cochlear nucleus, mammillary body and cerebral cortex contain a high density of capillaries. Areas with lower levels of glucose use and blood flow such as the hypothalamus, cerebellar cortex and medullary nuclei contain fewer capillaries.

1.1.2 Classification of ischaemic stroke

Diagnosis of a specific type of stroke is more difficult than diagnosis of coronary heart disease because there are fewer pathognomonic laboratory tests available for stroke detection. The timing of the available tests such as lumbar puncture, cerebral arteriography, CT scan, PET or SPECT scan or non invasive studies of cerebral patency are of critical importance for accurate diagnosis. Even at autopsy of fatal cases it may be difficult to differentiate accurately between intracerebral haemorrhage, haemorrhagic infarction and subarachnoid haemorrhage when there has been extensive destruction of the brain and vascular structures. Ischaemic brain infarction may be due to thrombosis or embolism and it is often difficult to distinguish between the two alternatives. However in most circumstances the clinical features and laboratory data suffice to differentiate accurately between haemorrhage and infarction and in many cases classification of the ischaemic stroke into subtypes can be done well enough to justify the treatment strategy. In general, laboratory data indicates that large artery atherothrombosis infrequently causes stroke and embolism is far more common. A schematic diagram of the classification of stroke is shown in figure 2. This particular section will only deal with the classification of ischaemic stroke and will not discuss haemorrhagic insults.

Figure 2 - Classification of stroke



Infarct with large artery thrombosis Atherosclerotic lesions (degenerative arterial changes associated with deposition of hard yellow plaques of lipid material in the intimal layers of the arteries) are found at bifurcations and curves of the larger vessels. Generally, the most severe atherothrombotic lesions are found in the more proximal locations of the vascular tree with primary occlusion of the arteries located distally over the cerebral surface being rare. Atherothrombosis can ultimately result in stroke by causing perfusion failure distal to the site of severe stenosis or occlusion of the major cerebral vessels (Hulquist 1942, Mohr 1979).

Infarct with tandem arterial pathology In certain cases of ischaemic stroke, the sudden onset of symptoms may suggest a diagnosis of embolism but in contrast the angiogram may show evidence of stenosis of a major extracranial vessel (Edwards *et al.* 1979, Imperato *et al.* 1979). The mechanism of infarction in this instance complicates the differential definition of thrombus and embolus. As a result, a separate category of ischaemic stroke has been defined as "tandem arterial pathology". Embolic fragments may arise from; extracranial arteries affected by stenosis or ulceration, stenosis of the stem of any major cerebral artery, the basilar artery or the stump of an occluded internal carotid artery. A variety of mechanisms account for intracerebral stenosis (Little *et al.* 1980).

Embolism attributed to cardiac or transcerebral source

It has previously been demonstrated that embolism accounts for between 15% and 30% of ischaemic strokes (Kunitz *et al.* 1984, Mohr 1979), the majority of which occur from an embolus lodging in the territory of the middle cerebral artery. The size of the embolized material determines the site at which it initially stops in the circulation but does not determine its final point of arrest. When the lumen diameter is too small to permit embolic material to pass, it will stop. This usually occurs at bifurcations or foci of atheroma at corners of the arteries (Adams & Fisher 1961). The use of angiography has demonstrated that mural thrombi and platelet aggregates are the materials most frequently embolized in the brain. Embolic fragments are found in the majority of cases examined angiographically within 48h of stroke onset but are no longer evident when the angiogram is repeated later (Mohr *et al.* 1978). This would suggest that the majority of embolic strokes recanalize within the first few days after the initial insult. Is this beneficial or detrimental to ischaemically-compromised brain tissue?

It has been reported that functional prognosis is poorer if the arterial occlusion persists (Kunitz *et al.* 1984) suggesting that reperfusion in this instance would be beneficial. However in general, information on the possible consequences of reperfusion into ischaemically compromised tissue is currently lacking. Early reperfusion through an occluded artery is obviously essential for the salvage of ischaemically compromised tissue and is known to be beneficial (Watson 1989). Delayed or late reperfusion is potentially

deleterious and may exacerbate cellular injury induced by the preceding ischaemia, due to such factors as an increased production of free radicals and prostaglandins, the initiation of an acute inflammatory response, haemorrhagic transformation, oedema formation and brain swelling (Hallenbeck & Dutka 1990, Ng & Nimmannitya 1970, Omar *et al.* 1991, Watson 1989). The nature of the reperfusion is another important aspect to consider (i.e. surge or gradual reperfusion). It has long been accepted that fragmentation and distal migration of an embolus will result in reperfusion of the vascular bed of ischaemic tissue perhaps culminating in haemorrhagic infarction (Fisher & Adams 1951). Presumably the full pressure of arterial blood into hypoxic capillaries results in a diapedesis of red blood cells through their hypoxic walls. The more intense the reperfusion the more severely damaged the capillary walls and the more confluent the haemorrhagic infarction. However in reality it is possible that reperfusion may be more gradual in nature. The most commonly documented means by which the lumen of a vessel is cleared of obstruction by an embolus is the insinuation of a column of blood between the embolus and the arterial wall. The column then enlarges eroding the embolus until the lumen is finally cleared. This process is documented to happen within hours to days after occlusion (Liebeskind *et al.* 1971, Zatz *et al.* 1965). At autopsy, it is common to find the vessels distended by the embolus although the wall is commonly histologically normal, suggesting that endothelial injury, vasospasm or necrotizing effects may have no role in the pathogenesis of infarction induced from embolism (Mohr & Barnett 1986). In addition, as it is common to find no evidence of haemorrhagic infarction this may suggest that surge reperfusion is the exception rather than the rule and reperfusion in the clinical situation is in general gradual in nature.

Infarction with normal angiogram

This diagnostic category was created to define cases with negative angiograms where there was no obvious cardiac or extracranial sources of ischaemic cerebral stroke. In these cases the CT scan is generally normal or shows low density or high density abnormalities consistent with haemorrhagic infarcts.

Lacunar infarcts

These infarctions are understood to reflect arterial disease of the vessels penetrating the brain to supply the capsule, basal ganglia, thalamus and paramedian regions of the brain stem (Fisher 1965). This may be caused by a tiny focus of microatheroma stenosing one of the deep penetrating arteries or rarely, stenosis of the middle cerebral artery.

Infarcts of undetermined cause

These may be due to :

1. Inappropriate laboratory studies
2. Inappropriate timing of the appropriate laboratory studies
3. No conclusive finding despite appropriate laboratory test and appropriate timing.

1.1.3 Epidemiology

Despite the fact that stroke is a major cause of morbidity and mortality, a variety of treatable risk factors as well as surgical and medical therapies for the primary and secondary prevention of stroke have been elucidated from epidemiological studies. However it is important to realise that the presence of a risk factor is not a prerequisite for stroke. In the same way, the absence of a risk factor is not a guarantee that stroke will not occur. Nevertheless, stroke risk factors most certainly influence the **probability** of stroke occurring (WHO/MNH Task force on stroke and other cerebrovascular disorders 1989). An excellent epidemiological study which documented important information on risk factors is the Framingham Study (Wolf *et al.* 1991).

Table 1 documents risk factors for atherothrombotic brain infarction (ABI) which was defined in the Framingham study and includes infarction secondary to large vessel atherothrombosis as well as lacunar infarction. According to Wolf and co-workers (1992) ABI documented in the Framingham study comprises the most common stroke subtype accounting for 44% of all strokes.

1.1.4 Prevention of stroke

*"When meditating over a disease, I never think of finding
a remedy for it, but instead a means of preventing it"*

Louis Pasteur 1884

Prevention of stroke by the treatment of modifiable atherogenic host and environmental risk factors is likely to be the most effective means of reducing morbidity and mortality in this fatal condition (Wolf *et al.* 1992). Even if risk factors are identified after a stroke, it is feasible that medication and surveillance may help prevent recurrence. Factors such as antihypertensive therapy, cessation of cigarette smoking, reduction in alcohol consumption and control of serum cholesterol levels should be considered as realistic measures which could prevent the occurrence of an atherothrombotic stroke. In addition because heart disease is an important precursor of stroke, prevention and treatment of coronary heart disease, congestive heart failure, left ventricular hypertrophy or atrial fibrillation would be anticipated to reduce the risk of stroke (Wolf *et al.* 1992).

Control of hypertension

Hypertension is the most significant risk factor associated with stroke. Indeed blood pressure elevation in the Framingham Study emerged as the strongest independent contributor to atherothrombotic stroke risk (Kannel *et al.* 1976). The authors stated that