WATER-SOLUBLE CONTRAST MEDIA

AND

THE BRAIN INTERFACES

BY

M.R. SAGE, MBBS (Adelaide)

DEPARTMENT OF SURGERY

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I certify that this thesis does not incorporate any material previously submitted for a degree or diploma in any University; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

MICHAEL R. SAGE

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SUMMARY

The blood, cerebro-spinal fluid (CSF) and the extracellular fluid of the brain parenchyma form the fluid compartments of the brain with three interfaces between, namely the blood-brain interface (BBB), the CSF-brain interface and the blood-CSF interface. One or more of these interfaces are exposed to water-soluble contrast medium (CM) following intra-arterial, intravenous, or intrathecal CM administration during cerebral angiography, computerised tomography (CT) or myelography.

Studies were performed in dogs to demonstrate that CT could be used to assess the distribution and the degree of BBB disruption after the intra-arterial injection of hypertonic solutions. Utilising this fact, further studies were performed to assess the effect of intra-arterial injections of various water-soluble CM on the permeability of the BBB. Whereas isotonic saline resulted in no change, hypertonic ionic methylglucamine iothalamate produced consistent, although variable breakdown of the BBB as demonstrated by both Evans' Blue staining and CT enhancement. Although this was thought to be due to the hyperosmolality of the methylglucamine iothalamate, a solution of 25% mannitol which has a similar osmolality to methylglucamine iothalamate did not produce the same degree of breakdown at a similar dose. This indicates that although the osmolality of particular solutions remains a major factor in producing BBB disruption following intra-arterial injections, other factors must play a part. The viscosity of methylglucamine iothalamate is more than twice that of 25% mannitol at 37 degrees centrigrade and it is postulated that this may be a factor in BBB

disruption by increasing the contact time with the endothelium of the cerebral capillaries following intra-arterial injection.

Using similar dose rates and iodine concentration as methylglucamine iothalamate, the non-ionic CM, metrizamide, iopamidol and iohexol failed to produce any evidence of BBB disruption. This is thought to reflect their lower osmolality when compared with methylglucamine iothalamate and 25% mannitol. As neurotoxicity appears to be at least partly related to the effect of CM on the blood-brain interface, the blood-brain barrier, these studies suggest that non-ionic CM with their lower osmolality should replace ionic CM for intra-arterial angiography.

Large intravenous doses of ionic CM are recommended for CT enhancement and digital intravenous angiography. Such large doses of ionic CM may briefly increase the osmolality of blood and hence lead to BBB disruption. However, studies with large doses of ionic sodium iothalamate in rabbits failed to demonstrate any quantitative evidence of BBB disruption. This probably reflects the rapid distribution of water-soluble CM into the plasma and also the extracellular fluid of non-neural tissue following intravenous injection resulting in rapid dilution of the CM within blood.

Intrathecally ionic CM are known to be more neurotoxic than equivalent iodine concentrations of non-ionic CM. It has been suggested that the toxicity of metrizamide is related to brain penetration and concentration. Studies were therefore carried out to compare the rate of brain penetration and concentration of ionic methylglucamine iothalamate and non-ionic metrizamide and iopamidol.

The depth of penetration and the concentration of CM in the brain parenchyma 60 minutes after intrathecal injection were found to be the same for each. This indicates that the relative difference in neurotoxicity is not due to a different rate of penetration across the CSF-brain interface but must be related to the molecular structure of the individual CM. Studies with iopamidol showed that there is rapid passage of CM across the CSF-brain interface and that a positive rate of diffusion across the CSF-brain barrier continues at least up to 60 minutes with an increase in concentration within the brain parenchyma.

Although there is no active CSF circulation in the spinal subarachoid space, a clinical study indicated that following lumbar myelography in which metrizamide was deliberately pooled in the lumbar thecal sac both during and after the examination, the CM passed cranially and by 6 hours was demonstrated in the intracranial CSF in the majority of patients with obvious brain penetration. This brain penetration of CM persisted for 24 hours, with an increase in depth of penetration and without a reduction in the concentration within the brain parenchyma. Gross brain penetration by metrizamide was demonstrated in the absence of any headache, neurological sequelae or post-myelographic EEG changes. No change in the density of the white matter was demonstrated in the absence of any intraventricular reflux to suggest penetration of CM into the white matter and there was no evidence of a reduction in density to suggest the development of brain oedema.

It was recently suggested that following a large intravenous dose of water-soluble CM that the CM may pass into the CSF across the

blood-CSF interface, allowing intravenous CT myelography to be performed. Studies with ionic sodium iothalamate showed that there was only minimal passage of CM across the blood-CSF interface after intravenous injection indicating that intravenous enhancement of the CSF is not possible.

1 BACKGROUND AND SUBJECT REVIEW

1.1 CONCEPT OF PROJECT

In neuroradiology, intra-arterial, intravenous and intrathecal injections of water-soluble contrast media (CM) are made. With the growing importance of digital and interventional angiography, water-soluble myelography and enhanced computerised tomography (CT) it is essential to have a clear understanding of the effect of such procedures on the nervous system.

The blood, cerebro-spinal fluid (CSF) and extracellular fluid of the brain parenchyma form the fluid compartments of the brain with three interfaces between, namely the blood-brain interface, the CSF-brain interface and the blood-CSF interface. One or more of these interfaces are exposed to water-soluble CM following intra-arterial, intravenous, or intrathecal CM administration during cerebral angiography, CT or myelography.

CM used for diagnostic purposes is probably the greatest and most frequent challenge to the interfaces between blood, brain and CSF. This project is designed to use an animal model, and utilising the ability of CT to demonstrate blood-brain barrier (BBB) disruption and measure changes in tissue density, to extend the knowledge of the pathophysiology of such procedures.

1.2 THE BLOOD-BRAIN BARRIER

1.2.1 Concept of the Blood-Brain Barrier

In most non-neural tissues, the endothelium of the capillary wall allows free passage of ions and poorly fat-soluble non-electrolytes up to the molecular size of albumin, between blood and interstitial fluid (Bradbury 1979). In the nervous system, the situation is very different. The endothelial cells of the cerebral capillaries restrict the entry of many substances from the blood to brain and in many instances, substances fail to equilibrate with the brain tissue water even under steady-state conditions (Katzman & Pappius 1973). This has given rise to the concept of the BBB, which is now known to be a complex physiological phenomenon (Katzman & Pappius 1973, Rapoport 1976, Bradbury 1979).

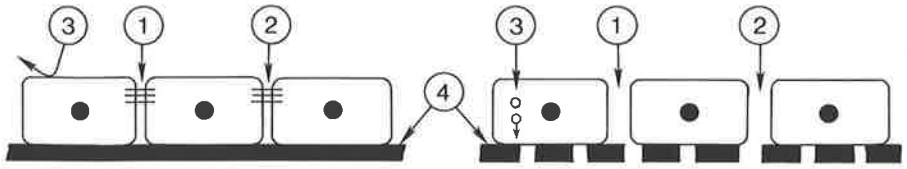
Historically, the concept of the BBB developed from observations that intravenous injections of certain dyes resulted in vital staining of various organs while the brain, except for the choroid plexus, remained unstained (Ehrlich 1885, Goldmann 1909, Lewandowsky 1900). Biedel and Krauss in 1898 also noted that the brain was not stained with bilirubin in cases of jaundice while many other tissues were saturated with the bile pigment. In contrast, Goldmann (1913) noted that Trypan blue introduced directly into the CSF did produce staining of the nervous system. Thus there appeared to be a barrier preventing the escape of dye from cerebral blood vessels into the brain but this barrier could

be circumvented by direct injection into the CSF.

The concept was expanded by Stern & Gautier in 1921 and they first introduced the term BBB. Further studies with dyes (Spatz 1933, Friedmann 1942, Broman 1949), bacterial toxins (Friedmann 1942), ions (Wallace & Brodie 1939), metabolites (Klein et al 1946), and drugs (Underhill & Dimick 1928) added further support to the fact that the permeability of the cerebral capillaries was different from that of other tissues.

1.2.2 Morphological Aspects of the Blood-Brain Barrier

Many authors have reviewed various theories proposed to explain the phenomenon of the BBB (Katzman & Pappius 1973, Rapoport 1976, Bradbury 1979, Friedmann 1942, Tschirgi 1962, Bakay 1956, Dobbing 1961, Lathja 1962, Edstrom 1964, Davson 1967). Four kinds of capillaries are found in the body (Davson 1967, Majno 1965, Karnovsky 1967, Karnovsky 1968). In most non-neural tissues, there appear to be large water-filled channels crossing capillary walls, formed both between adjacent endothelial cells, and by transient fusion of vesicles across their cytoplasm (Figure 1.2.2.1) (Bradbury 1979). The latter phenomenon is known as vesicular transport or pinocytosis. Capillaries in non-neural tissues often have a discontinuous or fenestrated basement membrane and also often have wide intercellular gaps (Figure 1.2.2.1). In contrast, the endothelium of cerebral capillaries, endoneurium, retina and inner ear have a continuous basement membrane, with cells being connected by a continuous belt of tight junctions (Reese & Karnovsky 1967) and vesicular transport



Neural

1. Tight junction
2. Narrow intercellular gaps
3. Paucity of pinocytosis
4. Continuous basement membrane

Non Neural

1. No tight junctions
2. Sometimes wide intercellular gaps
3. Pinocytosis
4. Discontinuous basement membrane

Figure 1.2.2.1. Comparison of neural and nonneural capillary endothelium characteristics.

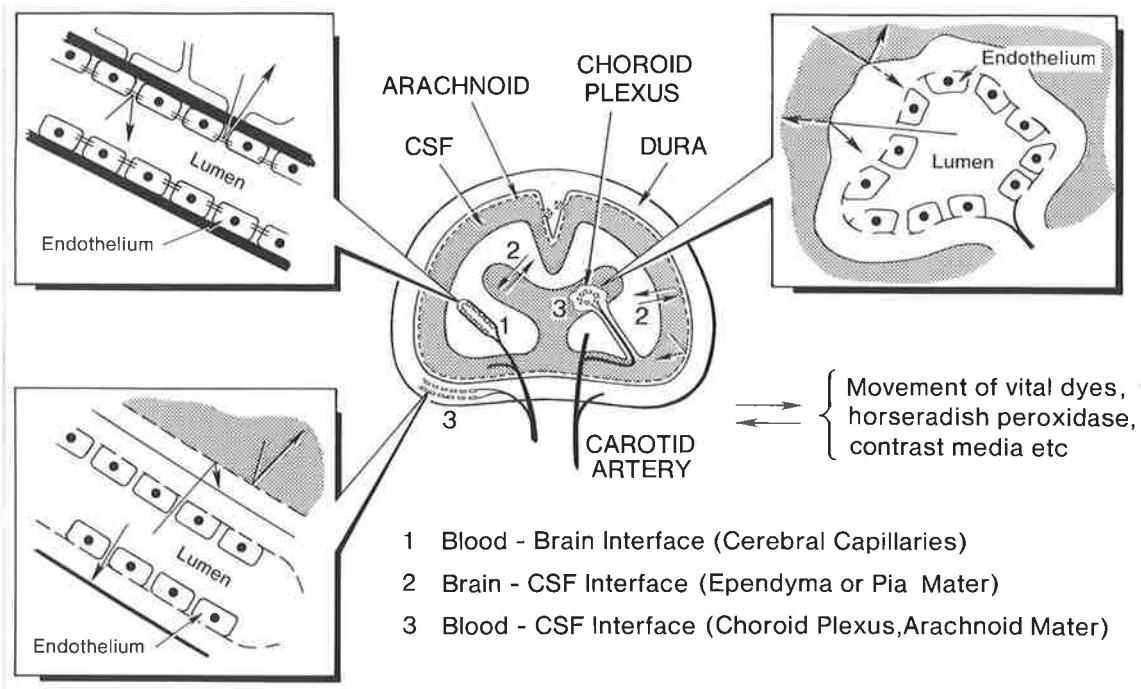


Figure 1.2.2.2. Major compartments of CNS and interfaces between blood, CSF, and brain tissue. Relatively free passage (straight arrows) of vital dyes, horseradish peroxidase and contrast media occurs between CSF and brain tissue and between vessels and tissues of choroid plexus. However such substances are prevented (deflected arrows) from passing freely between cerebral blood vessels and tissues by tight junctions: between dural vessels and CSF by arachnoid mater and between tissues of choroid plexus and CSF by ependyma.

(pinocytosis) is rare (Figure 1.2.2.1) (Majno 1965, Karnovsky 1967, 1968). Because of these morphological characteristics, the endothelium of cerebral capillaries has the permeability properties of an extended plasma membrane (Karnovsky 1968, Reese & Karnovsky 1967, Fromter & Diamond 1972). The continuous tight junctions prevent the passage of protein molecules from the capillary lumen through the endothelium into the extravascular space (Reese & Karnovsky 1967). Both horseradish peroxidase (molecular weight 43,000) and a micro peroxidase (molecular weight less than 2,000) when injected into the blood stream, remain within the cerebral capillary lumen (Reese & Karnovsky 1967, Feder 1971, Brightman et al 1971). On the other hand, when peroxidase is injected into the CSF, the molecule not only penetrates the ependyma and brain parenchyma, but also permeates the clefts between the perivascular glial end-feet, the basement membrane and the clefts between adjacent endothelial cells up to the tight junctions (Figure 1.2.2.2).

Unlike non-neural capillaries, cerebral capillaries have a closely investing glial sheath, composed of the "end-feet" astrocytes (Figure 1.2.2.3). Although the astrocytic end-feet are joined by discontinuous gaps and allow the passage of peroxidase (Brightman & Reese 1969), the close association between the astrocytes and the blood vessels suggest a functional relationship (Bradbury 1979). Davson & Oldendorf (1967) speculated that the physiological tightness of the barrier, namely tight junctions and a lack of pinocytosis, may be due to an inductive influence from such astrocytic end-feet (Bradbury 1979). This hypothesis has never been proven but has much substantial evidence to support it

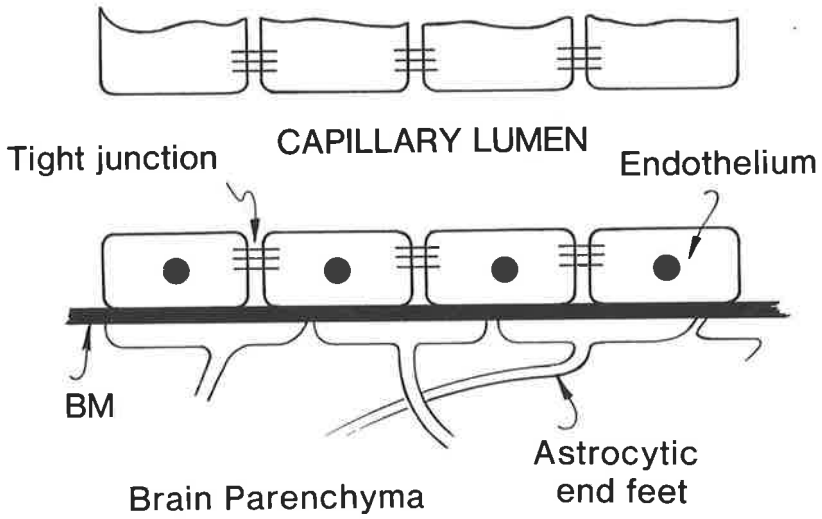


Figure 1.2.2.3. Neural capillary. Closely investing glial sheath of astrocytic "end feet". BM = basement membrane.

(Bradbury 1979).

In non-neural tissues, pinocytic vesicles are important sites for transferring macromolecules out of peripheral vessels (Karnovsky 1967, 1968, Bradbury 1979, Simionescu et al 1975). A characteristic feature of the endothelium of cerebral vessels is the paucity of such vesicles and it has been suggested that this represents another manifestation of the BBB (Katzman & Pappius 1973, Rapoport 1976, Bradbury 1979, Reese & Karnovsky 1967, Westergaard et al 1977, Torack & Barrnett 1964).

The BBB is notable for its resistance to a number of physical and chemical insults but its permeability may be increased in certain specific and fairly extreme circumstances (Bradbury 1979). Despite extensive research, there remains great division in the literature on the ultrastructural basis of any breakdown (Bradbury 1979). It was usually attributed to opening of the tight junctions between the endothelial cells but more recently, increased vesicular transport activity has been suggested. Perhaps different ultrastructural changes occur in response to different insults (Bradbury 1979).

1.2.3 The Properties and Purpose of the Blood-Brain Barrier

The function of the BBB is to maintain the homeostasis of the neuronal environment (Bradbury 1979). The continuity produced by the tight junctions between individual cells allows the endothelium of cerebral capillaries to behave like a plasma membrane (Rapoport 1976, Bradbury 1979, Krogh 1946, Crone 1965). Therefore, free entry and rapid equilibration occurs with substances with a high degree of lipid solubility (Katzman & Pappius 1973), a low degree of ionisation, physiological pH and a lack of plasma protein binding (Mayer et al 1959, Brodie et al 1960). On the other hand, substances poorly soluble in lipids (Klein et al 1946, Davson & Spazianai 1959), substances which are mostly ionized at physiological pH (Bakay 1956, Greenberg et al 1943), and substances bound to plasma proteins (Tschirgi 1950, Brodie & Hogben 1957) enter slowly and do not equilibrate. Being a near perfect semi-permeable membrane, the barrier allows water to move in either direction to maintain an equal osmotic concentration of solutes in cerebral extracellular fluid on the one hand, and in the blood on the other (Bradbury 1979). However, the volume of the brain is controlled in the face of prolonged osmotic disturbances while solutes such as potassium, calcium and magnesium are maintained in very constant CSF concentrations despite severe and prolonged disturbances in blood plasma concentrations (Katzman & Pappius 1973, Rapoport 1976, Bradbury 1979).

Cerebral capillaries are unique in comparison with other capillaries in the distribution of a variety of enzymes (Katzman &

Pappius 1973, Bradbury 1979). A lack of nucleoside phosphatase activity may represent one aspect of the BBB while the entry of glucose into the brain appears to be a mediated process (Klein et al 1946, Geiger et al 1954) and several carrier systems for the uptake of amino acids have been described (Lajtha 1968).

1.2.4 Sites of the Blood-Brain Barrier

The BBB separates the major compartments of the CNS, namely the brain and the CSF, from the third compartment, the blood (Katzman & Pappius 1973, Rapoport 1976, Bradbury 1979). Interfaces between the blood and these two compartments are found in the blood vessels of the brain and the subarachnoid space, the choroid plexus and the arachnoid membrane overlying the subarachnoid space (Figure 1.2.2.2).

Unlike the brain parenchyma, the choroid plexus does stain following intravenous vital dyes but it is the site of active transfer of some substances from the blood to CSF (Katzman & Pappius 1973) and acts as an effective barrier to the diffusion of some lipid insoluble substances (Figure 1.2.4.1). A blood-CSF barrier is sometimes proposed to explain why intravascular substances enter the CSF and brain at different rates (Davson & Welch 1971), but this may simply reflect the gross anatomical relationship between the three compartments (Rapoport 1976).

The ependyma and the pia constitute the brain-CSF interface (Figure 1.2.4.1). The ependyma allows rapid equilibration between the extracellular fluid of the cerebral tissues and the CSF

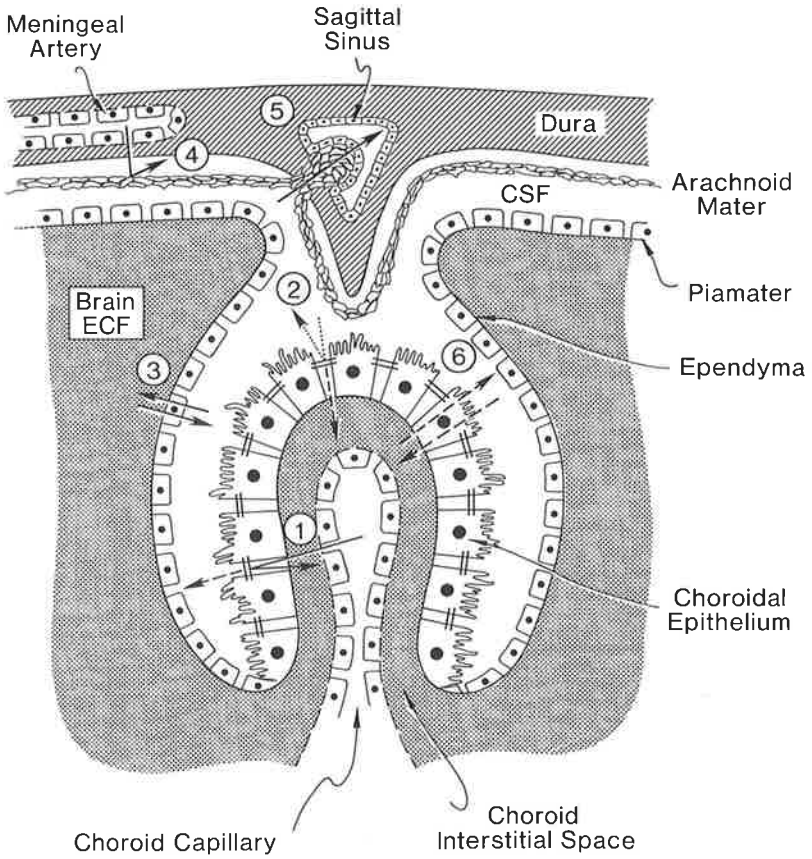


Figure 1.2.4.1. Diagrammatic representation of CSF pathway. Intravascular contrast medium (CM) passes freely through choroidal capillary endothelium but not tight junctions of overlying epithelium (1) (deflected arrow), which also prevent the reabsorption of inwrathecal CM by choroidal plexus (2) (deflected arrow). Intrathecal CM passes freely through ependyma (3) (straight arrows) and piamater by simple diffusion, but reabsorption depends on CSF absorption into arachnoid proliferations (5). Intravascular CM passes freely through fenestrated endothelium of dural capillaries, but is prevented from enwering the CSF by the arachnoid (4) (deflected arrow). CSF secretion and selective solute reabsorption across the choroidal epithelium is an active process (6) (straight arrows).

(Rapoport 1976, Bradbury 1979, Wallace & Brodie 1939, Davson 1955, Fencl et al 1966); the easy passage of horseradish peroxidase and other smaller molecules between the lining ependymal cells has been clearly demonstrated (Rapoport 1976, Bradbury 1979, Brightman & Reese 1969, Brightman et al 1970).

Certain specialised areas of the brain appear devoid of a BBB. These include the choroid plexus (Goldmann 1913), hypophysis (Schulemann 1912), tuber cinereum (Rachmanow 1913), area prostrema (Wislocki & Putnam 1920), paraphysis (Putnam 1922), pineal gland (Wislocki & Leduc 1952), and the pre-optic recess (Mandelstamm & Krylow 1927). Unlike other cerebral capillaries, vessels in these areas appear to have fenestrations, greater pinocytotic activity and differences in enzymes (Torack & Barrnett 1964, Becker et al 1968, Brightman 1968, Hashimoto & Hama 1968). The blood vessels of the dura also have a discontinuous endothelial cell layer and allow rapid diffusion of peroxidase and vital dyes into the tissue (Figure 1.2.4.1) (Rapoport 1976). However, although the three layers of the meninges are all mesodermal in origin, the outermost layer of the arachnoid has capillaries with tight junctions and peroxidase introduced into the CSF will not pass through this outer layer which therefore represents the barrier between the CSF and the mesoderm at this level (Schabo & Maxwell 1971, Nabeshima 1971, Nabeshima & Reese 1972). Similarly, vital dyes injected intravenously stain the dura that covers the arachnoid membrane but do not penetrate into the CSF (Katzman & Pappius 1973).

1.3 CSF KINETICS

1.3.1 The CSF-Brain Interface

The interface between the CSF and the brain is at the pia mater overlying the brain surface and the ependyma lining the ventricular system (Figure 1.2.4.1). While a physiological barrier between the blood and the brain parenchyma, the BBB, has been well documented (Section 1.2) there is not an apparent diffusion barrier between the CSF and the extracellular fluid of the brain (Bradbury 1979, Oldendorf & Davson 1967). No obvious barrier appears to exist at the pia mater and the ependyma, to the passage of small water-soluble molecules (Bradbury 1979, Oldendorf & Davson 1967, Dunker et al 1976, Cserr 1974) and evidence suggests that they enter the brain parenchyma by simple diffusion into the extracellular space (Figure 1.3.1.1) (Winkler & Sackett 1980).

1.3.2 CSF Circulation

In an adult man, the total volume of CSF is about 140mls with 25-35mls in the ventricles and a similar amount in the lumbar sac (Bradbury 1979, Last 1953). The production rate is between 0.4 and 0.6% of the total volume per minute (Davson 1967) or approximately 0.35ml/min (Lups 1954) and about 500mls is secreted over 24 hours, being a slower rate than in most other mammals (Rubin et al 1966). Most CSF is secreted by the choroid plexus

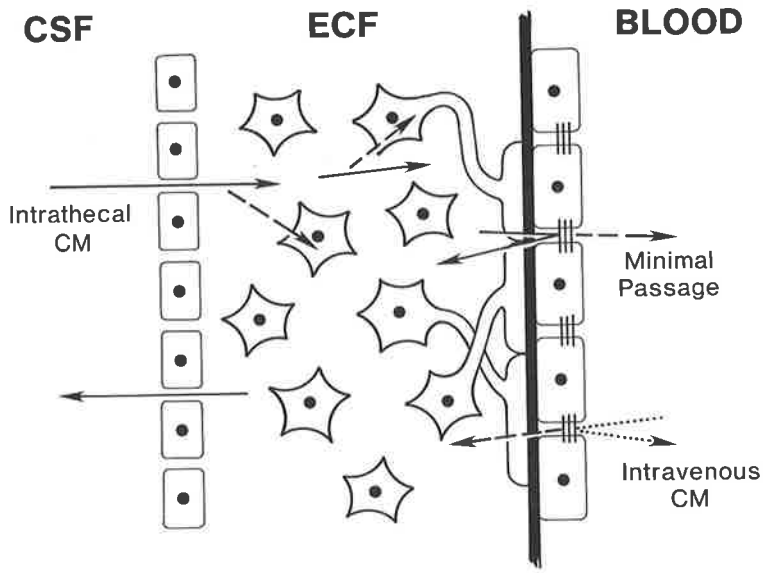


Figure 1.3.1.1. Diagrammatic representation of the relationship of intrathecal and intravenous contrast media (CM) to the CSF-brain and blood-brain interfaces. Free passage (straight arrow): Restricted passage (deflected arrow).

(Bradbury 1979, Potts et al 1977), but the ependyma may contribute a fraction of the total production (Bradbury 1979, Bering & Sato 1963, Pollay & Curl 1967). The movement of CSF is secondary to small hydrostatic pressure changes (Bradbury 1979) with the net formation of CSF by the choroid plexuses plus the bulk absorption into the venous sinuses resulting in a net movement or circulation of CSF (Cutler & Spertell 1982, Katzman & Pappius 1973). Pulsation of arteries in the skull, changes in venous pressure due to respiration and other factors (Williams 1976) and cilia on the ependyma presumably help with mixing but probably only contribute a small amount to actual circulation. There is no evidence to indicate any consistent flow or circulation of CSF within the spinal subarachnoid space (Bradbury 1979). Although there is experimental evidence to suggest a spinal circulation in cats (Grundy 1962), this does not appear to be the case in man (Bradbury 1979, Katzman & Pappius 1973). Isotopes introduced into the subarachnoid space in animals kept immobile in a horizontal position spreads evenly cranially and caudally (Williams 1976). Differences in the venous pressure in the skull and the vertebral column are obviously transmitted to the CSF and therefore changes in posture, coughing and hyperventilation will contribute to the mixing of the CSF (Williams 1976).

Most CSF absorption is by the cranial arachnoid villi and granulations and only a relatively small fraction (15%) occurs through the arachnoid villi and granulations in the spine (Potts et al 1977, Kido et al 1976). CSF appears to pass from the subarachnoid space into the venous system via the arachnoid villi and granulations, through pathways that act as open passages,

large enough to transmit large protein molecules without significant resistance (Figure 1.2.4.1) (Potts et al 1977, Davson et al 1970). A pressure difference of approximately 6.5cms of water normally exists betwen the subarachnoid space and the adjacent venous sinuses (Potts & Deonarin 1973) giving a "driving pressure" which is the major factor in the rate of CSF absorption. The rate of CSF absorption has been shown to increase linearly with CSF pressure once a critical opening pressure of about 70mm of water has been exceeded (Cutler & Spertell 1982). The arachnoid villi and granulations appear to function as valves, permitting relatively free flow of CSF towards veins but not in a reverse direction even if venous pressure is increased (Potts et al 1977, Cutler & Spertell 1982. However the exact mechanism by which CSF and its constituents are absorbed by "bulk" flow through the arachnoid proliferations remains unknown (Cutler & Spertell 1982). Human arachnoid granulations are large and are commonly situated lateral to the superior sagittal sinus in the lacunae laterales (Potts et al 1977). Granulations and villi do occur in the spine in various animals including man (Kido et al 1976,Davson et al 1970), being related to spinal nerve roots where they are surrounded by the small extensions of the subarachnoid space. The relationship between the venous system and the arachnoid granulations in the spine is similar to the cranial structures (Newhouse 1977). In man, spinal arachnoid proliferations occur in relationship to most spinal nerves at all levels throughout the spinal column (Potts et al 1977, Kido et al 1976). The intracranial "driving pressure" between the subarachnoid space and the nervous system appears to remain constant in dogs no matter what position (Potts & Deonarin 1973) and it is probable that the

pressure difference in the spine is also constant in all positions (Potts et al 1977).

1.4 WATER-SOLUBLE CONTRAST MEDIA

1.4.1 Development of Water-Soluble Contrast Media

The evolution of water-soluble CM began in 1923 when faint visualisation of the urinary tract was noted after the therapeutic administration of intravenous sodium iodide (Osborne et al 1923). Since this time, iodine has remained the one element suitable for injection into the circulation as a radiographic contrast agent (Grainger 1982). Sodium iodide, an inorganic iodine salt was far too toxic. The development of organic iodine compounds led to the clinical introduction of Uroselectan in 1929 (Swick 1929), which was soon superceded by two other improved pyridine products, Diodrast (Diodone) and neo-iopax (Iodoxyl, Uroselectan B) (Swick 1929, 1933, Binz 1931). In these early CM,the pyridone nucleus was used to carry one or two atoms of iodine (Figure 1.4.1.1). It was not until the early 1950's that the work of Wallingford (1953) and others (Hoppe et al 1956) led to the first tri-iodinated CM and since then, the benzene nucleus has become the established iodine carrier in modern CM (Figure 1.4.1.2). The basic molecule arising from Wallingford's work was 2,4,6- tri-iodo-benzoic acid. Various substitutions at the 3 and 5 positions are responsible for affecting the solubility and lowering the toxicity of the parent compound (Figure 1.4.1.2). All conventional ionic CM are ionic monomeric salts of tri-iodinated substituted benzoic acid.

The group principally responsible for water-solubility is the carboxylic group at position 1. For water-solubility the carboxyl

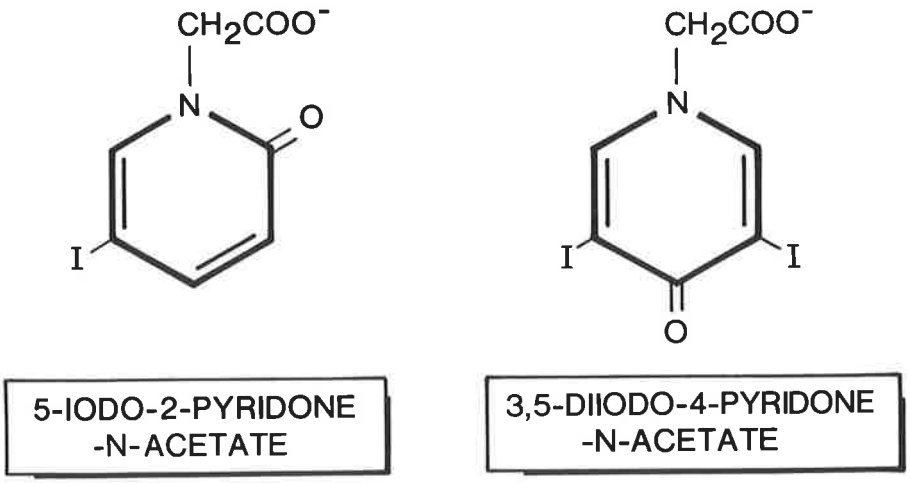
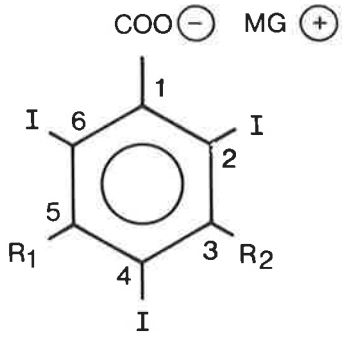


Figure 1.4.1.1. Early mono- and diiodo compounds based on the pyridine nucleus.



2,4,6 — triiodobenzoic acid

R_1	R_2	COMPOUND
H	$-NHCOCH_3$	Acetrizoate
$-NHCOCH_3$	$-NHCOCH_3$	Diatrizoate
$-CONHCH_3$	$-NHCOCH_3$	Iothalamate
$-NHCOCH_2CH_3$	$-NHCOCH_2CH_3$	Diprotrizoate
$-N(CH_3)COCH_3$	$-NHCOCH_3$	Metrizoate

Figure 1.4.1.2. The structure of conventional ionic contrast media, being monomeric salts of tri-iodinated substituted benzoic acid.

group must be present in an ionized form ie. as a salt. The most common salts of CM are the sodium and methylglucamine salts. Methylglucamine (MG) is a highly water-soluble secondary amine, similar in structure to a glucose molecule with the oxygen on C-1 replaced by methylamine (Evill 1980) (Figure 1.4.1.3). MG is not appreciably metabolised by mammals (Ryan et al 1976) and has almost no pharmacological effect apart from its osmotic action. Such ionic CM dissociate in solution to form an anion and a cation; only the anion carries iodine atoms and is radiopaque. The cation has no function except as a solubilising agent.

Modifications to these ionic tri-iodobenzoic salts have been aimed at increasing iodine content and hence radiopacity and/or reducing osmotic activity and hence making them more physiological (Almen 1969). Following the suggestions of Almen (1969) there have been various approaches to this problem. These are:

(a) Linking of two and three tri-iodobenzoic acid derivitives into one molecule which would therefore contain six and nine iodine atoms per molecule respectively (Hilal 1966, 1970, Bjork et al 1969). This led to the development of a dimer of iothalamic acid, iocarmate (Dimer X) (Figure 1.4.1.4).

(b) Replacement of the carboxyl group by a strongly hydrogen bonding, non-ionizing functional group. The removal of non-radiopaque solutes gives a molecule with three iodine atoms and no ionizing group (Almen 1969). This led to the development of metrizamide, iopamidol and iohexol (Figure 1.4.1.5); the so-called non-ionic water-soluble CM.

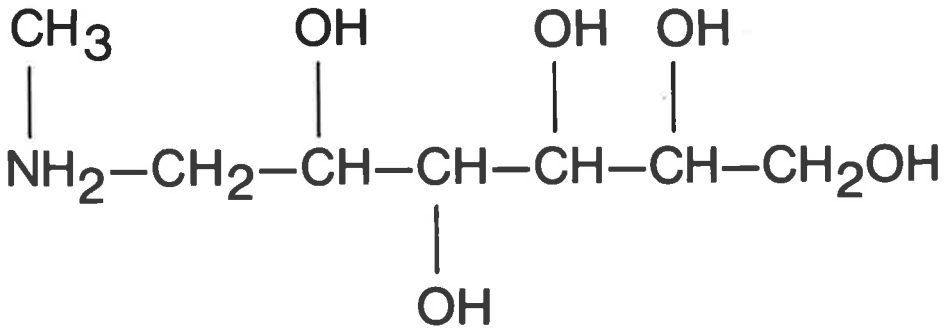
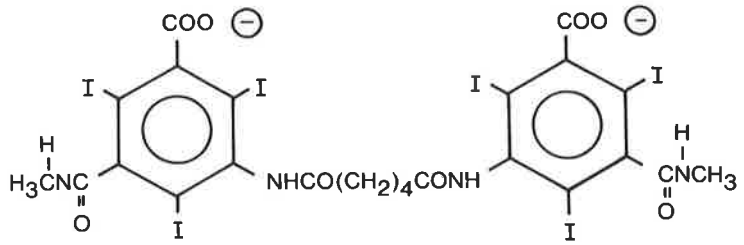
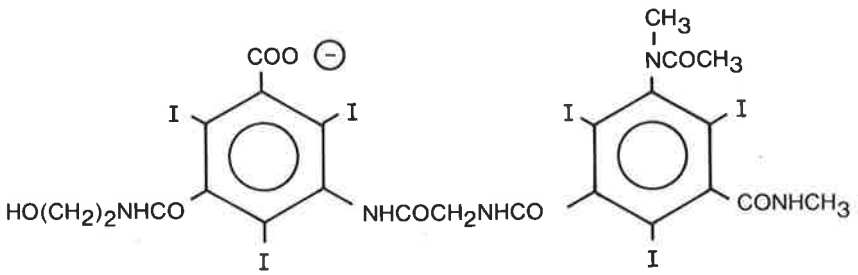


Figure 1.4.1.3. Methylglucammonium ion.



IOCARMATE (Dimer X)



IOXAGLATE (Hexabrix)

Figure 1.4.1.4. The structures of ionic dimer contrast media, with six atoms of iodine per molecule.

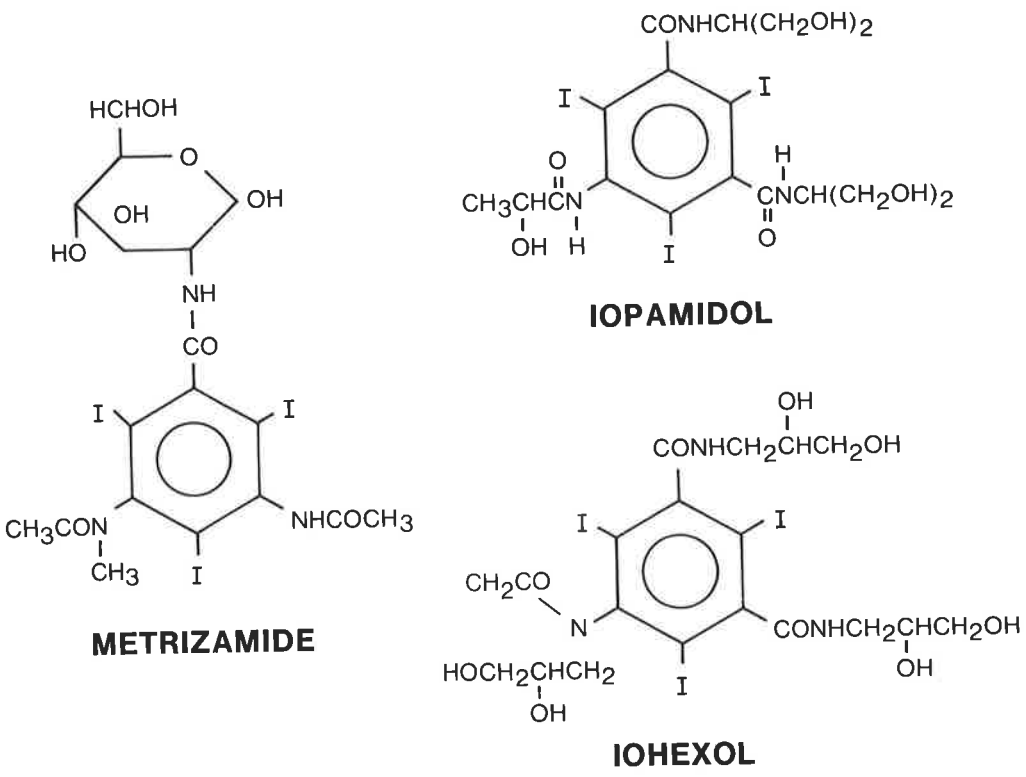


Figure 1.4.1.5. Modern non-ionic contrast media.

(c) Replacement of one of th oups in tri-iodobenzoic acid (Figure 1.2.1.2) by suitably substituted tri-iodobenzene ring to produce ule with six iodine atoms and one ionizing group (.4.1.4). This led to the development of ioxaglate (Hex

1.4.2 Osmotic Activity and Iodine Content of Contrast Media

Ideally, any foreign solution introduced into the body should be isotonic. The osmotic activity of CM is therefore a major factor in determining the response of tissues to its presence. In the following discussion osmotic activity will be described in relation to CM and their iodine content.

Osmotic activity is exerted by every particle in solution. Under "ideal" conditions each solute particle exerts unit osmotic effect regardless of size (molecular weight) or charge. Thus, for any particular mix of solutes and solution the osmotic activity of the solution will be dependent on the total number of solute particles per volume of solvent regardless of the chemical composition of the solution. This situation will be modified by interactions of solute particles with themselves and with the solvent. In determining the osmotic properties of a solution the important relationship is the ratio of solute particles to solvent molecules. Osmotic activity is commonly expressed in milliosmoles per kilogram of solvent (mO/kg). An osmole is defined as 6.023x10-23 osmotically active particles. Hence the osmotic activity of a solution is usually expressed in terms of the number of solute particles for a given weight of solvent rather than for a given volume of solution.

The degree of radio-opacity resulting from CM depends on the concentration of iodine atoms. Iodine concentration in turn depends on the concentration of contrast molecules and the number of iodine atoms which each solution carries. Thus for a given

molecular concentration, a molecule which carries two iodine atoms will give superior radiographic density to a molecule carrying only one iodine atom. The production of osmotically active particles by solvation of a given number of iodine atoms will depend on the molecular structure of various CM. Comparison of the solute particles produced in solution for the solvation of an equivalent number of iodine atoms is given in Figure 1.4.2.1 (Evill 1980). Table 1.4.2.1 summarises the data on solute particles produced in solution by various CM and the number of iodine atoms per molecule (Evill 1980). The ratio of solute particles to iodine atoms in solution is calculated. The lower this value, the lower the osmotic activity will be of CM to give a similar iodine concentration in solution.

All solute particles contribute to the osmotic activity of a particular solution. Conventional ionic water-soluble CM are salts with either sodium or methylgucamine cation. The cations, which do not contribute to iodine concentration and hence radiopacity, do however contribute to the osmolality of these solutions. Non-ionic CM do not require such a cation to ensure water-solubility and they therefore have a lower osmolality in solution than equivalent iodine concentrations of ionic CM (Table 1.4.2.2) (Haavaldsen 1980, Almen & Golman 1979).

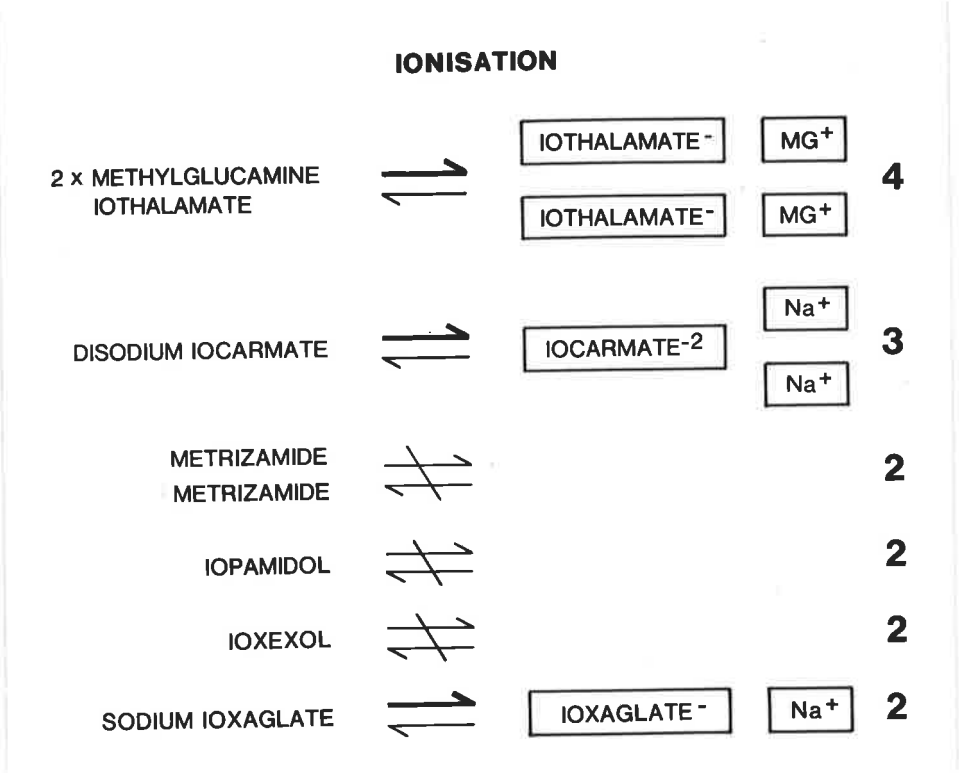


Figure 1.4.2.1. Solute particles produced in solution for the solvation of 6 atoms of iodine with various CM (Evill 1980).

TABLE 1.4.2.1 Solute Particles per Iodine Atom for Contrast Molecules

COMPOUND	IODINE ATOMS PER MOLECULE	SOLUTE PARTICLES PER MOLECULE	SOLUTE PARTICLES PER IODINE ATOM
Iodohippurate	1	2	2
Diodone	2	2	1
Iothalamate	3	2	0.67
Iocarmate	6	3	0.5
Metrizamide	3	1	0.33
Iopamidol	3	1	0.33
Iohexol	3	1	0.33
Ioxaglate	6	2	0.33

TABLE 1.4.2.2 Viscosity and Osmolality of Various Water-Soluble Contrast Media Used in Neuroradiology.

CONTRAST MEDIUM	CONCENTRATION (mgI/ml)	VISCOSITY (37^{O}) (mPa.S)	OSMOLALITY (mol/kg H_2O)
IONIC			
MEGLUMINE IOTHALAMATE	280	4.3	1.46
IOCARMATE	280	7.2	1.04
IOXAGLATE	280	7.5	0.58
NON-IONIC			
METRIZAMIDE	280	5.0	0.43
IOPAMIDOL	280	3.8	0.57
IOHEXOL	280	4.8	0.62

Measurements derived from Haavaldsen J 1980, Cutler & Pollay 1977.

1.4.3 General Kinetics of Intravenous CM

After intravenous bolus injection, there is a rapid distribution of the CM throughout the vascular and extracellular spaces of the non-neural tissue (Kormano 1976). Therefore, peak blood levels are reached almost immediately after injection and there is a rapid fall during the next two minutes as the CM equilibrates between the plasma and the extracellular fluid of the non-neural tissue (Cattell et al 1967). Following this, there is a more gradual fall in plasma level which is related to renal excretion. In normal tissues, practically no water-soluble CM is bound to cell membranes or taken up by the cells. Instead they are rapidly and extensively distributed outside blood vessels into volumes approaching that of the extracellular fluid (McChesney & Hoppe 1957, Kormano & Dean 1976, Newhouse 1977, Dean & Kormano 1977).

When the CM is given as an infusion rather than as a bolus, no rapid peak level of CM in the blood is obtained but instead there is a gradual rise in blood levels until the rate of renal excretion equals that of the rate of infusion (Cattell et al 1967). The plasma level will only be maintained as long as the rate of infusion equilibrates with renal excretion. Once the infusion is stopped, the plasma level will drop in a similar fashion to a bolus injection, depending on the rate of renal clearance (Cattell et al 1967).

As peak blood levels are only maintained for a short time

following a bolus injection, for conventional enhanced CT studies, there appears to be little advantage in giving the CM as an infusion instead of a bolus injection.

1.5 CAROTID ANGIOGRAPHY

Carotid angiography involves the injection of a radiopaque substance directly into the carotid artery. The initial technique was to expose the artery which was then punctured (Moniz 1927). This was followed by percutaneous puncture of the carotid artery (Loman & Myerson 1936, Shimidzu 1937). Percutaneous direct puncture remained the method of choice for carotid angiography and subsequently direct percutaneous puncture of the vertebral artery was also developed (Lindgren 1950).

Following various attempts at indirect catheterisation, percutaneous catheterisation of the cerebral vessels via the femoral artery was eventually developed (Lindgren 1956) and this now remains the universal method of choice for introducing the radiopaque substance directly into the vessels.

Although these developments were important in the evolution of cerebral angiography, the development of a suitable CM was by far the most important single factor. Moniz (1927) initially experimented with strontium bromide for carotid injection and after this proved to be too toxic, he changed to colloidal thorium dioxide (Thorotrast) (Moniz 1931, 1932). However, this produced local tissue damage as well as fibrotic change in the reticulo-endothelial system.

Because of the recognised risks of thorium dioxide, various other substances including colloidal ethyl tri-iodostearate (Vasoselectan) Diodrast (Gross 1939) and other similar preparations of the di-iodized diethanolamine salt type (Perabrodil, Umbradil) were used.

All of these agents proved to be somewhat neurotoxic and were shown to produce significant damage to the BBB (Broman & Olsson 1948, Broman et al 1950).

It was therefore not until the introduction of water-soluble tri-iodinated CM that carotid angiography, using the percutaneous puncture technique, came into more general use. With progressive improvement, the neurotoxicity of such water-soluble CM has been reduced, and research on contrast media continues.

Following the intra-carotid injection of water-soluble CM, various effects have been measured including a fall in blood pressure, a slowing of heart rate and changes in EEG patterns (Fischer 1974). Cerebral blood flow is increased and the cerebral vessels become dilated in the post injection period (Schmidt 1955, Kagstrom et al 1958, Lindgren 1959). Delta activity or generalised slow activity on the EEG related to the dose of injected CM has been reported (Bloor et al 1951) but others have found little change in the EEG (Ingvar 1957).

Complications may follow cerebral angiography and, although these may be due to the surgical technique, anaesthesia, or the clinical state of the patient, complications are sometimes attributed wholly or partly to the CM. A feeling of warmth or subjective distress and even pain at the site of injection (Broadbridge & Leslie 1958) may occur, but sometimes more important complications such as muscle twitching, minor and major convulsions, hemiparesis, transient or permanent hemiplegia, transient decerebrate state, blindness, general deterioration and death may occur (Abbott et al 1952, Brendler &

Hayes 1959, Kendall 1964, Fischer 1974, Howieson 1974). The extensive literature on comparative toxicity of CM in experimental animals was summarised by Fischer (1974). He concluded that for cerebral angiography, the pure methylglucamine agents in 60% concentration are best tolerated when considering ionic water-soluble CM. Evidence was insufficient to indicate an advantage for diatrizoate, iothalamate or metrizoate (Figure 1.4.1.2) as an anion. A direct chemotoxic reaction is suggested by some investigators (Sessions et al 1962, Margolis & Yerasimides 1966) but the hypertonicity of the CM on the BBB is also considered a significant factor (Grainger 1980). Increased permeability of the BBB following carotid injection of various CM has been well documented in both experimental animals (Harrington et al 1966, Jeppson & Olin 1970, Rapoport et al 1974, Salvesen et al 1967, Waldron et al 1974, Waldron & Bryan 1975) and in humans (Sage et al 1981) and the neurotoxicity of such CM is probably related to this (Doust & Fischer 1971, Hilal 1966, Bassett et al 1953).

The normal circulation time from injection of the CM into the common carotid artery to its disappearance from the veins of the brain varies with the individual and averages from 5-7 seconds (Sutton 1969). The angiogram may be divided into four phases. The first, the arterial phase, lasts for 1-2.5 seconds and this is followed by the second or capillary phase which lasts for about 1 second or less. This is rarely clearly defined on the x-ray film as there is usually some late arterial or early venous filling superimposed. The third and fourth phases are characterised by venous filling and last 4 or 5 seconds. They include the early and late venograms respectively. The early venogram outlines the superficial veins of the hemisphere

and the late venogram shows the deep veins.

1.6 ENHANCEMENT IN COMPUTERISED TOMOGRAPHY

1.6.1 The Blood-Brain Barrier and Contrast Enhancement in Computerised Tomography

The BBB renders cerebral capillaries impermeable to iodinated CM (Caille et al 1980b) and therefore, intravenous injection of CM only demonstrates vascular structures. The cerebral parenchyma only shows a slight increase in density since the cerebral blood volume represents only 4-5% of total brain volume (Caille 1980b, Ambrose 1975). Both white and grey matter show a slight increased attenuation in the normal contrast enhanced CT (average enhancement of 1.9HU for grey matter and 1.4HU for white matter (Arimitsu et al 1977)).

The dural vessels are fenestrated and allow passive diffusion of contrast agents into their extracellular space. Therefore, in the normal CM enhanced CT, intense enhancement of the dural folds of the falx and tentorium occurs (Figure 1.6.1.1) (Naidich et al 1977). The choroid plexus vessels also lack a significant barrier between capillaries and the extracellular space and show marked enhancement (Naidich et al 1977). Naidich et al (1977) also report that at times there is a distinct blush of the lateral walls of the lateral ventricles after CM enhancement. The explanation for this is unknown but it may be due to the caudate nucleus and the subependymal veins. It has been shown that an iodine concentration of 1mg/ml raises the average attenuation of a solution by 24 to 30HU (Gado et al 1975, Riding et al 1975,

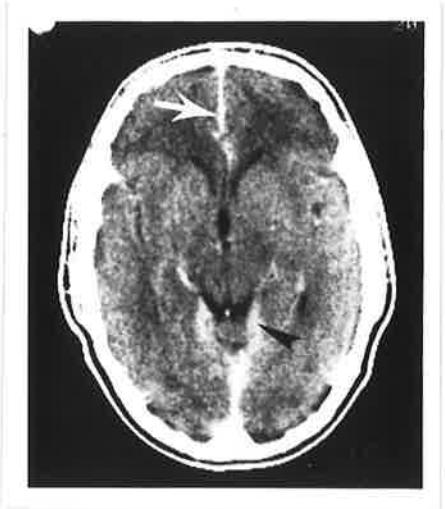
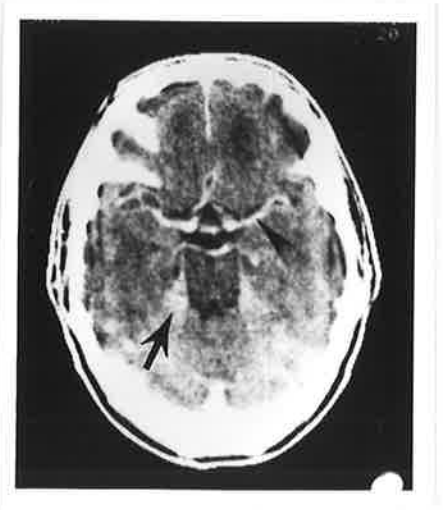


Figure 1.6.1.1. Normal enhanced CT scan. Enhancement of tentorial incisura (arrowhead) and falx (arrow).

Figure 1.6.1.2. Normal enhanced CT scan. Vessels of Circle of Willis (arrowhead) due to iodinated contrast media in blood pool. Normal enhancement of tentorium (arrow).



Kricheff & Lin 1980). The high values of the tissue-blood ratio of enhancement in pathological conditions in CT cannot be explained by intravascular CM alone (Gado et al 1975). Following recent studies (Gado et al 1975, Handa et al 1978), it must be concluded that cerebral CM enhancement by iodinated material in CT is to a great extent a reflection of the integrity of the BBB, except where large vascular channels contain a sufficiently large pool of iodinated material to be visualised directly (Figure 1.6.1.2) (Kricheff & Lin 1980).

Unlike CM, xenon is lipid soluble and passes freely across the normal BBB. Therefore, specific damage to the BBB cannot be detected by xenon enhancement because it passes freely into the normal extracerebral space (Hayman et al 1980). However, because it has high atomic number (Mandelstamm & Krylow 1927), xenon in the extravascular tissue results in enhancement, and experimental studies suggest that such enhancement may have a place in blood flow studies (Drayer et al 1980, Meyer et al 1980). Recently, xenon, as inhaled gas, has been employed for CM enhancement in CT of the brain, spinal cord and other tissues (Meyer et al 1980, Coin & Coin 1980, Foley et al 1978, du Boulay 1980). Theoretical considerations and possible use of further contrast agents other than those incorporating iodine in CT have recently been discussed (du Boulay 1980).

1.6.2 Intravenous CM and the Blood-Brain Interface

In contrast to non-neural tissue, the BBB prevents distribution of water-soluble CM into the brain extracellular fluid following intravenous administration (Section 1.2). Unlike non-neural tissues which show generalised marked enhancement, brain tissue only shows a minimum increase in density, corresponding to its vascular pool, once the dynamic phase is finished (Section 1.6.1). Following intravenous infusion, CM is delivered to the brain via the carotid arteries. Direct injection of CM into the carotid arteries has been shown in certain circumstances to lead to breakdown or disruption of the BBB (Section 1.5). Therefore, the suggested use of high doses of intravenous CM for enhanced CT (Hayman et al 1979) could potentially lead to an increase in the osmolality of blood in the cerebral circulation and hence BBB breakdown. Increased vesicular transport (pinocytosis) has been demonstrated in the cerebral capillaries following intravenous CM in experimental animals (Burns et al 1980). Increased permeability of the BBB has also been demonstrated experimentally using both qualitative Evans' Blue and quantitative CT assessment following large doses of intravenous ionic CM (Zamani et al 1981). Such breakdown appeared to be dose-related and it was suggested that the study raised a question about the advisability of using high doses of ionic CM for enhanced CT.

Normal brain tissue is thought to be protected from intravenous CM by the BBB. However, the abnormal enhanced CT depends on leakage of CM across a damaged BBB into pathological tissue. The pathological brain tissue and, more importantly perhaps, the

adjacent normal tissue is no longer protected from the CM by an intact BBB. The presence of a water-soluble CM within the extracellular fluid of the brain may have in fact a direct toxic effect. Recent reports that the prognosis of cerebral infarction is worse in patients who have undergone enhanced CT (Pullicino & Kendall 1980, Kendall & Pullicino 1980) indicates the need for further consideration of this.

Lalli has suggested that the effect of intravenous CM on areas of the brain without a BBB may explain contrast reactions (Lalli 1980). Certainly, the BBB protects the brain from the effects of CM and the absence of the BBB would presumably allow water-soluble contrast media to be distributed into the extracellular fluid of those areas without a BBB in a similar way to that of non-neural tissue.

1.6.3 Intravenous CM and the CSF-Blood Interface

The interface between the blood and CSF occurs principally in the choroid plexus (Figure 1.2.4.1) (Bradbury 1979), being richly vascularised epithelial tissue that projects into each of the four major ventricles. The choroid plexuses are responsible for the secretion of at least 85% of CSF production and also the clearance of certain solutes such as foreign anions and weak organic acids such as p-aminohippuric acid and prostaglandins from CSF back into the blood (Bradbury 1979, Wright 1979).

Unlike the cerebral capillaries, the capillaries of the choroid plexus are fenestrated and therefore allow ultrafiltration of

plasma (Potts et al 1977, Davson 1967) and the passage of other substances such as CM between the blood and the choroidal interstitium (Figure 1.2.4.1). Because of this, enhancement of the choroid plexuses occurs during CT (Sage 1982, Naidich et al 1977). The interstitium on the other hand is enclosed by a specialised epithelium of cuboidal cells resting upon a thin basement membrane and each cell is joined to its neighbour at the apical surface by tight junctions (Figure 1.2.4.1) (Bradbury 1979, Wright 1979, Potts et al 1977). The epithelium forms an imperfect barrier between the CSF and the choroidal interstitium, restricting the passage of solutes, and of CM in and out of the CSF. Cerebrospinal fluid is secreted by an incompletely understood two step process (Cutler & Spertell 1982, Segal & Pollay 1977). Fluid is filtered through the highly permeable core capillary into the extracellular space and then sodium is actively transported across the choroidal epithelium into the CSF and water follows down an osmotic gradient (Cutler & Spertell 1982). The primary process in CSF production is therefore an active process resulting in secretion of CSF composed of sodium (Na+), chloride (Cl-), bicarbonate (HCO3-), calcium (Ca++) and water from blood, to the ventricles, while at the same time a variety of solutes are absorbed from the CSF back into the blood (Bradbury 1979, Wright 1979). The passage of substances from blood to CSF depends largely on their lipid-solubility (Almen & Golman 1979). Water-soluble CM are highly lipid insoluble and therefore their CSF:blood ratio after intravenous injection would be expected to be low, as only small amounts would be transported into the CSF (Almen & Golman 1979). McClennan and Becker demonstrated a CSF:blood ratio of 0.05 at one hour following intravenous ionic

iothalamate in dogs (McClennan & Becker 1971). A ratio of 0.04 at one hour has been demonstrated following intravenous non-ionic metrizamide in rabbits (Golman & Dahl 1973). These studies indicate that the suggestion that intravenous CT myelography may be possible, is incorrect (Coin et al 1979).

Another potential site of exchange between the CSF and blood is the arachnoid membrane (Figure 1.2.4.1) (Bradbury 1979, Wright 1979). The capillaries of the dura are fenestrated and allow the free passage of CM into the dural extracellular space, producing CT enhancement (Naidich et al 1977). However, the outer-most layers of the arachnoid have capillaries with tight junctions that do not allow the free passage of solutes, including CM and therefore this represents another CSF-blood barrier (Shabo & Maxwell 1971, Nabeshima & Reese 1972). A number of diverse solutes, amino acids and weak bases, are in fact cleared by the arachnoid membrane from the CSF back into blood (Wright 1979).

A secondary site of possible exchange between blood and CSF is via the brain tissue capillaries (Davson 1978). If materials actually escape across the BBB into the brain extracellular fluid they may diffuse freely across the ependyma or pia mater into the CSF. In the absence of disruption of the BBB following intravenous CM this is unlikely but as indicated previously, such disruption may in fact occur to a variable degree, depending on the osmotic load produced by such an injection (Burns et al 1980, Zamani et al 1981).

1.6.4 Pathological Alterations in the BBB and their Relevance to CT Enhancement

1.6.4.1 Intra-Axial Tumours

Normally, the cerebral capillary endothelium has a close investment by a glial sheath, namely the "end feet" of astrocytes (Figure 1.2.2.3) (Bradbury 1979), and the physiological tightness of the BBB may be due to an inductive influence from these astrocytic end-feet (Bradbury 1979, Davson & Oldendorf 1967). Disruption of this glial sheath by mitotic activity may increase the permeability of these capillaries (Bradbury 1979).

Tumours stimulate the proliferation of abnormal capillaries by releasing specific angiogenic factors in the brain (Torack 1961, Long 1970, Folkman 1971) and the nature of these capillaries in glial tumours is not predictable (Caille et al 1980b). In low grade gliomas, such as grade 1 astrocytoma, new capillaries resemble normal cerebral capillaries with maintenance of the BBB and therefore no CT enhancement is demonstrated (Figure 1.6.4.1.1) (Caille et al 1980b). On the other hand, in more malignant tumours, the capillaries are fenestrated with vesicular formation (pinocytosis) and these changes lead to disturbance of the BBB and hence CT enhancement after CM (Figure 1.6.4.1.1) (Caille et al 1980b, Steinhoff & Aviles 1976). Long (1970) suggested that it was more correct to speak of a complete absence of the BBB rather than a

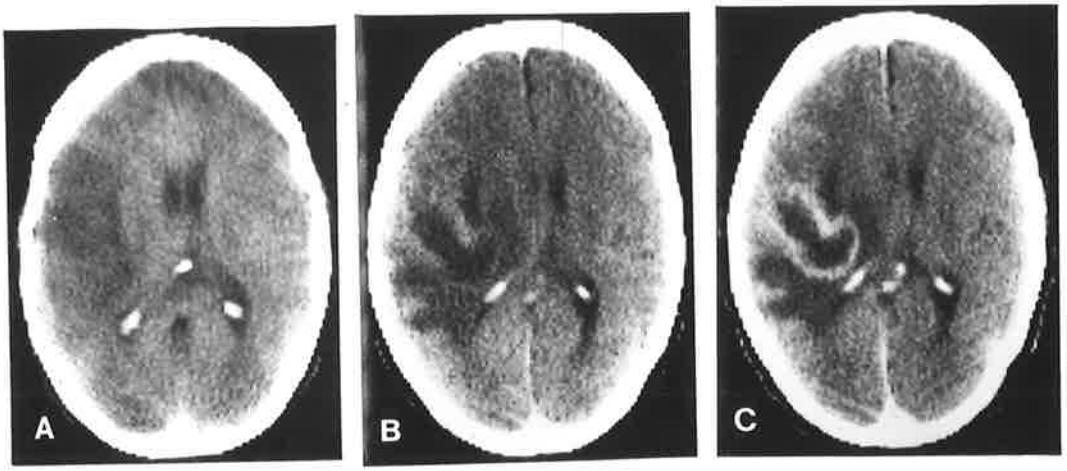


Figure 1.6.4.1.1. A. Enhanced CT scan of a patient with a low grade astrocytoma of temporal lobe. Extensive area of hypodensity but no enhancement becase new tumour capillaries resemble normal cerebral capillaries with maintenance of BBB. B,C. Glioblastoma multiforme. B, plain study. Extensive vasogenic oedema. C, after contrast. Intense enhancement of tumour due to disturbance or even complete absence of BBB.

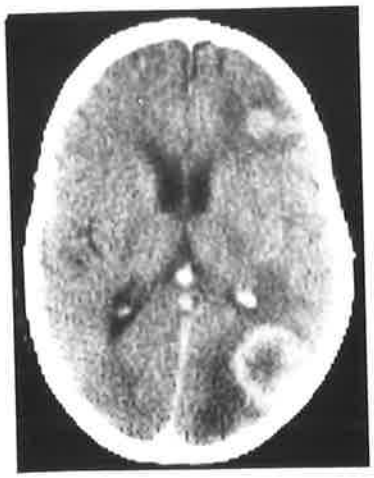


Figure 1.6.4.1.2. Enhancement of two metastatic tumours surrounded by rim of oedema. Proliferation of non-neural capillaries, similar to tissue of origin, allows passage of contrast from blood pool into tumour tissue.

breakdown, in malignant tumours showing endothelial hyperplasia, extensive vesicle formation, and attenuation and irregularity of cells. The basement membrane of such an endothelium is often difficult to define and glial processes are often absent. Fenestrated capillaries have been demonstrated in experimental tumours (Brightman et al 1971) while rupture of the endothelial cells themselves has been postulated with tumour growth (Brightman et al 1972). Barrier breakdown allows protein and other blood solutes to be taken up by the astrocytes and in the case of tumours (Brightman et al 1971b, Raimondi & Evans 1964), this may also upset the postulated influence of the astrocytic end-feet on barrier integrity.

There is no relationship between the vascular structure and the angiographic aspects of a particular tumour on the one hand and enhancement at CT on the other hand (Caille et al 1980b, Gado et al 1975). The major influence on enhancement of such lesions is a change in the BBB due to the factors above. Gado suggested that the vascular pool of angioblastic tumours represents at the best only 20-30% of enhancement (Gado et al 1975).

Metastatic tumours of the brain induce proliferation of non-neural capillaries, characteristic of the tissue of origin. For example, capillaries of cerebral metastatic lymphomas have regular endothelial cells with pinocytotic activity and fenestrations are characteristic of a normal lymph gland rather than of cerebral capillaries (Hirano et al 1974). Such

capillaries have no BBB and therefore show CT enhancement (Figure 1.6.4.1.2). Although the change in the BBB is the most important factor in enhancement, the volume of the extracellular space in both primary and secondary tumours is also important (Gado et al 1975).

1.6.4.2 Extra-Axial Tumours

Meningiomas arise from arachnoid rests in the dura. Because of the mesenchymal origin of such tumours, the capillaries are fenestrated and therefore have no significant BBB. This absence explains the intense enhancement seen, particularly with meningiomas (Figure 1.6.4.2). Similarly, the anterior lobe of the pituitary normally has no BBB and the normal gland therefore enhances uniformly (Syvertsen et al 1979), being isodense with cerebral vessels. Therefore, typically, pituitary macroadenomas appear hyperdense compared to brain tissue following CM enhancement (Caille et al 1980). On the other hand, the density and CM enhancement in prolactin-secreting microadenomas is often less than that of the surrounding normal gland (Syvertsen et al 1979, Cusick et al 1980).

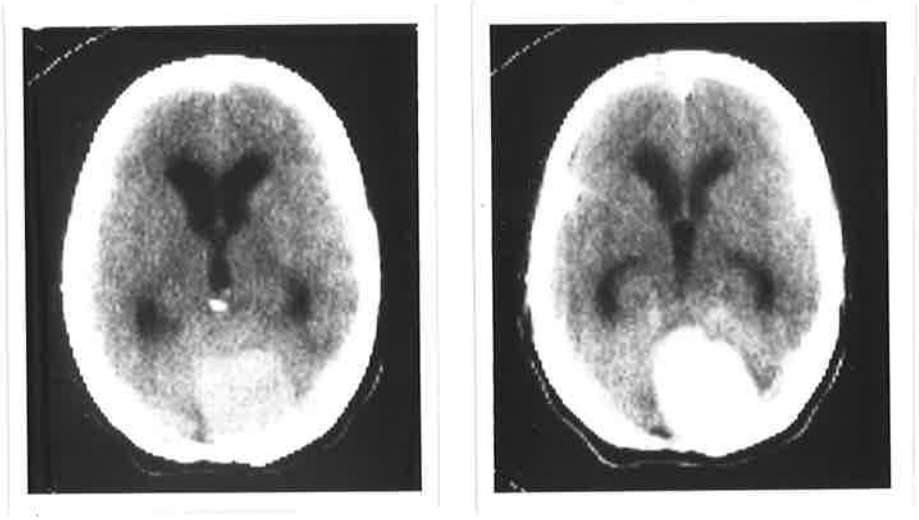


Figure 1.6.4.2. Obstructive hydrocephalus due to meningioma in posterior fossa. Hyperdense lesion in plain study (left) shows intense enhancement after contrast (right), illustrating absence of BBB in these tumours.

1.6.4.3 Hypoxia, Ischaemia and Infarction

Maintenance of the integrity of the BBB in the face of hypoxia and ischaemia has been confirmed by many authors (Bradbury 1979, Eich & Weimers 1950, Bakay & Bendixen 1963). This resistance is attributed to a differential sensitivity to oxygen lack, of the endothelial cells on one hand and neurons and glial cells on the other (Rapoport 1976, Bradbury 1979, Hossmann & Olsson 1971, Broman & Lindberg-Broman 1945). However, eventually, periods of ischaemia lead to focal necrosis and infarction (Ito et al 1976, Blackwood et al 1963), and neurons, glia and capillary endothelia fall out in sequence (Rapoport 1976). Oedema develops at the periphery of the infarcted area, where increased BBB permeability is demonstrated, reaching a maximum at 4-5 days and remaining for 20 days or more (Rapoport 1976). Invading phagocytes eventually remove necrotic tissue which is replaced by a fluid-filled cyst with normal blood vessels (Blackwood et al 1963).

CT findings in acute cerebral infarction have been well documented (Yock & Marshall 1975, Davis et al 1975, Wing et al 1976, Davis et al 1977). Usually no enhancement is seen in the first few days (Inoue et al 1980) but 60-93% of infarcts show enhancement between 7 and 30 days (Yock & Marshall 1975, Davis et al 1975, Inoue et al 1980). After 3-4 months 50% show enhancement (Davis et al 1977, Inoue et al 1980) and it has been observed as late as six months (Norton et al 1978, Masdeu et al 1977). The actual appearance of the enhancement is variable. It may be heterogenous with a finger-like pattern, particularly in the cortical area (Figure 1.6.4.3), or homogenous over the entire low density area demonstrated in the pre-contrast series (Palmers et al 1978). Sometimes localised enhancement is noticed in an area apparently isodense on the

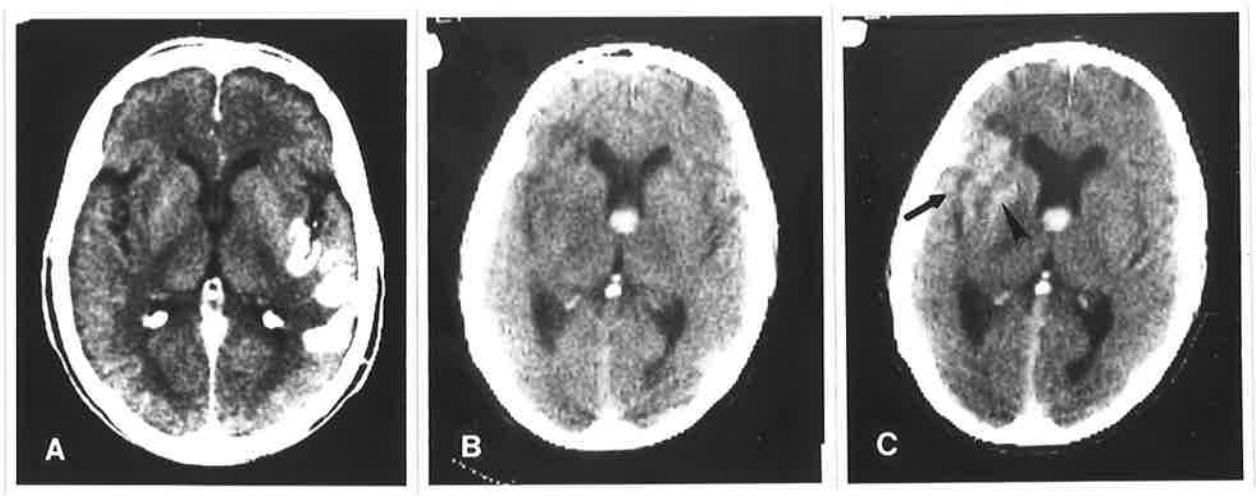


Figure 1.6.4.3. A. Enhanced scan 7 days after cerebrovascular accident. Intense cortical enhancement in area of recent infarction. Luxury perfusion and stasis may be factors in such enhancement transiently but increased permeability of BBB is dominant factor.
B,C. Patient with known colloid cyst who developed left hemiplagia 10 days before. B, plain series. Vague areas of low density in anterior temporal region on right side. B, after contrast. Enhancement of both cortical mantle (arrow) and deep central gray matter of basal ganglia (arrowhead) indicates extensive area of infarction.

pre-contrast scan (Palmers et al 1978).

Close inspection of a post-contrast scan performed soon after injection, shows enhancement corresponding to the irregular margin around the periphery and the normal dense bands inside the area of low density of the pre-contrast scan, corresponding exactly to the cortical grey matter of the cerebral mantle and deep central grey matter (Figure 1.6.4.3) (Hayman et al 1980, Inoue et al 1980). On the other hand, delayed scans 3 hours after CM, show a diffuse spreading of CM and the diffuse pattern is thought to indicate spread of the extravasated CM in the extracellular space (Hayman et al 1980, Davis et al 1977). The patho-physiological basis of enhancement in cerebral infarction still remains speculative but destruction of, or increased permeability of the BBB (Bland 1971, Yamaguchi et al 1971), luxury perfusion (Soin & Burdine 1976) and new capillary growth (Inoue et al 1980, Yamaguchi et al 1971) play a part. Hayman et al (1980) recently investigated iodine in enhanced CT patterns after cerebral arterial embolisation in baboons. No enhancement was demonstrated with total absence of perfusion. While stasis with slow flow demonstrated a transient focal cortical enhancement, and luxury perfusion with increased blood volume and blood flow at the margin of the infarct may result in enhancement, disturbance of the BBB was thought to be the major factor. Usually enhancement due to increased permeability of the BBB obscured enhancement due to luxury perfusion or stasis. Delayed CM scans are therefore necessary to distinguish BBB damage, associated with progressively increasing enhancement, from flow abnormalities, characterised by transient enhancement

1.6.4.4 Inflammatory Diseases

In pyogenic meningitis, bacteria multiply within the CSF or meninges. The blood vessels of the subarachnoid space are involved in the inflammatory reaction, and migration of the polymorphs across cerebral vessels is accompanied by increased vascular permeability to substances like albumin, mannitol and antibiotics (Prockop & Fishman 1968, Som et al 1972). Protein in the CSF is elevated because of this barrier damage. The inflammatory process may spread into the brain parenchyma but the increased barrier permeability appears to be limited to the blood-CSF barrier, as it is usually not possible to demonstrate any enhancement of the CT or isotope uptake on brain scans (Moseley et al 1977). On the other hand, a heterogenous appearance with vaguely defined hyperdense areas following CM has been described (Dupont et al 1978). In tuberculous meningitis, the effect on the BBB is poorly understood and various CT appearances have been described (Moseley et al 1977, Dupont et al 1978).

The CT appearance of brain abscesses has been well documented (Moseley et al 1977, Dupont et al 1978, Stevans et al 1978, Whelan & Hilal 1980, Chiu et al 1977, Enzmann et al 1979). Enhancement is thought to be uncommon in the cerebritis stage prior to capsule formation (Whelan & Hilal 1980, Chiu et al 1977). However, recently in experimental abscess evolution, ring enhancement was seen in the cerebritis stage prior to capsule formation (Enzmann et al 1979). In fact, maximum ring enhancement correlated best with the area of cerebritis, particularly with delayed scans; the diameter of the ring of enhancement decreased as cerebritis receded. Typical ring enhancement is seen in most cases, with some variation in configuration (Figure 1.6.4.4). In the acute

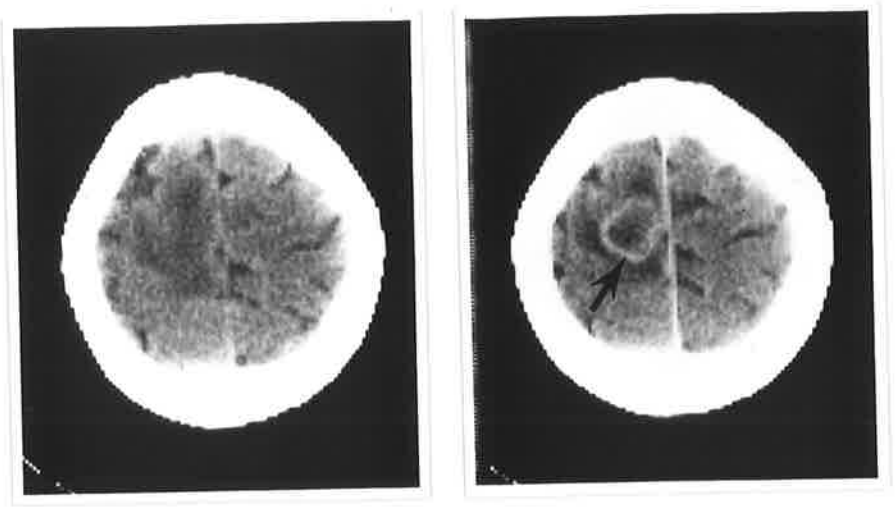


Figure 1.6.4.4. Cerebral abscess in parietal region. Plain study (left). After contrast (right). Typical rim enhancement (arrow) within area of oedema in plain study due to inflammation and capsule neovascularity.

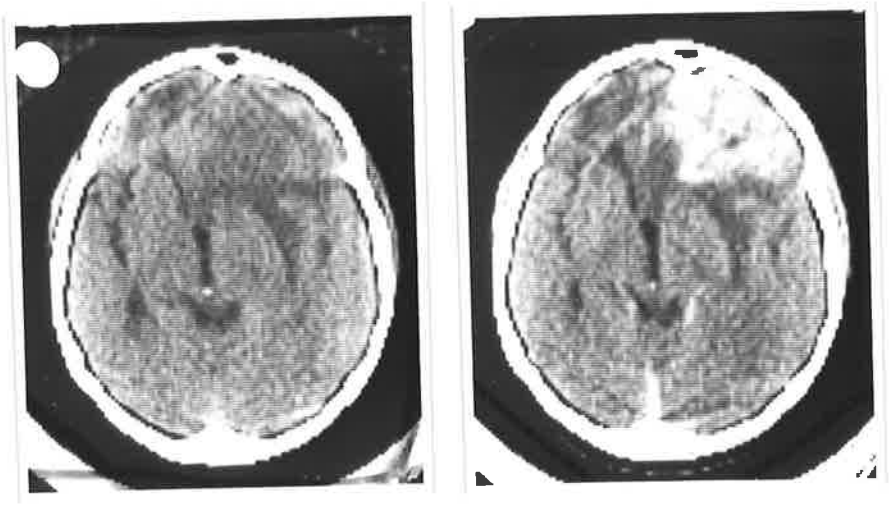


Figure 1.6.4.5. Follow up study after radiotherapy for a glioma of the frontal lobe. Plain (left) and enhanced (right) scans. Significant mass effect, oedema and intense enhancement of the left frontal lobe. At craniotomy there was no obvious tumor recurrence but extensive radiation necrosis was found.

phase, such enhancement is thought to represent inflammatory neovascularity although, later, increased blood flow through the capsule may be a factor. With follow-up, brain abscesses show regression of the BBB disruption as demonstrated by enhancement (Whelan & Hilal 1980). An increase in enhancement after withdrawal of steroids has been observed, perhaps illustrating the protective effect of the steroids on the BBB (Whelan & Hilal 1980).

Much work has been done on the effect of viral encephalitis on the BBB (Rapoport 1976). Experimental encephalomyelitis has been shown to increase the BBB permeability to various markers including vital dyes and horseradish peroxidase (Barlowz 1956).

In uncomplicated cases of viral encephalitis, perivascular cuffs of lymphocytes surround the blood vessels but do not apparently damage the brain or interfere with function. However, in more fulminating infections, lymphocytes penetrate deeply into the brain parenchyma where they destroy myelin sheaths on direct contact and provoke an inflammatory reaction (Austin 1970, Waksman 1960). BBB permeability increases either just before or at the same time as the lymphocyte migration across cerebral vessels (Barlowz 1956).

1.6.4.5 Radiation

Various forms of ionizing radiation have been shown to increase the permeability of the BBB (Bradbury 1979a). Large doses of x-rays and alpha particle irradiation will break down the barrier within 72 hours and produce a severe white matter oedema (Miguel & Haymaker 1965). The total dose needed to produce acute breakdown of the BBB is large (Nair &

Roth 1964).

Lower experimental and clinical therapeutic doses of radiation may not produce an acute effect on the BBB permeability (Levin et al 1979). However, they initiate changes that result in BBB breakdown one month to five years after exposure, and delayed necrosis of the brain following exposure to high doses of radiation is well recognised (Kramer 1968, Kramer et al 1974, Compton & Layton 1961, Mikhael 1978). Brain oedema may develop in conjunction with this increased permeability and lead to swelling of the involved tissues and therefore increased pressure (Mikhael 1978, Brismar et al 1976).

Recently, the CT appearance of such necrosis has been described (Mikhael 1978, Brismar et al 1976). A low density mass is demonstrated which shows enhancement following CM (Figure 1.6.4.5) even when the original tumour before radiation therapy showed no CM enhancement. In the absence of tumour regrowth, radiation necrosis alone may result in significant mass effect (Figure 1.6.4.5) (Mikhael 1978, Brismar et al 1976). Presumably the CT appearance is explained by an increased permeability of capillaries leading to enhancement, while a mass effect results from oedema due to this increased permeability.

Recently it has been suggested that BBB function may be altered by cranial radiation, to allow increased permeability to chemotherapeutic agents such as methotrexate (Griffin et al 1977, Seshadri et al 1979).

1.6.4.6 Brain Trauma and Cerebral Oedema

The accumulation of fluid through oedema may be intracellular, extracellular, or a combination of the two. Cerebral oedema has been divided into cytotoxic and vasogenic (Klatzo 1967, 1972). Cytotoxic oedema is where the primary lesion and accumulation of fluids is in the parenchymatous cells, neurons, and glia and is seen in hypoxic and ischaemic damage. BBB breakdown is therefore unusual and occurs late if at all. Most other types of oedema are vasogenic and are due to excessive exudation of fluid from the cerebral capillaries (Broman 1949). Most insults that rupture the barrier will cause cerebral oedema if applied with sufficient intensity (Bradbury 1979a, Davson 1967). Direct injury to the brain, such as a stab wound, results in local destruction of the brain cells and blood vessels (Hirano et al 1969). The damaged endothelial cells separate from each other, rupturing tight junctions and leaving gaps in capillary linings, while pinocytosis increases in the remaining cells (Rapoport 1976). During this stage therefore, some enhancement with CT would be expected. With healing, phagocytes remove destroyed tissue while proliferating astrocytes lead to the formation of a glial scar. Eventually, after one month, the region is invaded by new normal capillaries with normal barrier properties (Broman 1949, Macklin & Macklin 1920, Bjorklund et al 1969). Therefore in the healed phase, such injuries would not show enhancement.

1.6.5 Miscellaneous Causes of Changes in the Permeability of the BBB

1.6.5.1 Multiple Sclerosis

CT has demonstrated a spectrum of abnormalities in patients with multiple sclerosis, including small focal areas of CM enhancement (Morariu et al 1980, Aita et al 1978, Reisner & Maida 1980, Lebon et al 1978). The CM-enhancing lesions, typically periventricular in distribution, are usually demonstrated in the presence of acute, active or exacerbating disease (Morariu et al 1980, Aita et al 1978, Lebon et al 1978) and represent areas of active demyelination. This association has been pathologically proven (Lebon et al 1978, Wuthrich et al 1976, Davis et al 1976).

Such enhancement presumably results from changes in the integrity of the BBB produced by acute demyelination which allows extravasation of CM (Aita 1980). This is supported by the fact that such lesions are better visualised by the use of an expanded dose of CM (Aita 1980, Davis et al 1979) and also delayed CM examinations (Morariu et al 1980, Aita 1980). With clinical improvement, CM enhancing lesions become isodense (Morariu et al 1980, Aita et al 1978) while corticosteroids may also reduce enhancement (Sears et al 1978), presumably by re-establishing an intact BBB (Morariu et al 1980, Reisner & Maida 1980, Sears et al 1978).

1.6.5.2 Epilepsy

Using vital dyes and radio-active tracers, increased permeability or breakdown of the BBB has been demonstrated after induced convulsions (Bradbury 1979a, Bauer & Leonhardt 1956, Lending et al 1959). The increase in permeability correlates with the duration of seizure activity (Bradbury 1979a), while experimentally, low blood pressure, pentobarbitone (Lee & Olszewski 1961) and glucocorticoids (Eisenberg et al 1970) have a protective effect. The degree of neuronal activity (Sokoloff 1977) and vasodilation (Bradbury 1979b) may play a part in the degree of opening, and all changes are reversible within one hour after cessation of the convulsions (Bradbury 1979a, Lorenzo et al 1972). Structural abnormalities have been identified in the enhanced CT in half of epileptic foci identified by EEG and clinical evaluation, while further lateralisation is possible using the region of interest technique (Oakley et al 1979).

Seizures induced by intravenous CM for enhanced CT have been described (Scott 1980), particularly in the presence of metastases. This may be partly due to the effect of the CM itself; possibly, the underlying lesion alters the BBB and allows CM to leak into the brain parenchyma (Fischer 1980).

1.6.5.3 Disturbance of Normal Auto-Regulation

Experimentally-induced severe hypertension leads to increased permeability of the BBB (Byrom 1954), susceptibility being increased by irradiation (Blomstrand et al 1975a) and reduced by dexamethasone (Blomstrand et al 1975b). In hypertensive encephalopathy, characterised by an acute rise in blood pressure above 200mm Hg, increased BBB permeability and clinical disorders of the nervous system have been described (Rapoport 1976).

Other conditions including hypercapnia, profound metabolic and respiratory acidoses, cerebral concussion and intracranial hypertension may also alter normal autoregulation (Rapoport 1976). Increased luminal pressure results in capillary dilatation and this may widen intercellular tight junctions leading to increased BBB permeability and brain oedema. When autoregulation is restored, changes are reversed.

Similarly, certain other metabolic disturbances may alter the permeability of the BBB in experimental animals, including thiamine deficiency (Manz & Robertson 1972) and porto-caval anastomosis (Zamorra et al 1973), while clinically, heavy metal poisoning such as by lead, may lead to an encephalopathy characterised by increased BBB permeability (Bradbury 1979a).

1.6.5.4 Hypertonic Solutions, Including Contrast Media

Various hypertonic solutions of electrolytes and non-electrolyes have been shown to disrupt or increase the permeability of the BBB, often in a reversible fashion (Chiueh et al 1978, Hasegaiva et al 1979, Neuwelt et al 1980, Pappius et al 1979, Pollary 1975, Rapoport et al 1971, Rapoport et al 1978). Recent studies using hypertonic mannitol suggests that reversible osmotic BBB disruption may increase markedly the delivery of chemotherapeutic agents to the cerebral parenchyma (Neuwelt et al 1980).

Increased permeability of the BBB following carotid injection of various CM has been documented (Harrington et al 1966, Jeppsson & Olin 1970, Rapoport et al 1974, Salvesen et al 1967, Waldron et al 1974, Waldron & Bryan 1975) and the neurotoxicity of such media is probably related to this. This subject will be further investigated in this project.

1.7 MYELOGRAPHY

1.7.1 Myelography

The ideal myelographic medium should have the following characteristics (Shapiro 1975):

1. Thorough miscibility with CSF;
2. Complete re-absorbability from the CSF;
3. Absence of local and systemic toxicity;
4. Pharmacologic inertness;
5. Satisfactory radiopacity.

Following the classic description of pneumoencephalography (Dandy 1919), the use of intrathecal injections of air to diagnose spinal tumours soon followed (Jacobeus 1921, Dandy 1925). The accidental injection of iodized poppyseed oil (Lipiodol) into the subarachnoid space (Sicard & Forestier 1922) led to its use in the localisation of spinal cord tumours and Lipiodol soon supplanted air as the myelographic medium of choice. However, it had many disadvantages including a relatively high viscosity, poor miscibility with spinal fluid, and significant neurotoxicity, particularly to the pia mater. In 1932 colloidal thorium dioxide (Thorotrast) was used for myelography (Radovici & Meller 1933) but this also proved far too toxic and its use was eventually abandoned. Abrodil, a water-soluble organic iodine compound was introduced in 1931 (Arnell & Lidstrom 1931) and it received wide acceptance in Scandinavia because of its ready miscibility with spinal fluid, and its rapid, complete absorption from the subarachnoid space. However, it failed to

achieve universal popularity because of its irritating effect on the meninges and spinal cord.

In 1944, the oily CM, Pantopaque was introduced (Ramsey & Strain 1944), proving considerably less irritating to the meninges than either Lipiodol or Abrodil. However, being an oil, it was not miscible with spinal fluid and necessitated attempts to remove it from the subarachnoid space following each examination. Although occasionally leading to arachnoiditis (Haughton 1978) it became the myelographic medium of choice in the majority of countries, apart from Scandinavia. The poor miscibility of Pantopaque with CSF and the need to remove the CM at the end of the examination, led to the search for a suitable water-soluble CM with a low toxicity. The conventional ionic CM, methylglucamine iothalamate was used (Campbell et al 1964) but this proved to be far too toxic and its use was abandoned. This was followed by the development of methylglucamine iocarmate (Dimer X) and although initial clinical trials were encouraging (Grainger et al 1971) it did not gain universal use, again because of neurotoxicity.

The major breakthrough in myelographic CM, that led to the development of the non-ionic CM metrizamide followed the proposal by Almen (1969). Not only was metrizamide freely miscible with CSF and re-absorbed spontaneously from the CSF, it also proved to be far less toxic than other water-soluble CM. Following clinical trials (Sage et al 1981a), it has become the universal CM for myelography. Subsequently, further non-ionic CM have been developed, including iopamidol and iohexol. It is hoped these will prove even less neurotoxic than metrizamide. A mono-ionized dimer, ioxaglate, has

also been developed. It is important that the kinetics of these new CM within the CSF be clearly

1.7.2 Intrathecal CM and the CSF-Brain Interface

Brain penetration by the water-soluble CM, non-ionic metrizamide, after intrathecal injection has been documented experimentally (Drayer & Rosenbaum 1977, Golman 1973, Dubois et al 1981) and clinically (Drayer & Rosenbaum 1977, Caille et al 1980a, Hammer 1981).

The brain distribution of metrizamide after subarachnoid injection is thought to be primarily extracellular (Golman 1973, Fenstermacher et al 1980). Inulin, horseradish peroxidase, and other lipid insoluble, metabolically inert molecules have been shown to be distributed in the extracellular fluid following subarachnoid administration and for practical purposes do not cross the BBB (Bradbury 1979). They have been shown to pass through the astrocytic end-feet surrounding the capillary endothelium but are prevented from entering the lumen of the capillaries by the tight junctions between the cells (Figure 1.3.1.1). This is likely to hold for the water-soluble CM as long as the integrity of the BBB is maintained.

Certain metabolically active molecules such as cycloserine and 2-deoxyglucose are distributed intra- as well as extracellularly (Caille et al 1980a, Davson 1982) after subarachnoid injection. Although the brain distribution of subarachnoid metrizamide is predominantly extracellular, deoxyglucose is part of the metrizamide molecule (Figure 1.4.1.5) and it has been suggested that metrizamide

may compete with glucose for ultimate penetration into the intracellular space (Caille et al 1980a). After their extensive study, Fenstermacher et al concluded that nothing precise could be said about the intracellular penetration of metrizamide at this stage. However, they did conclude that metrizamide passes through grey matter by simple diffusion, being largely distributed in the extracellular fluid and that back movement across the BBB is small.

Brain penetration by the newer non-ionic CM such as iopamidol has not been well documented.

It has been suggested that the complications following intrathecal metrizamide are directly related to the cerebral concentration reached (Cala 1981, Caille et al 1980, Drayer & Rosenbaum 1977). However quantitative assessment of such penetration has not been carried out. In particular, the rate and depth of penetration is unknown and also the concentration reached in the brain parenchyma is poorly documented.

The neurotoxicity of contrast media is thought to be related to both their chemical structure and osmolality (Section 1.5). Water-soluble ionic meglumine iothalamate (Conray 280) is known to be more neurotoxic than non-ionic metrizamide, at the same iodine concentration.

A comparison between a hypertonic ionic CM, non-ionic CM, and isotonic saline would be appropriate to assess the significance of the osmolality and ionic nature of a particular CM in determining its degree, depth and concentration of brain penetration.

The neurotoxicity of CM within the brain parenchyma would depend on the local reaction of cerebral tissue. In particular, the recent suggestion that significant cerebral oedema may occur (Caille et al 1980a, Cala 1981) requires clarification.

Penetration of CM into the brain parenchyma is thought to cause EEG changes with a frequency depending on the amount administered (Hindmarsh 1977, Drayer et al 1977, Drayer & Rosenbaum 1977, Hammer 1981). A strong correlation between the maximum density of brain penetration and maximum EEG changes has been reported by Drayer et al (1977) but has not been confirmed by others (Hammer 1981). EEG changes have been reported following intrathecal metrizamide in about 50% of patients (Hammer 1981), rarely before 2 hours, frequently after 5 hours and have usually disappeared after 24 hours.

The association between EEG change and clinical morbidity is not understood.

1.7.3 Clearance of Intrathecal Contrast Media

The clearance of intrathecal CM depends on mixing of the media within the CSF and its rate of CSF absorption. Unlike blood to CSF transfer, the transfer of substances from CSF to blood is not related to lipid solubility (Arimitsu et al 1977) but more to simple "bulk flow" reabsorption of CSF, particularly above a certain molecular size (Inoue et al 1980).

As discussed, there appears to be an absence of active circulation of CSF within the spinal subarachnoid space - mixing depending more on variations of venous pressure and diffusion. In monkeys, water-soluble CM injected into the lumbar region is eliminated predominantly directly into the vascular circulation, presumably through spinal arachnoid villi into epidural veins (Norton et al 1978).

Reabsorption of water-soluble CM in humans has been shown to commence almost immediately after lumbar injection (Masdeu et al 1977) and therefore some CM is reabsorbed by the spinal villi and granulations (Nabeshima 1971, Masdeu et al 1977). The transfer is delayed slightly if the patient is maintained in an erect position but is reduced significantly if maintained in a supine position immediately after the examination (Masdeu et al 1977). The slight delay in the erect position is presumably due to the poor mixing of the water-soluble CM within the CSF, allowing it to stay in the lumbar region only and therefore only those arachnoid granulations or villi related to the CM would take part in the reabsorption. In the horizontal position, however, mixing brings water-soluble CM in

contact with the proliferations throughout the spinal canal, and hence, gives more rapid clearance.

Therefore the absorption of CM from CSF to blood is not only dependent on the rate of CSF production and reabsorption but also upon the location and volume injected. Absorption appears to take place through arachnoid proliferations nearest the site of injection (Arimitsu et al 1977).

When mixing is more complete, as occurs during total myelography, the passage of CM into the cranial cisterns is more rapid (Nabeshima 1971).

The effects of leakage at the lumbar puncture site following myelography with water-soluble CM probably depends on the concentration of CM in the fluid leaving from the subarachnoid space (Nabeshima 1971). In view of the role of the spinal arachnoid proliferations in the clearance of water-soluble CM from the CSF, the presence of arachnoiditis would be expected to decrease the rate of excretion. Following repeat water-soluble myelography, the transfer from CSF to blood was slowed in monkeys, presumably due to arachnoiditis affecting elimination (Eldevik et al 1980, Norton et al 1978). In the same animals, this led to an increase in intracranial CM concentrations and a prolongation of the exposure of the lumbar arachnoid to the CM. Meglumine iocarmate was shown to have a more significant effect than metrizamide. In monkeys, dehydration also apears to slow the elimination of CM in the lumbar subarachnoid space (Palmers et al 1978) and dehydration has been shown to be a factor in human toxicity (Bland 1971).

The rate of CSF formation is reduced with hypothermia, alkalosis, and drugs including acetazolamide, frusemide, ouabain, spirolactone, amphotericin and vasopressin (Eich & Weimers 1950, Yamaguchi et al 1971). A drop in CSF pressure of only 4cms of H20 in monkeys has been shown to cause cessation of CSF absorption (Nabeshima 1971, Soin & Burdine 1976) and it is therefore possible in man also, that a small reduction in CSF pressure would reduce the rate of absorption to zero and hence cause stagnation of fluid in the subarachnoid space. Therefore, in myelography, if a large amount of CSF is removed, greater than the amount of water-soluble CM introduced, this could lead to a drop in pressure and therefore a drop in the rate of reabsorption (Nabeshima 1971).

Transfer of water-soluble CM from CSF to blood starts almost immediately after intrathecal injection in humans (Masdeu et al 1977), being slightly delayed in the erect position when compared with the supine position. However subsequent transport is similar with a maximum blood concentration being reached at 1-3 hours with a half life of 3.9 $\pm$ 2.4 hours. There is therefore considerable individual variation in the rate of reabsorption. Two half-lives have been demonstrated because of renal excretion from blood. The velocity of actual transport does not appear to be influenced by the position (Masdeu et al 1977) apart from the initial delay in the erect position. With a mean half-life of 4 hours, the greater part of CM has been transferred to blood by 12 hours after adminstration and less than 5% of CM remains after 24 hours (Masdeu et al 1977). In experimental rabbits, rats and cats 97-98% of CM is cleared in the urine and feces within 48 hours after injection into the cisterna magna (Procrop & Fishman 1968). In humans, most is excreted in the

urine within 2-3 days (Som et al 1972) and less than 5% enters the feces. A very small amount appears to remain in the body being excreted slowly over 7 days (Som et al 1972).

2 MATERIALS AND METHODS

2.1 DEVELOPMENT OF MODEL FOR INTRA-ARTERIAL STUDIES

2.1.1 Animal Preparation

As CT of the brain was to be performed, the dog was selected as the experimental model in preference to a smaller animal in order to limit partial volume effects. Quantitative evaluation of the canine brain by CT has been shown to have a high precision for densitometry both within and among animals (Fike et al 1982).

Studies were performed on anaesthetised adult greyhound dogs of either sex weighing between 18kg and 33kg. Anaesthesia was induced with intravenous pentobarbital (Pentothal) 25mg/kg. The dogs were intubated and anaesthesia was maintained using 1% halothane in a 1:1 mixture of nitrous oxide and oxygen. Respiration was controlled at 8 breaths/min by an Oxford ventilator.

2.1.2 Test Solutions

Table 2.1.2 lists the test solutions used for intra-arterial injection in the preliminary and definitive studies described. Methylglucamine iothalamate is widely used for selective carotid angiography, having an iodine concentration of 280mgI/ml. To obtain a similar iodine concentration, iopamidol and iohexol at a supplied concentration of 300mgI/ml were diluted appropriately with distilled water to a concentration of 280mgI/ml. Metrizamide at a concentration of 280mgI/ml was prepared by adding the appropriate amount of solvent (as indicated by the manufacturer) to the dried preparation.

A solution of 25% mannitol has an almost similar osmolality to methylglucamine iothalamate at a concentration of 280mgI/ml (Table 2.1.2). This was prepared by dissolving anhydrous mannitol in warm distilled water and passing the solution through a 0.22 micron membrane filter to remove particulate matter. The osmolality of the mannitol obtained was checked by freezing-point depression, using an Advanced Osmometer (Advanced Instruments) while the values for water-soluble CM were obtained from references (Haavaldsen 1980, Almen & Golman 1979).

TABLE 2.1.2 Osmolality of Test Solutions

SOLUTION	CONCENTRATION (mgI/ml)	OSMOLALITY (mol/kg H_2O)	VISCOSITY (37°) (mPa.S)
METHYLGLUCAMINE IOTHALAMATE	280	1.46	*4.5
METRIZAMIDE	280	0.43	5.0
IOPAMIDOL	280	0.57	3.8
IOHEXOL	280	0.62	4.8
ISOTONIC SALINE		.3	*1.01
MANNITOL (25%)		1.52	*1.91

Measurements by Haavaldsen J 1980, *Sage & Wilcox 1983.

2.1.3 Selective Canine Angiography

The anatomy of the cranial vessels of the domestic dog was summarized by du Boulay (1974), based on descriptions by others (Hoffman 1900, Daniel et al 1953, de la Torre et al 1959) (Figure 2.1.3.1). The common carotid artery divides into external and internal carotid arteries beyond the origin of the thyroid artery. The external carotid artery is much larger, representing the continuation of the main stem of the common vessel. It gives off extracranial branches including the occipital artery, the lingual artery, the external maxillary (facial) artery, the posterior auricular artery and the superficial temporal artery, and then continues as the internal maxillary artery. Two branches of the internal maxillary artery provide an anastomosis between the external and internal carotid vessels. The middle meningeal branch of the internal maxillary artery not infrequently sends a large ramus anastomoticus to join the extra dural course of the internal carotid artery while the large orbital ramus of the internal maxillary artery within the orbit gives off an arteria anastomotica which turns backwards into the head to join the internal carotid artery.

Although the internal carotid artery often shows a dilated infundibulum at its origin, it quickly narrows down to a small vessel, particularly when compared with the larger external branch. It runs cranially to reach the medial wall of the tympanic bulla, receiving the anastomotic ramus of the middle

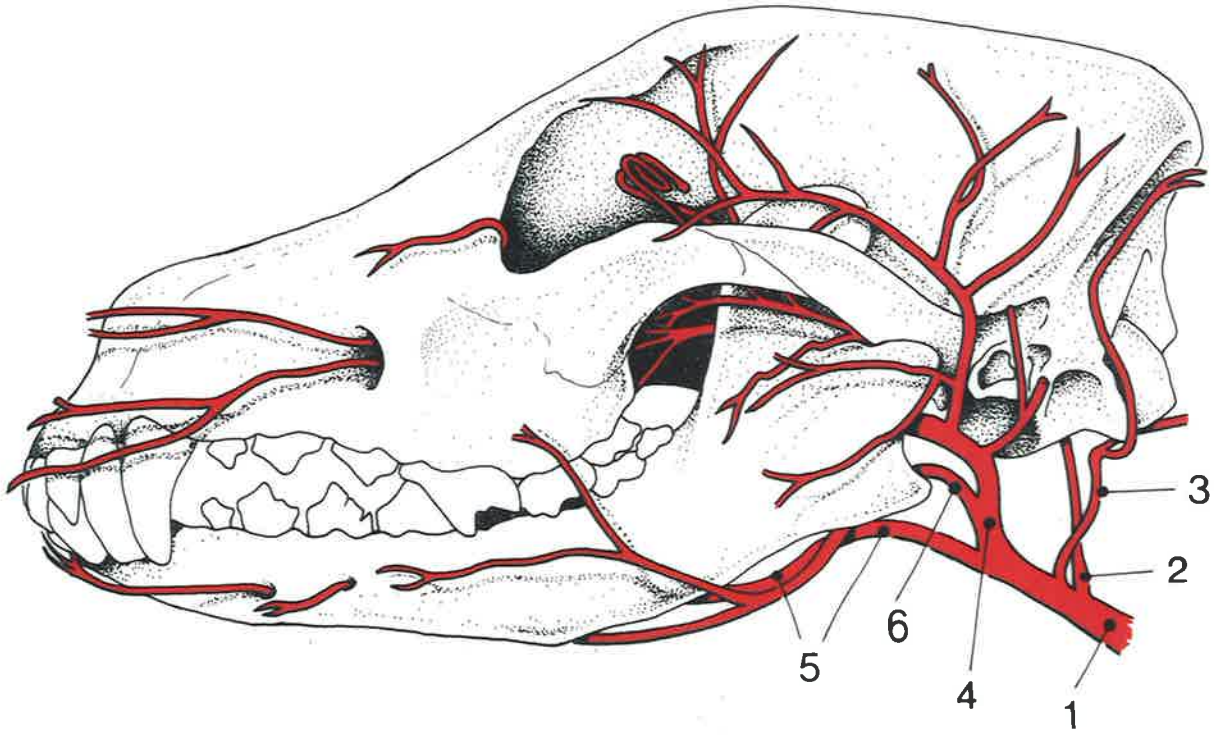


Figure 2.1.3.1. 1. Common carotid artery. 2. Internal carotid artery. 3. Occipital artery. 4. External carotid artery. 5. Lingual artery. 6. Facial artery. (Popseko, P. Atlas of Topographical Anatomy of Domestic Animals. Philadelphia, W.B. Saunders, 1977; 192).

meningeal artery at the apex of the petrous pyramid and shortly afterwards it is joined by the arteria anastomotica (Figure 2.1.3.1).

The cerebral internal carotid artery divides into a large cranial artery and a similar caudal branch after perforating the dura. The cranial branch, after giving off a relatively large internal opthalmic branch, sends out the large artery of the sylvian fissure (the middle cerebral artery), and finally continues as a narrow anterior cerebral artery to join the other side via a short wide anterior communicating artery.

The caudal branch of the internal carotid artery joins its opposite number to form the basilar artery, giving off along the way numerous branches including the posterior cerebral artery.

Five preliminary angiographic studies were performed to gain an understanding of the canine cerebral circulation, and to establish a technique which would allow selective perfusion of one internal carotid artery with test solutions. Selective catheterisation of the internal carotid artery via the femoral artery is difficult because of the narrow lumen of the internal carotid artery. Direct dissection of the carotid artery was therefore performed and retrograde catheterisation of the lingual artery was carried out using 1mm i.d. polyethylene tubing (Dural Plastics, Dural, N.S.W.). The tip of the catheter was manipulated to the level of the bifurcation of the common carotid artery so that it was opposite the infundibulum of the internal carotid artery. Following this, the thyroid artery and the external carotid artery distal to the lingual artery were identified and ligated. Thus, apart from the lingual artery containing the catheter, the internal carotid artery remained the only patent branch of the common carotid artery.

Angiography confirmed that this technique resulted in perfusion of the internal carotid artery without the risk of occlusion of the lumen of that vessel.

Previous studies (Neuwelt et al 1979, 1980) had demonstrated that an intracarotid injection of test solutions at a rate of 1.5mls per second for a period of 30 seconds was sufficient to blanch the cortical surface in dogs. Using a trephine technique, the cortical surface was observed during three preliminary angiographic studies and isotonic saline injected at this rate was shown to blanch the cortical surface in all three animals. It was therefore decided to use this rate of injection in the definitive studies.

In several initial angiographic studies using this injection rate of 1.5mls/sec., CM passed retrograde down the common carotid artery with some resultant reflux into the contralateral side. For the definitive studies, it was therefore decided to occlude the lumen of the proximal common carotid artery for the duration of the injection of test solutions i.e. 30 seconds.

2.1.4 Computerised Tomography of Canine Brain

Contrast enhancement demonstrated by CT reflects increased permeability or disruption of the BBB (Section 1.6). Sixteen preliminary studies were performed to develop a technique for obtaining CT scans of the canine brain after removal. A special perspex container (Figure 2.1.4.1) was constructed to allow the brain to be suspended in water for the duration of scanning. The container was positioned in the CT scanner (EMI model 5005) to allow coronal scans to be performed at 1cm

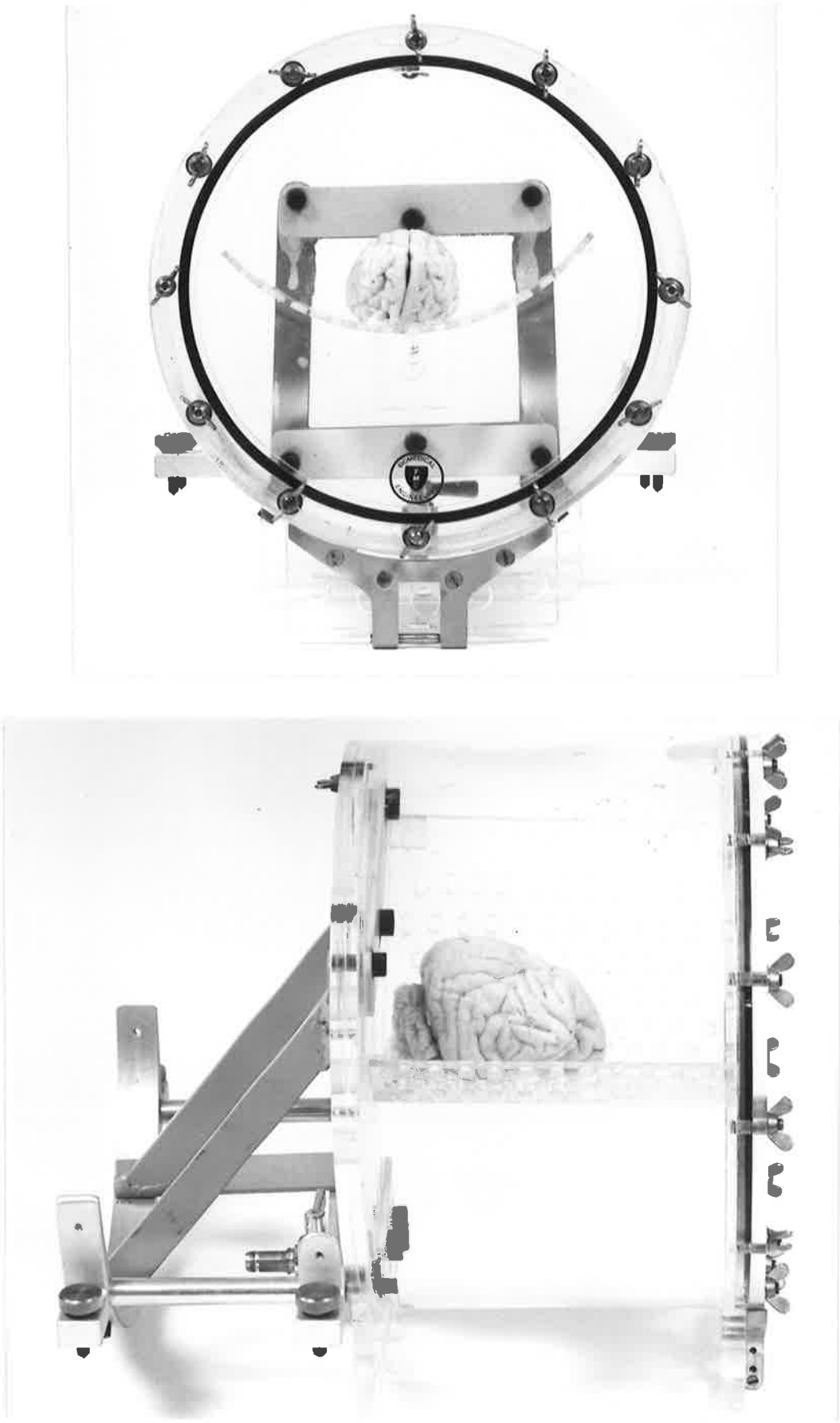


Figure 2.1.4.1. Special perspex container to allow suspension of brain in water to enable coronal CT scans to be performed.

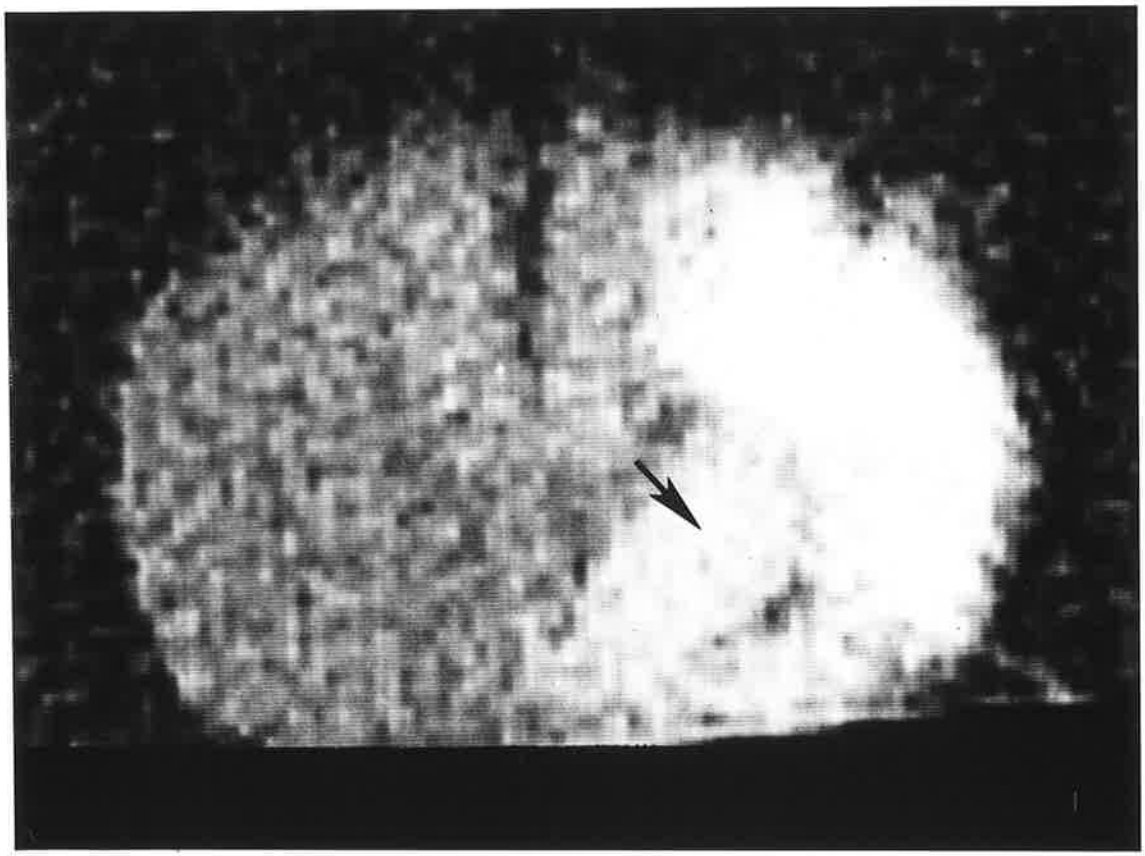


Figure 2.1.4.2. Coronal CT scan of canine brain after intracarotid of methylglucamine iothalamate and intravenous injection of sodium iothalamate. Intense enhancement in the distribution of the middle cerebral artery, including the basal ganglia (arrow) is demonstrated.

13mm collimation (Figure 2.1.4.2). Prior to each examination, the CT scanner was calibrated, using a water bath to ensure accuracy and consistency of attenuation measurements.

In conventional human studies, intravenous water-soluble CM is given to demonstrate any increased permeability or breakdown of the BBB (Section 1.6). Therefore, in the canine model, immediately after the intracarotid injection of saline or mannitol, sodium iothalamate (Conray 420, May & Baker Ltd.) was administered intravenously at a dose of 1.6ml/kg. Since carotid injections of water-soluble CM contributed to the total plasma iodine concentration compared to saline or mannitol, the intravenous dose of sodium iothalamate was reduced in these studies by an amount sufficient to achieve an equivalent total iodine dose for body weight for all solutions. In all studies a second intravenous injection of sodium iothalamate (1.6ml/kg) was administered 20 minutes after the first injection to maintain plasma iodine levels. If increased permeability or disruption of the BBB resulted from intracarotid injection of the test solution, circulating CM would cross the BBB into the brain parenchyma in the areas of breakdown. This CM would be demonstrated as an area of increased attenuation (enhancement) in the coronal scans of the brain performed after removal (Figure 2.1.4.2).

2.1.5 Assessment of BBB Disruption by Evans' Blue Staining and CT Enhancement

It was proposed to use both qualitative and quantitative assessment of BBB disruption, by Evans' Blue staining and CT enhancement respectively. Sixteen preliminary studies, four with isotonic saline and six with each

of methylglucamine iothalamate and 25% mannitol were therefore performed to establish these methods of assessment and their relationship to each other.

2.1.6 Evans' Blue Assessment of BBB Disruption

Historically, the concept of the BBB developed from observations that intravenous injections of certain dyes resulted in staining of various organs while the brain, except for the choroid plexus, remained unstained (Ehrlich 1885, Goldmann 1909, Lewandowsky 1900). Since those early observations, the acid azo dyes trypan blue and Evans' Blue have been used experimentally to demonstrate increased permeability or disruption of the BBB (Rapoport et al 1972, Chiueh 1978, Neuwelt et al 1979, 1978). In the preliminary studies, 3mls/kg of Evans' Blue solution (2% in 0.9% saline, membrane filtered) was administered intravenously 5 minutes prior to intracarotid injections. Following removal of the brain, its surface was rinsed with Hartmann's solution and then inspected for evidence of any Evans' Blue staining. Following the CT examinations, (Section 2.1.4) coronal sections of the brain were cut in the same plane and at corresponding levels to the CT scans. Qualitative assessment of intensity and distribution of staining in the brain sections was performed by two independent observers. Although certain researchers have used up to 10 grades of Evans' Blue staining in experimental animals , the preliminary experiments indicated that a smaller number of grades led to less observer variation. Therefore visible staining in the distribution of each of the anterior, middle and

posterior cerebral arteries and also in the basal ganglia was graded on a scale from 0-3 as follows (Figure 2.1.6):

0 No staining

\+ Just visible staining

++ Easily visible diffuse staining

+++ Diffuse dark staining.

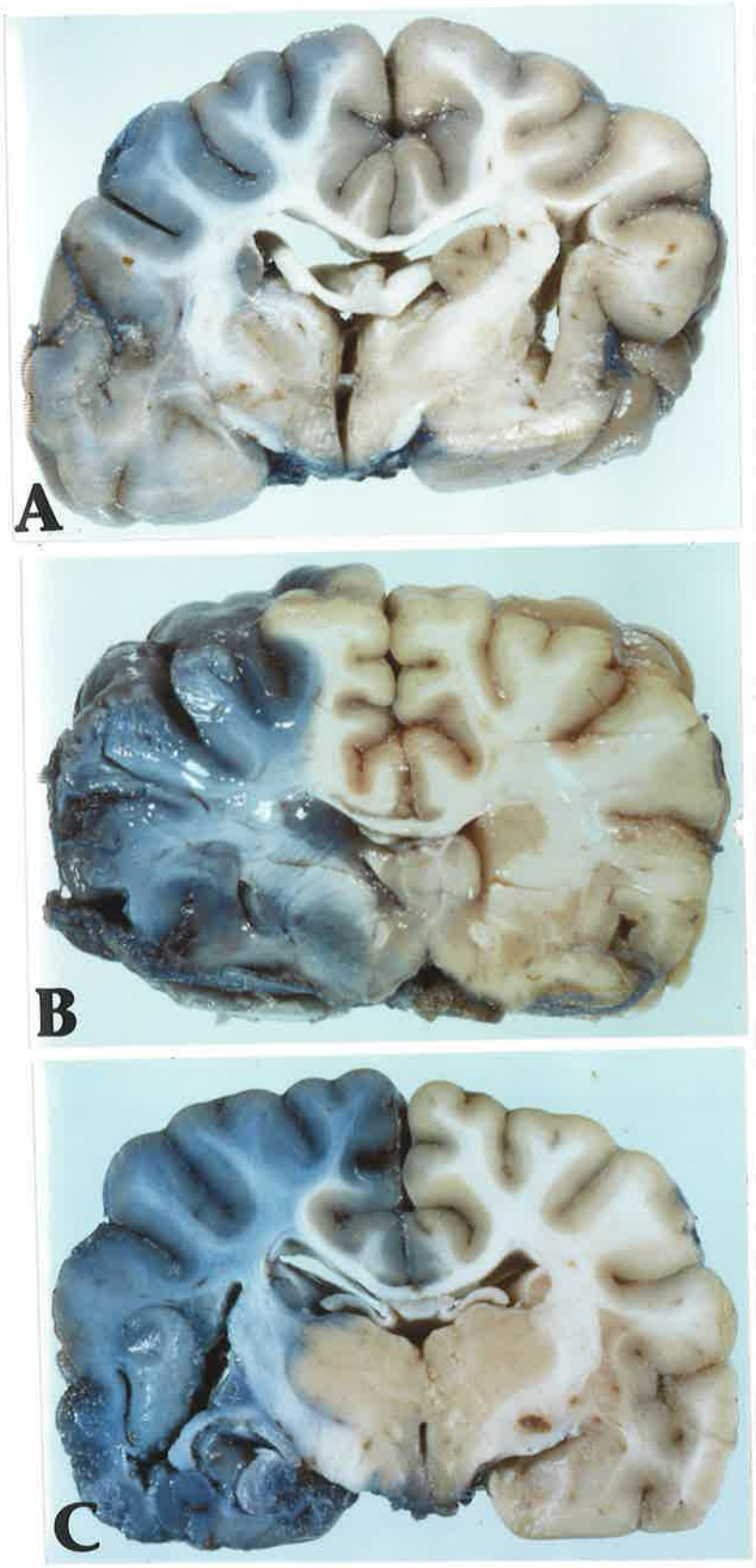


Figure 2.1.6. Coronal sections of three canine br after intracarotid injection of methylglucamine iothalamate on side. Evans' Blue staining, indicating BBB disruption is demons d. A. Grade I staining (+); B. Grade 2 staining (++) in dis tion of middle cerebral artery with sparing of the anterior cere artery (arrow); C. Grade 3 staining (+++).

2.1.7 CT Assessment of BBB Disruption

CT scans were performed as described in Section 2.1.4. Printouts of EMI numbers for the sections of interest were obtained. The region of interest was located and an area consisting of 100 pixels was selected. From this the mean numerical value (EMI units) was determined. In all studies, the injected hemisphere was compared with the contralateral hemisphere. Thus, any contribution from the intravenous water-soluble CM would be cancelled out.

The mean difference between the injected and control hemispheres represented the mean density of CM that had crossed the disrupted BBB. Previous studies by others have indicated that there is a linear relationship between CT attenuation values and iodine concentrations up to 10mgI/ml (Wilson & Evill 1981). Thus it is possible to calculate the CM concentration in the brain.

2.1.8 Distribution of BBB Disruption

The degree and distribution of Evans' Blue staining in the twelve preliminary studies is given in Table 2.1.8. Whenever staining was demonstrated, the area of the distribution of the middle cerebral artery was always involved . On the other hand, variable staining of the anterior cerebral and posterior cerebral artery distributions was observed. This presumably reflected variation in the Circle of Willis in individual animals. For this reason, the distribution of the middle cerebral artery was selected for comparison, between the injected and control hemispheres in the definitive studies.

TABLE 2.1.8 Intensity of Evans' Blue Staining of Cerebral Parenchyma in the Distributions of the Anterior (ACA), Middle (MCA) and Posterior (PCA) Cerebral Arteries and the Basal Ganglia in Preliminary Studies

INTRACAROTID SOLUTION		STUDY NO.	ACA	MCA	PCA	BASAL GANGLIA
METHYLGLUCAMINE IOTHALAMATE	PRELIMINARY STUDIES	1	0	+++	0	++
		2	++	+++	0	++
		3	0	++	0	+
		4	+++	+++	+	+++
		5	+	++	0	++
		6	+	+++	0	++
MANNITOL 25%	PRELIMINARY STUDIES	7	0	0	0	0
		8	++	++	++	++
		9	0	+	0	+
		10	0	++	0	0
		11	+	+	+	0
		12	+++	+++	+++	+++

* No staining was observed with isotonic saline (4 studies)

2.1.9 Relationship Between BBB Disruption by Evans' Blue Staining and Contrast Enhancement

In the preliminary studies, the degree of Evans' Blue staining in the distribution of the middle cerebral artery on the injected side, was compared with the mean attenuation number in a region of interest in the same area. The mean for the control side was subtracted from the mean for the injected side to produce a number indicating the amount of CM iodine within the brain parenchyma (enhancement). Figure 2.1.9 shows the mean attenuation number in the region of distribution of the middle cerebral artery in relation to the intensity of Evans' Blue staining assessed visually, in the same region of sectioned dog brain.

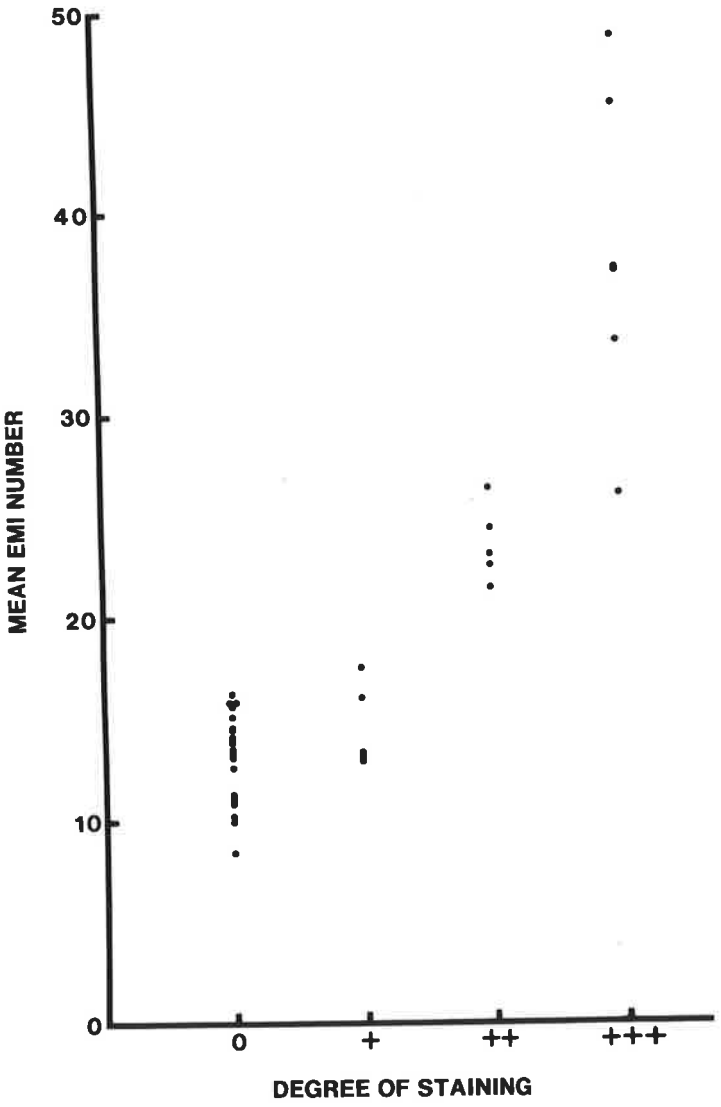


Figure 2.1.9. Mean EMI number in the region of the distribution of the middle cerebral artery in relation to intensity of Evans' Blue staining assessed visually in the matching region of sectioned dog brain.

2.2 DEFINITIVE INTRA-ARTERIAL EXPERIMENTS

2.2.1 Definitive Experimental Model

Studies were performed on adult greyhound dogs of either sex weighing between 20kg and 32kg. Anaesthesia was induced as described in Section 2.1.1.

The surgical preparation was carried out as described in Section 2.1.3.

In all studies both Evans' Blue (visual) and CM iodine were used as markers of BBB disruption. Three millilitres per kg of Evans' Blue solution (2% in 0.9% saline, membrane filtered) was administered intravenously 5 minutes prior to intracarotid injections.

Intracarotid injections of test solutions were administered at body temperature at a rate of 1.5ml/sec for a period of 30 seconds. For the duration of the injection the common carotid artery was occluded to prevent retrograde flow ad ensure delivery of the test volume into the internal carotid artery. Immediately after the intracarotid injection of saline or mannitol, sodium iothalamate (Conray 420, May & Baker Ltd.) was administered intravenously at a dose of 1.6ml/kg. Since carotid injections of water-soluble CM contributed to the total plasma iodine concentration compared to saline or mannitol, the intravenous dose of sodium iothalamate was reduced in these studies by an amount sufficient to achieve an equivalent total iodine dose for body weight for all solutions. In all studies a second

intravenous injection of sodium iothalamate (1.6ml/kg) was administered 20 minutes after the first injection to maintain plasma iodine levels.

Forty minutes after intracarotid injections the animals were killed by an intravenous injection of 10-15mls of saturated potassium chloride solution. The brain was then removed and suspended in water in a sealed, cylindrical, perspex container and positioned in the CT scanner (EMI model 5005). Coronal scans were performed at 1cm intervals , using 13mm collimation.

The coronal CT sections were assessed for evidence of enhancement and quantitative measurements were made. The mean EMI number was calculated in a region of interest of 100 pixels in the distribution of the MCA in the control and injected hemispheres. The mean from the control side was subtracted from the mean for the injected side to produce a number indicating the amount of CM iodine taken up by the brain. For each injected solution the mean difference $\pm$SD mean was calculated for each series of studies. The mean differences were then compared by "Student's" t-test.

After obtaining CT scans the brain was placed in 10% buffered formalin overnight, prior to sectioning. Initial experience had shown that fresh brain tissue was too difficult to cut accurately and that formalin preservation caused only superficial leaching of stain during this period. Sections were cut in the same plane and at corresponding levels to the CT scans. Qualitative assessment of intensity and distribution of staining in the brain sections was performed by two independent observers. Visible staining in the

distribution of the middle, cerebral arteries, and also in the basal ganglia, was graded on a scale of 0-3 (Section 2.1.6).

2.2.2 Definitive Studies

Two series of studies were carried out:

Series I. Using 12 dogs, four studies with each of methylglucamine iothalamate (280mgI/ml), 25% mannitol and isotonic saline were carried out.

Series II. Using 18 dogs, six studies with each of methylglucamine iothalamate, metrizamide, iopamidol, and iohexol at an iodine concentration of 280mgI/ml were carried out.

2.3 DEVELOPMENT OF MODEL FOR INTRATHECAL STUDIES

2.3.1 Animal Preparation

Preliminary animal preparation and anaesthesia was as described in Section 2.1.1.

2.3.2 Test Solutions

Table 2.3.1 lists the water-soluble CM used for intrathecal injection in the studies described. Methylglucamine iothalamate has an iodine concentration of 280mgI/ml. To allow comparison, iopamidol at a supplied concentration of 300mgI/ml was diluted appropriately with water to a concentration of 280mgI/ml while metrizamide at a concentration of 280mgI/ml was prepared by adding the appropriate amount of solvent, as indicated by the manufacturer, to the dried preparation.

2.3.3 Canine Cisternal Puncture

In the definitive studies it was proposed to inject the various test solutions by cisternal puncture. Four preliminary studies were therefore performed to develop a technique to allow an atraumatic puncture to be performed. The vertebral spinous processes in the dog project cranially rather than caudially. By performing the puncture with a 22 gauge needle angled slightly caudally, a clean puncture could be performed . During the puncture a 5cc syringe was connected to the needle and gentle suction applied. Puncture of the thecal sac

was immediately detected by the aspiration of a small amount of CSF. Following the injection of the water-soluble CM, control radiographs were performed to demonstrate the presence of the CM in the subarachnoid space. During injection of the CM, the body of the dog was elevated slightly to prevent retrograde flow caudally, and immediately after injection, the dog was placed head-down for two minutes to allow the CM to enter the cranial subarachnoid space.

2.3.4 CT Demonstration and Assessment of the Presence of Water-Soluble CM in the Brain Parenchyma

2.3.4.1 In Vitro Studies

The technique for performing a CT examination of the canine brain after removal had been previously developed (Section 2.1.4). Six preliminary studies were performed without an intrathecal injection to establish the normal density of the cortex in the dog. Following coronal CT examination, a region of interest of 100 pixels was assessed in the temporal lobe of both hemispheres and the mean attenuation values established for normal brain tissue.

Four further preliminary studies were performed, 2 each with methylglucamine iothalamate and metrizamide. One hour after intrathecal injection of the test CM, the brain was removed and a coronal CT examination was performed (Section 2.1.4). These preliminary examinations established that intrathecal penetration of water-soluble CM could be clearly demonstrated in the cerebral

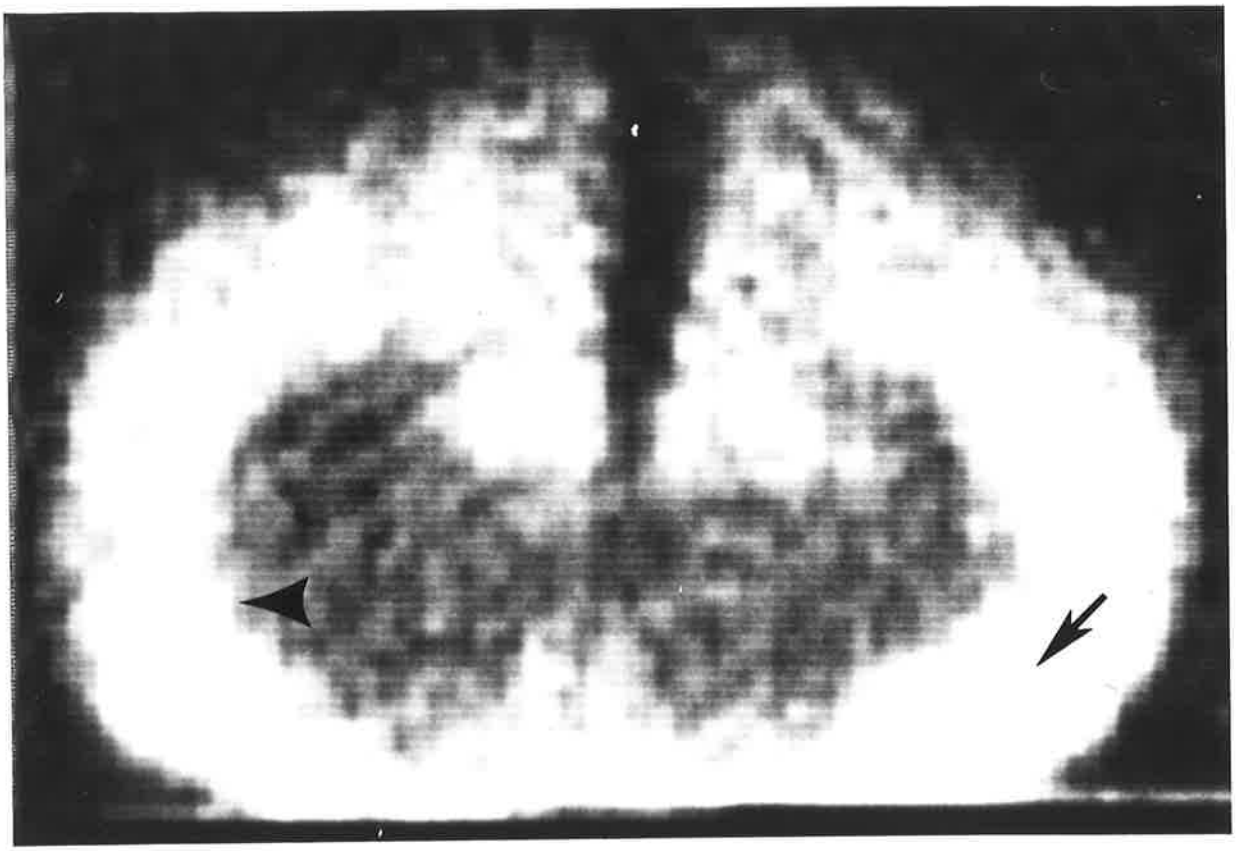


Figure 2.3.4.1. Coronal CT scan of canine brain after intrathecal metrizamide (280mgI/ml) has been in contact with the cortical surface for 60 minutes. Hyperdense grey matter is due to contrast penetration through CSF-brain interface. Region of interest sites for concentration assessment (arrow) and level of depth of penetration measurement (arrowhead) are indicated.

cortex at one hour and that the injection technique and positioning allowed a uniform distribution of the CM into the subarachnoid space. Consistent penetration was demonstrated in the anterior temporal and parietal region bilaterally and therefore, this area was selected for assessment in the definitive studies (Figure 2.3.4.1).

The actual iodine concentration in both hemispheres at one level could be compared by the region of interest technique. The following method of assessing the depth of penetration was established. The window width of the diagnostic display console was placed on "measure'" setting and the level set at 24 EMI units. This was the maximum value of the normal canine brain, established in the six preliminary studies without CT. The depth of attenuation values above that of normal cortex could then be measured directly at right angles to the brain surface in the same region of the temporal lobe for each study (Figure 2.3.4.1). In these preliminary studies, blind subjective assessment of the depth of penetration of CM into both temporal lobes was assessed at two adjacent levels by two independent observers and this established the reproducibility of this method of assessment.

2.3.4.2 In Vivo Assessment

In the preliminary in-vitro studies (Section 2.3.4.1) it was found that it took 10-15 minutes to remove the brain after the dog was killed with intravenous potassium chloride. To establish that brain penetration by intrathecal CM was not simply a post mortem phenomenon, four in-vivo studies were performed and following an in-vivo coronal CT at one hour the dog was killed, the brain removed and a coronal CT of the brain performed. The in-vivo and in-vitro studies confirmed that in-vivo

penetration of CM occurred and that there was a good qualitative relationship between the in-vivo and in-vitro studies. A close correlation between the quantitative assessment of CM concentration in-vivo and in-vitro was not to be expected because of the overlying bone and CM in the CSF in the in-vivo studies which alters attenuation values.

2.3.4.3 Evans' Blue Assessment of BBB Disruption

The BBB is presumably exposed to water-soluble CM which passes from the CSF into the brain parenchyma after intrathecal injection. To establish whether this led to any disruption of the BBB, similar to an intra-arterial injection, Evans' Blue was administered intravenously in all intrathecal studies, being administered immediately following the intrathecal injection of water-soluble CM. The surface of the brain was inspected immediately after removal and subsequently following the CT examinations, the brain was sectioned (in the coronal plane) in the same plane and at corresponding levels to the CT scans. The brain tissue in areas showing significant CM penetration in the CT examinations, was inspected by two independent observers to assess the presence or absence of any staining.

2.4 DEFINITIVE INTRATHECAL EXPERIMENTS

2.4.1 Defintive Experimental Model

Adult greyhound dogs of either sex, weighing between 20kg and 30kg were anaesthetised as previously described (Section 2.1.1).

With the dog in a prone position with the neck flexed, cisternal puncture was performed using a 22 gauge needle. Five millilitres of test solution were injected into the subarachnoid space slowly over 2 minutes. Radiographs were performed to determine successful intrathecal injection and if a clean puncture was not obtained, the study was abandoned.

Following intrathecal injection of the CM, the dog was placed in a head-down position for 2 minutes during which time the head was rolled gently from side to side and following this, the dog was returned to a supine position to encourage the CM to lie adjacent to the surface of the cerebral cortex. Evans' Blue solution (2% in 0.9% saline, membrane filtered) was administered intravenously at a dose of 3ml/kg immediately after the intrathecal injections.

Fifteen or sixty minutes from the end of the intrathecal injection the animals were killed by an intravenous injection of 10-15mls of saturated potassium chloride solution. Within 15 minutes, the brain was removed and the surface rinsed with an isotonic solution (Hartmann's solution: Travenol Labs. Sydney) to remove overlying CSF and CM. The brain was then suspended in water in a sealed ,

cylindrical, perspex container and positioned in the CT scanner (EMI model 5005). Following phantom studies to ensure the accuracy of attenuation values, coronal scans were performed at 1cm intervals, using 13mm collimation (Figure 2.4.1.1). After obtaining CT scans, the brain was sectioned in the coronal plane and qualitative assessment of the presence of any Evans' Blue staining was performed by two independent observers.

The coronal CT sections were assessed for evidence of brain penetration by the CM. Preliminary studies (Section 2.3.4.1) indicated that consistent and maximum penetration was demonstrated in the anterior and mid parietal region bilaterally. Therefore, quantitative measurements were made in these regions by calculating the mean attenuation number in a 100 pixel region of interest in the middle of the grey matter in both parietal lobes (Figure 2.3.4.1). The mean of the mean EMI number of both temporal lobes at two adjacent levels for each of four studies with each CM was calculated. The mean result for each CM was compared by "Student's" t-test.

Preliminary studies without an intrathecal injection had demonstrated that the maximum attenuation value of the normal canine grey matter was never greater than 24 EMI units. With this information, blind subjective assessment of the depth of penetration of CM into both temporal lobes was made at two adjacent levels in each of the eight studies. The window width of the diagnostic display console was placed on "measure" setting and the level set at 24 EMI units. The depth of attenuation values above that of normal grey matter could then be measured directly at right angles to the brain surface in the same region of the parietal lobe for each study (Figure 2.4.1.2).

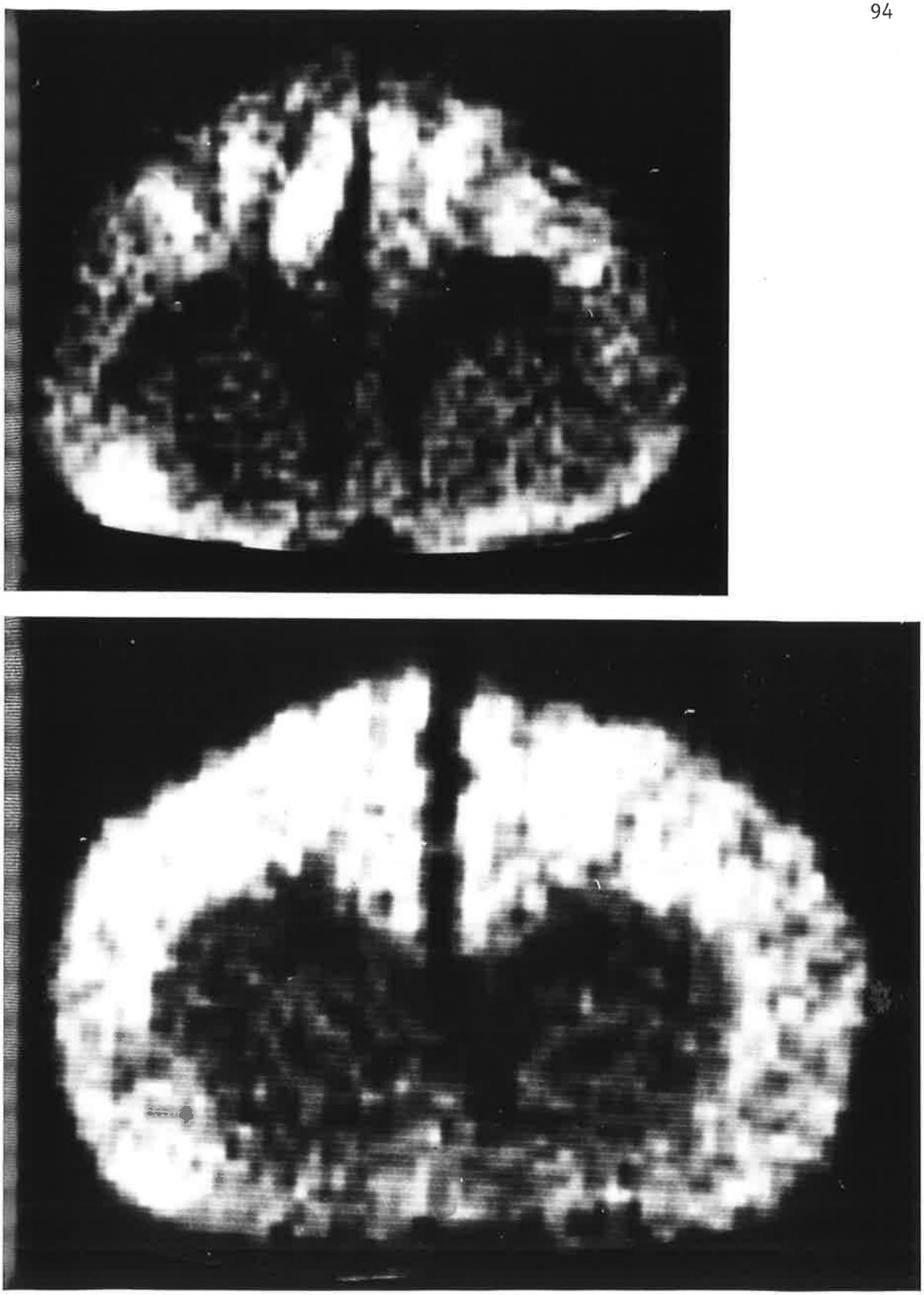


Figure 2.4.1.1. Coronal CT scans of two canine brains after intrathecal Iopamidol (280mgI/ml) has been in contact with the cortical surface for 15 minutes (A) and 60 minutes (B) respectively.

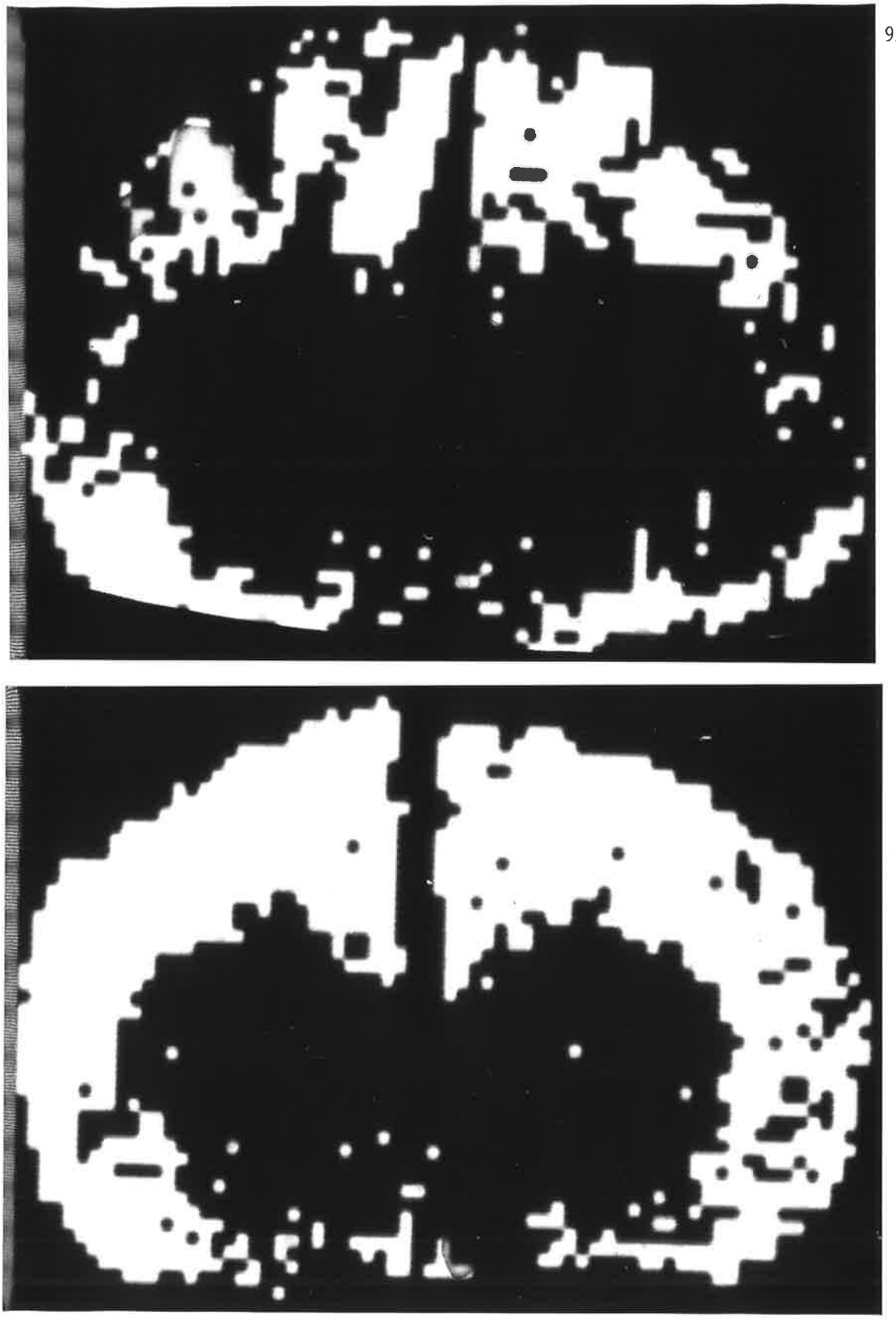


Figure 2.4.1.2. Coronal CT scans in figure 2.4.1.1 at a "measure" setting of 26 EMI units. The distribution of CM is demonstrated. The patchy brain penetration at 15 minutes (A) is related to the cortical sulci but by 60 minutes (B) relatively even distribution of the CM throughout the grey matter has occurred.

2.4.2 Definitive Studies

Two series of studies were carried out:

Series I: Using eight dogs, four studies with each of methylglucamine iothalamate and metrizamide at an iodine concentration of 280mg/ml were carried out. Dogs were killed 60 minutes after intrathecal injection.

Series II: Using twelve dogs, twelve studies with iopamidol at an iodine concentration of 280mg/ml were carried out. In six studies the dogs were killed 15 minutes after injection after injection and in six studies, they were killed 60 minutes after injection.

2.5 INTRATHECAL BRAIN PENETRATION IN HUMANS

2.5.1 Aims of Study

This in-vivo study was designed to assess the degree of brain penetration and concentration by the non-ionic water-soluble CM, metrizamide, following lumbar myelography.

2.5.2 Research Method

Thirteen patients, 9 males and 4 females with an age range of 22-64 years, who presented for lumbar radiculography because of back problems, were included in the study. Apart from their back problem, all patients were healthy and free of any history of symptoms related to the central nervous system. Prior to the examination, they were interviewed and examined to gain a baseline for assessment of morbidity. Ten millilitres of metrizamide at a concentration of 190mgI/ml were used in each patient. Following the examination, the patient remained in bed with the head elevated 30-40 degrees for the first 6 hours. Following this, they remained in bed with toilet privileges for a further 18 hours.

A baseline CT examination at the level of the basal ganglia was performed the day prior to the examination and further studies were performed at the same level at 6 and 24 hours after myelography (Figure 2.5.2). Prior to each CT study, phantom studies were performed to ensure the accuracy of attenuation values obtained.

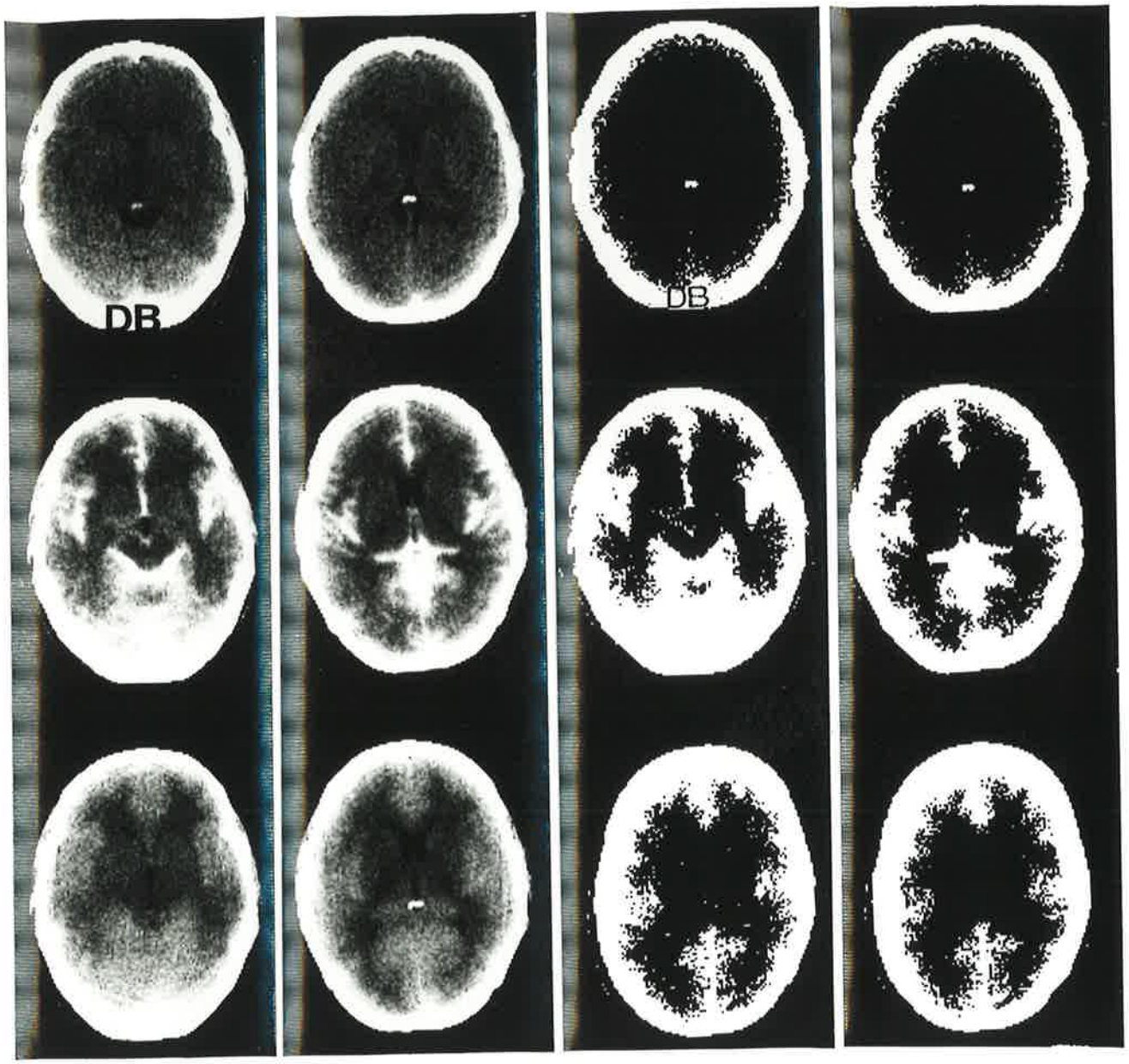


Figure 2.5.2. CT scans performed on a patient before (top) and at 6 (middle) and 24 (bottom) hours after lumbar myelography. A. Window setting of 30 EMI units. B. "Measure" setting similar to density of grey matter in control scan. At 6 hours, dense CM is noted in basal cisterns and sylvian fissures, without reflux into the ventricles. Brain penetration is noted. At 24 hours, dilute CM obscures the definition of the basal cisterns and sylvian fissures and CM brain penetration persists.

Subjective assessment of the distribution of metrizamide and the depth and degree of brain penetration was carried out, using the "measure" setting of the window width control of the diagnostic display console of the CT scanner. The depth of penetration was assessed adjacent to the interhemispheric fissure at the level of the anterior horns of the lateral ventricles in each case. The window width of the diagnostic display console was set on the "measure" setting at a level corresponding to the maximum value for grey matter established by the numerical printout of the control scan. Numerical printouts of each scan were obtained and quantitative assessment of brain penetration was performed by calculating the mean EMI number in a 100 pixel point region of interest in the grey matter of each temporal lobe. Using the same technique, quantitative assessment of the white matter adjacent to the anterior horns of the lateral ventricles was also made.

Patients were interviewed at 6 hours and at 24 hours after the lumbar radiculogram to record the presence of any significant morbidity. In particular, the severity of headache was recorded as absent, mild or severe. A neurological assessment was also made at the same time.

In eight of the thirteen patients, a baseline EEG was performed prior to the procedure and repeat examinations performed at 6 and, where possible, 24 hours. Independent blind assessment of the EEG studies was carried out by two neurologists.

2.6 DEVELOPMENT OF MODELS FOR INTRAVENOUS STUDIES

2.6.1 Intravenous CM and the CSF-Blood Interface

The passage of CM into the CSF following intravenous injection was studied in dogs. Dogs were used rather than a smaller animal to ensure clean cisternal puncture and adequate, uncontaminated CSF samples. The method of animal preparation (Section 2.1.1) and the technique of cisternal puncture in dogs (Section 2.3.3) was similar to that used in the intrathecal studies.

2.6.2 Intravenous CM and the Blood-Brain Interface

The effect of intravenous CM on the integrity of the BBB was studied in rabbits. Rabbits were used because of the large number of studies to be performed and the use of a radioisotope quantitative marker.

2.6.2.1 Test Solutions

At present because of cost factors, ionic water-soluble CM are used for enhancement in CT and intravenous bolus for digital angiography rather than non-ionic CM. Sodium iothalamate at a supplied concentration of 420mgI/ml (Conray 420) is widely used for these purposes in Australia. Therefore ionic sodium iothalamate was selected as the test CM with isotonic saline as the control.

2.6.2.2 Method of Assessment of BBB

Evans' Blue was selected as a qualitative marker of the BBB (Section 2.1.6) while Technetium-99m (99mTc) was selected as a quantitative marker. 99mTc pertechnetate is widely used in radioisotope imaging of the brain (Davies 1980). Pertechnetate is concentrated in the stomach, urinary bladder, thyroid and salivary glands (Wagner & Rhodes 1968, Atkins 1976) after intravenous injection but is excluded from the normal brain parenchyma by the intact BBB (Atkins 1976). Some concentration does occur in the choroid plexus (Atkins 1976) but this may be blocked by oral potassium perchlorate (Davies 1980). If the integrity of the BBB is disrupted the 99mTc-pertechnetate crosses freely into the extracellular fluid of the brain parenchyma. The gamma radiation from 99mTc may be readily measured in tissue and body fluids using a scintillation counter. If a sample of brain tissue is counted, 99mTc in both the brain tissue itself and the brain blood-pool will contribute to the activity recorded. If a sample of blood is also counted a ratio of brain to blood can be established in a normal situation where the intact BBB prevents the radionuclide from entering the brain extracellular fluid. However, if the BBB is disrupted, pertechnetate will cross into the brain parenchyma and alter the brain to blood ratio. The technique of calculating the ratio of blood to brain avoids the problems of closely matching pertechnetate doses, and making corrections for radioactive decay and blood volumes.

2.6.2.3 Preliminary Assessment of Evans' Blue and Technetium-99m-Pertechnetate

To determine whether increased permeability or disruption of the BBB in the rabbit could be detected using Evans' Blue and pertechnetate, a series of preliminary studies were carried out using intra-arterial injections of hypertonic water-soluble CM, sodium iothalamate. The cerebro-vascular anatomy of the rabbit was reviewed (Scremin et al 1982) and a technique of catheterising the common carotid artery was developed. Rabbits (IMVS strain, either sex) were anaesthetised with a 25% solution of urethane in 0.9% saline injected intravenously at a dose of 7mls/kg. This provided a satisfactory state of anaesthesia for the duration of the examination without requiring intubation. A fine polythene catheter (PE90) was inserted into the vessel and directed cranially. As a rule, the catheter completely obstructed the vessel and therefore in all cases, the common carotid artery, proximal to the catheter was tied off. Following this, the external carotid artery was tied off at a point just distal to its origin. A burr hole in the vault of the skull on the same side as catheterisation was made to expose the superficial pial veins. Intra-arterial injections of either isotonic saline or sodium iothalamate (Conray 420) were made over 30 seconds, the rate of injection being adjusted to maintain clearance of blood from the pial vessels.

Immediately after the intra-arterial injection, an intravenous injection of 2% Evans' Blue in 0.9% saline (membrane filtered) at a dose of 3mls/kg was administered to allow qualitative assessment

of gross breakdown of the BBB. This was also followed by an intravenous injection of 99mTc-pertechnetate (approximately 100uCi in 0.2mls of saline). This dose of 99mTc gave a count rate of approximately 10,000cpm in one hemisphere of the brain by the time counting was carried out. Sixty minutes after the pertechnetate injection, 1ml of cardiac blood was removed and the animal killed immediately with 5-10mls of saturated potassium chloride solution injected intravenously. The brain was then removed as quickly as possible and rinsed with Hartmann's solution to remove superficial blood and CSF. Subjective assessment of the degree of Evans' Blue staining was then made. The brain was carefully bisected and the hemispheres weighed. Then the blood sample and each half of the brain were counted separately in a Searle gamma counter using a "well" attachment. Following correction for background radiation a ratio of blood (cpm/ml) to brain (cpm/gm) was calculated. By calculating the ratio of blood to brain, variations in blood volume and initial isotope injections for individual animals as well as radioactive decay was accounted for. Results of these preliminary studies are given in Table 2.6.2.3. Control studies were performed with one common carotid artery ligated and no arterial injections. This did not lead to significant breakdown in the BBB either qualitatively or quantitatively. Following intra-arterial sodium iothalamate, various degrees of BBB disruption were demonstrated both qualitatively by Evans' Blue staining and also quantitatively by increasing isotope in the brain tissue on the side of injection (Table 2.6.2.3). In these preliminary studies, it was determined that using 99mTc, differences equivalent to $\pm$0.06mls of blood per gram of brain could be detected.

TABLE 2.6.2.3 Preliminary Intra-arterial Rabbit Studies using Technetium to Demonstrate Alterations in Blood-Brain Barrier Integrity. The Mean Difference of Counts between the Test and Control Hemispheres is given.

		RATIO OF BRAIN TO BLOOD
		Mean Difference ± SD
CONTROLS (One Carotid Tied Off) No Injection	(n = 7)	0.0021 ± 0.0013
INTRACAROTID SALINE	(n = 10)	0.0024 ± 0.0026
INTRACAROTID METHYLGLUCAMINE IOTHALAMATE	(n = 10)	0.0089 ± 0.0027
CONTROL vs SALINE	$p > 0.1$	
CONTROL vs METHYLGLUCAMINE IOTHALAMATE	$p < 0.01$	
SALINE vs METHYLGLUCAMINE IOTHALAMATE	$p < 0.05$	

2.7 DEFINITIVE INTRAVENOUS EXPERIMENTS

2.7.1 Intravenous CM and the CSF-Blood Interface

Studies were performed on four adult greyhound dogs weighing between 21kgs and 35kgs. Anaesthesia was induced with intravenous pentobarbital (Pentothal) 25mg/kg. Dogs were intubated and anaesthesia was maintained using a mixture of halothane (Fluothane), nitrous oxide and oxygen. Respiration was controlled at 8 breaths per minute by an Oxford ventilator.

An intravenous catheter was inserted in a leg vein and a control sample of blood removed. Dogs were placed in a prone position and cisternal puncture performed using a 22 gauge needle. A control sample of CSF was then obtained.

A bolus injection of sodium iothalamate (Conray 420) at a dose of 4.2gI/kg (10ml/kg) was administered manually through the catheter. Injection was completed within 3-4 minutes. Blood and CSF samples were removed at 30 minutes and 60 minutes from the beginning of the contrast injection.

Using pre-injection samples as blanks, contrast medium concentrations were measured spectrophotometrically on deproteinised plasma and whole CSF at a wavelength of 239nm using a Gilford 250 UV-VIS spectrophotometer. A mean iodine concentration was calculated for CSF, and for plasma for each collection time. The iodine concentrations of each collection time for both CSF and plasma were

then compared by "Student's" t-test.

2.7.2 Intravenous CM and the Blood-Brain Interface

Rabbits (IMVS strain) of either sex were anaesthetised with a 25% solution of urethane in 0.9% saline injected intravenously at a dose of 7mls/kg. After satisfactory anaesthesia was established, the test solution at a dose of 8mls/kg was injected manually as a bolus into an ear vein within 60 seconds. In the event of dyspnoea injections were ceased until breathing returned to normal. The test solutions were either isotonic saline or sodium iothalamate (Conray 420). Evans' Blue and pertechnetate were injected as described in Section 2.6.2.3.

Sixty minutes after the pertechnetate injection 1ml of cardiac blood was removed and the animal killed immediately with 5-10mls of saturated potassium chloride solution injected intravenously. The brain was then removed as quickly as possible and rinsed with Hartmann's solution to remove superficial blood and CSF. Subjective assessment of the degree of Evans' Blue staining was then made as described in Section 2.1.5.

The brain was weighed and then the blood sample and brain were counted separately in a Searle gamma counter using a "well" attachment. The blood/brain ratio was then calculated as described previously (Section 2.6.2.3).

A ratio of blood to brain for normal brain tissue was established by performing twenty control studies, ten with no intravenous injection

and ten with intravenous injections of isotonic saline. Ten studies were also performed with intravenous sodium iothalamate. The difference between the blood to brain ratio for each series of studies was compared by "Student's" t-test.

3 RESULTS

3.1 INTRA-ARTERIAL STUDIES

3.1.1 Series I: Methylglucamine Iothalamate, 25% Mannitol and Isotonic Saline

Visual assessment of the presence and degree of Evans' Blue staining in the distribution of the middle cerebral artery by two independent observers is given in Table 3.1.1.1. Evans' Blue staining was demonstrated in all of the methylglucamine studies, a proportion of mannitol studies but none of the isotonic saline studies. This qualitative assessment reflected varying degrees of disruption of the BBB.

CT results for the definitive studies comparing methylglucamine iothalamate, 25% mannitol and isotonic saline are given in Table 3.1.1.2. The mean attenuation numbers for a 100 pixel region of interest in the middle cerebral artery distribution in the control and injected hemispheres for each study are shown. In every study with methylglucamine iothalamate or mannitol the difference between control and injected hemispheres was statistically significant ($p<0.05$). The difference was not statistically significant in any of the saline studies ($p>0.1$) In two of the mannitol studies a significant difference between hemispheres was demonstrated by CT attenuation despite the absence of Evans' Blue staining.

TABLE 3.1.1.1 Intensity of Evans' Blue Staining of Cerebral Parenchyma in the Distribution of the Middle Cerebral Artery.

INTRACAROTID SOLUTION	STUDY NUMBER	DEGREE OF STAINING
METHYLGLUCAMINE IOTHALAMATE	1	++
	2	+
	3	+++
	4	++
MANNITOL 25%	5	+
	6	0
	7	0
	8	++
ISOTONIC SALINE	9	0
	10	0
	11	0
	12	0

TABLE 3.1.1.2 Degree of Enhancement as Determined by Measuring Mean CT Number (EMI Units) for Comparable Regions of Interest (100 Pixels) in the Distribution of the Middle Cerebral Artery in Both Injected and Control Hemispheres.

INTRACAROTID SOLUTION	STUDY NUMBER	CORTEX : MCA				DIFFERENCE (EMI Units)	MEAN DIFFERENCE (EMI Units)	SD MEAN DIFFERENCE (EMI Units)
		INJECTED HEMISPHERE		CONTROL HEMISPHERE				
		MEAN EMI NO	SD	MEAN EMI NO	SD			
METHYLGLUCAMINE	1	29.79	7.10	12.42	3.76	17.37		
IOTHALAMATE	2	19.85	3.76	12.94	3.64	6.91		
	3	42.34	5.85	13.63	3.79	28.71	15.01	6.45
	4	22.23	3.82	15.16	3.34	7.07		
MANNITOL 25%	5	15.54	4.26	12.40	4.51	3.14		
	6	13.99	3.18	11.45	3.42	2.54		
	7	14.67	3.82	12.27	3.39	2.40	4.00	5.53
	8	18.95	5.29	11.04	2.79	7.91		
SALINE	9	14.36	3.88	13.44	4.62	0.92		
	10	12.75	4.71	12.15	4.20	0.60		
	11	14.61	3.39	13.89	3.06	0.72	0.65	5.61
	12	13.38	4.33	13.02	3.16	0.36		

The mean difference between control and injected hemispheres, measured with each of the injected solutions is also given in Table 3.1.1.2. With saline the mean enhancement was not significantly different from zero ($p>0.1$). Of the two hypertonic solutions methylglucamine iothalamate gave the greater difference ($p<0.01$) between control and injected hemispheres.

3.1.2 Series II: Methylglucamine Iothalamate, Metrizamide, Iopamidol and Iohexol at Iodine Concentrations of 280mgI/ml

Visual assessment of the presence and degree of Evans' Blue staining in the distribution of the middle cerebral artery was made by two independent observers and the higher value was used. A variable degree of staining was demonstrated in five studies with methylglucamine iothalamate but no staining was demonstrated with metrizamide, iopamidol or iohexol.

CT results for the definitive studies for the four CM are given in Tables 3.1.2.1, 3.1.2.2 and 3.1.2.3. The mean attenuation numbers for a 100 pixel region of interest in the middle cerebral artery distribution in the control and injected hemispheres for each study are shown. With methylglucamine iothalamate, the difference between control and injected hemispheres was statistically significant ($p<0.05$) but this was not the case with metrizamide, iopamidol or iohexol ($p>0.1$).

TABLE 3.1.2.1 Degree of Enhancement as Determined by Measuring Mean CT Number (EMI Units) for Comparable Regions of Interest (100 Pixels) in the Distribution of the Middle Cerebral Artery in Both Injected and Control Hemispheres.

INTRACAROTID SOLUTION	STUDY NUMBER	CORTEX : MCA				DIFFERENCE (EMI Units)	MEAN DIFFERENCE (EMI Units)	SD MEAN DIFFERENCE (EMI Units)
		INJECTED HEMISPHERE		CONTROL HEMISPHERE				
		MEAN EMI NO	SD	MEAN EMI NO	SD			
METHYLGLUCAMINE IOTHALAMATE	1	22.16	4.29	14.67	3.77	7.49		
		21.24	4.45	12.49	4.66	8.75		
	2	33.43	5.93	14.52	3.49	19.91		
		32.49	4.12	15.92	4.02	17.57		
	3	26.03	4.67	13.78	3.02	12.25	11.01	5.79
		30.41	3.64	14.70	3.92	15.71		
	4	22.23	3.82	15.16	3.34	7.07		
		20.95	4.13	12.68	4.46	8.27		
	5	19.85	3.76	13.46	3.71	6.39		
		20.72	3.87	13.04	3.91	7.68		
METRIZAMIDE	6	15.42	3.08	14.80	3.61	0.62		
	7	10.84	3.66	11.35	4.02	-0.51	0.33	5.48
	8	14.35	4.60	13.73	4.15	0.62		
	9	14.45	4.26	13.86	3.39	0.59		

* In Metrizamide studies, only one scan was used for assessment, as in Series I. For subsequent Methylglucamine Iothalamate, Iohexol and Iopamidol studies two adjacent scans were assessed.

TABLE 3.1.2.2 Degree of Enhancement as Determined by Measuring Mean CT Number (EMI Units) for Comparable Regions of Interest (100 Pixels) in the Distribution of the Middle Cerebral Artery in Both Injected and Control Hemispheres.

		CORTEX : MCA						
	STUDY	INJECTED HEMISPHERE		CONTROL HEMISPHERE		DIFFERENCE	MEAN DIFFERENCE	SD MEAN DIFFERENCE
INTRACAROTID SOLUTION	NUMBER	MEAN EMI NO	SD	MEAN EMI NO	SD	(EMI Units)	(EMI Units)	(EMI Units)
IOPAMIDOL	10	14.96	3.58	14.72	3.94	0.24		
		13.28	4.65	12.70	3.88	0.58		
	11	14.92	3.88	13.99	4.74	0.93		
		13.94	4.39	14.98	4.37	-1.04		
	12	15.02	3.56	15.11	3.59	-0.09	0.07	5.41
		14.20	3.16	14.31	4.28	-0.11		
	13	16.52	3.61	15.82	3.62	0.70		
		16.25	3.35	16.54	3.60	-0.29		
	14	16.85	3.27	16.60	3.58	0.25		
		15.72	3.42	16.27	3.49	1.45		
IOHEXOL	15	12.43	4.79	12.12	4.13	0.21		
		12.32	4.12	12.93	4.15	-0.71		
	16	12.64	3.92	11.91	4.26	0.73		
		12.54	3.90	12.93	4.17	-0.39		
	17	11.37	3.74	11.25	3.96	0.12	0.27	5.83
		11.01	3.39	10.62	4.07	0.39		
	18	11.62	4.05	11.04	3.93	0.58		
		11.34	4.10	11.06	4.44	0.28		
	19	11.30	4.43	10.96	4.50	0.34		
		11.59	3.84	10.65	4.32	0.94		

* In Metrizamide studies, only one scan was used for assessment, as in Series I. For subsequent Methylglucamine Iothalamate, Iohexol and Iopamidol studies two adjacent scans were assessed.

TABLE 3.1.2.3 Summary of Intracarotid Studies in Series 3.1.2

INTRACAROTID SOLUTION	MEAN DIFFERENCE BETWEEN INJECTED AND CONTROL HEMISPHERES (EMI UNITS)	SD MEAN DIFFERENCE (EMI UNITS)
METHYLGLUCAMINE IOTHALAMATE	11.01	5.79
METRIZAMIDE	0.33	5.48
IOPAMIDOL	0.07	5.41
IOHEXOL	0.27	5.83

3.2 INTRATHECAL STUDIES

3.2.1 Series I: Methylglucamine Iothalamate and Metrizamide at an Iodine Concentration of 280mgI/ml. Brain penetration after 60 minutes

CT attenuation values comparing methylglucamine iothalamate and metrizamide are given in Table 3.2.1.1. The mean of the mean attenuation number for a 100 pixel region of interest for each series of experiments with each CM agent are shown, being 37.0 for methylglucamine iothalamate and 39.2 for metrizamide. Analysis using "Student's" t-test showed no significant difference between the concentration of iodine obtained with either of the CM within the brain parenchyma ($p>0.1$).

The depth of penetration of both methylglucamine iothalamate and metrizamide into the brain was similar after one hour. For methylglucamine iothalamate it was 13.7 ± 1.6mm; for metrizamide it was 13.4 ± 0.9mm ($p>0.01$). There was no evidence of Evans' Blue staining of the parenchyma in the parietal region of either hemisphere in any of the studies, despite the obvious presence of CM in these areas, as indicated by iodine concentration demonstrated by CT.

TABLE 3.2.1.1 Mean CT Attenuation (EMI Units) in the Cortex in the Temporal Region of Both Hemispheres in Two Adjacent CT Scans (ROI = 100 pixels), following either Intrathecal Methylglucamine Iothalamate or Metrizamide at an Iodine Concentration of 280mgmI/ml, 60 Minutes after Intrathecal Injection.

INTRATHECAL CM	STUDY NUMBER	LEFT HEMISPHERE MEAN (EMI UNITS)	LEFT HEMISPHERE SD (EMI UNITS)	RIGHT HEMISPHERE MEAN (EMI UNITS)	RIGHT HEMISPHERE SD (EMI UNITS)	MEAN (EMI UNITS)	SD (EMI UNITS)
		35.43	7.05	36.33	7.72		
	1	30.44	5.97	32.96	5.93		
		35.45	8.95	33.37	6.24		
METHYLGLUCAMINE	2	33.77	7.85	34.29	8.43	36.99	8.31
IOTHALAMATE		36.08	8.40	44.31	8.59		
	3	38.33	7.79	41.62	6.42		
		41.63	11.68	40.24	10.83		
	4	40.94	9.21	36.64	9.40		
		34.47	7.95	39.77	6.83		
	5	35.49	7.04	34.30	7.98		
		33.89	7.56	35.01	7.67		
METRIZAMIDE	6	36.51	10.55	39.48	8.68	39.15	9.72
		45.69	9.71	45.85	12.41		
	7	49.76	12.46	45.09	11.37		
		36.32	9.13	41.62	11.79		
	8	35.75	7.61	37.36	13.16		

3.2.2 SeriesII: Iopamidol at an Iodine Concentration of 280mgI/ml. Brain Penetration After 15 and 60 Minutes

CT attenuation values for each series of experiments are given in Table 3.2.2.1. The mean EMI number for a 100 pixel region of interest for each series was 27.9±6.7 at fifteen minutes and 36.7±6.6 at sixty minutes. Analysis using "Student's" t-test showed a significant increase in the concentration of CM at sixty minutes compared to fifteen minutes ($p<0.05$).

The maximum depth of penetration of CM into the grey matter was similar after the two time periods, being 13.4±1.3mm at fifteen minutes and 13.7±0.8mm at sixty minutes. However the distribution of CM was very patchy at fifteen minutes without even distribution throughout the grey matter (Figure 2.4.1.1, 2.4.1.2). The maximum penetration appeared to correspond to the site of cortical sulci with some sparing of grey matter between. After sixty minutes, the distribution of CM was uniform throughout the grey matter without an obvious increase in the maximum depth of penetration (Figures 2.4.1.1, 2.4.1.2).

The results of the series of experiments at 60 minutes were compared with the results of the methylglucamine iothalamate and metrizamide studies, at a similar iodine concentration and after a similar time period (Section 3.1.1). Analysis using "Student's" t-test showed no significant difference between the concentration of iodine obtained by the three CM within the brain parenchyma ($p>0.1$).

TABLE 3.2.2.1 Mean CT Attenuation (EMI Units) in the Cortex in the Temporal Region of Both Hemispheres (ROI = 100 pixels), either Fifteen or Sixty Minutes following Intrathecal Iopamidol at an Iodine Concentration of 280mgmI/ml.

TIME POST INJECTION	STUDY NUMBER	LEFT HEMISPHERE MEAN (EMI UNITS)	LEFT HEMISPHERE SD	RIGHT HEMISPHERE MEAN (EMI UNITS)	RIGHT HEMISPHERE SD	MEAN (EMI UNITS)	SD MEAN (EMI UNITS)
	1	31.54	9.92	30.60	6.73		
	2	23.05	3.71	25.55	4.73		
15 MINUTES	3	26.07	6.75	29.41	6.80	27.86	6.72
	4	31.68	7.63	31.38	7.84		
	5	25.67	5.40	23.61	5.62		
	6	38.29	7.71	43.13	6.64		
	7	34.65	7.03	38.30	9.81		
60 MINUTES	8	33.68	5.19	36.34	7.01	36.72	6.62
	9	43.25	6.16	37.17	6.40		
	10	33.26	4.21	29.11	3.99		

3.2.3 Series III: Clinical Study with Metrizamide at an Iodine Concentration of 190mgI/ml

In six patients (Patients 1-6) dense contrast medium was demonstrated in the CSF in the basal cisterns and sylvian fissures at 6 hours but there was no reflux into the lateral or third ventricles (Figure 2.5.2, 3.2.3.1). Penetration of CM into the cortex was demonstrated to a similar degree in all six cases as demonstrated subjectively with the diagnostic display console (Figure 2.5.2, 3.2.2.1) and also in the digital printouts (Table 3.2.3, Figure 3.2.3.2). At 24 hours, there was a loss of definition between the CSF and the brain surface and there had been an increase in the depth of penetration in all six patients (Figure 2.5.2, 3.2.3.1). The concentration of CM within the grey matter at 24 hours had not changed significantly since the 6 hour study (Table 3.2.3; Figure 3.2.3.2). There was no CM in the ventricles of these patients at 24 hours and there had been no significant change in the density of the white matter adjacent to the anterior horns of the lateral ventricles betwen any of the CT examinations (Table 3.2.3; Figure 3.2.3.2).

One patient (Patient 7) showed transient reflux of CM into the lateral ventricles at 6 hours at a lower concentration than the CSF in the basal cisterns but the degree and depth of penetration at both 6 and 24 hours was similar to patients 1-6 (Table 3.2.3). At 24 hours, no CM could be detected in the ventricular system.

In two patients (patients 8 & 9) a similar pattern to patients 1-6

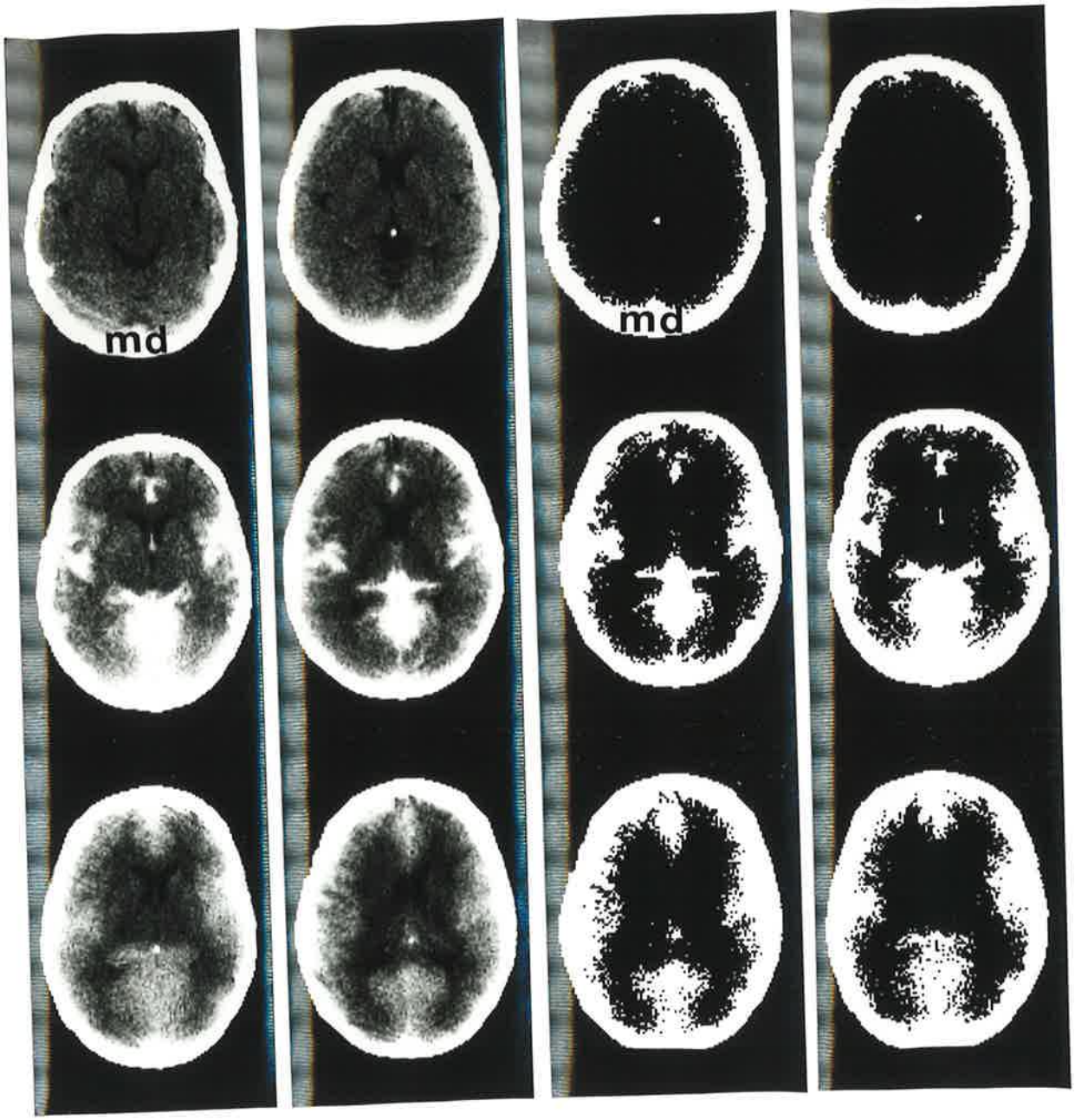


Figure 3.2.3.1. CT scans performed on a patient before (top) and at 6 (middle) and 24 (bottom) hours after lumbar myelography. A. Window setting of 30 EMI units. B. "Measure" setting similar to density of grey matter in control scan. At 6 hours, dense CM is noted in basal cisterns and sylvian fissures, without reflux into the ventricles. Brain penetration is noted. At 24 hours, dilute CM obscures the definition of the basal cisterns and sylvian fissures and CM brain penetration persists.

TABLE 3.2.3 Mean CT Attenuation (EMI Units) in the Cerebral Cortex and White Matter (ROI = 100 pixels), before and after Lumbar Myelography

PATIENT NUMBER	CORTEX						WHITE MATTER					
	CONTROL		6 HOURS		24 HOURS		CONTROL		6 HOURS		24 HOURS	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
1	21.35	4.07	28.27	4.95	27.27	3.28	17.81	3.49	17.54	3.58	18.28	3.91
2	22.05	3.96	25.49	4.38	27.59	3.72	18.31	3.64	19.34	3.34	19.23	3.34
3	22.09	1.85	27.27	3.09	29.15	3.24	17.53	1.86	17.77	1.96	17.49	1.63
4	22.49	2.38	28.70	3.55	29.90	2.18	19.13	2.28	18.60	2.89	21.56	2.53
5	22.10	2.05	-	-	24.89	2.11	18.95	1.60	-	-	20.13	2.00
6	24.74	2.65	33.19	3.45	29.58	2.64	20.25	1.95	21.17	1.92	20.55	1.83
7	21.20	2.34	26.30	3.05	25.23	2.28	17.87	2.14	17.79	1.62	17.24	1.44
8	21.71	2.82	23.57	2.25	25.29	2.36	19.49	2.13	18.66	2.82	19.18	2.35
9	19.99	1.80	22.02	2.28	23.60	2.14	17.13	1.80	17.37	1.98	17.73	2.34
10	20.28	2.04	20.28	2.65	23.18	2.47	15.97	1.89	17.64	1.78	19.16	2.10
11	18.70	1.88	18.79	1.90	20.23	2.11	15.97	1.63	15.69	2.00	16.04	1.75
12	21.90	2.11	23.67	2.15	21.90	2.16	18.86	1.71	19.10	2.17	17.72	1.90
13	20.85	1.96	24.96	3.47	28.98	3.35	16.67	1.95	19.84	3.82	19.86	2.16

was demonstrated except that the concentration of CM in the CSF at 6 hours was less together with the concentration of contrast medium in the grey matter at both 6 and 24 hours (Table 3.2.3; Figure 3.2.3.3 a & b).

In two patients (Patients 10 & 11) no CM was demonstrated in the basal cisterns at 6 hours but at 24 hours, a similar pattern to patients 1-6 was demonstrated apart from a reduced concentration of contrast medium within the parenchyma (Table 3.2.3; Figure 3.2.3.3 a & b). The depth of penetration however was similar.

In one patient (Patient 12) dilute CM was demonstrated in the basal cisterns at 6 hours with slight brain penetration but at 24 hours, no increase in density of the CSF or grey matter compared with the control study could be demonstrated suggesting rapid clearance of the CM (Table 3.2.3; Figure 3.2.3.3 a & b).

In one patient (Patient 13) with obvious ventricular dilatation and cerebral atrophy in the control study, there was marked dense reflux of CM into the lateral ventricles at a similar concentration as that in the basal cisterns at 6 hours, and at 24 hours the subarachnoid CSF and ventricles were obscured by the persisting dilute CM (Figure 3.2.3.4). This patient demonstrated an increase in the density of both the grey and white matter indicating penetration of CM across both the piamater and ependyma (Table 3.2.3; Figure 3.2.3.3 a & b).

Seven of the thirteen patients complained of a mild headache after 6 hours, including five in patients 1-6. By 24 hours, the headache had resolved in all seven patients. Patient number 7 who showed transient

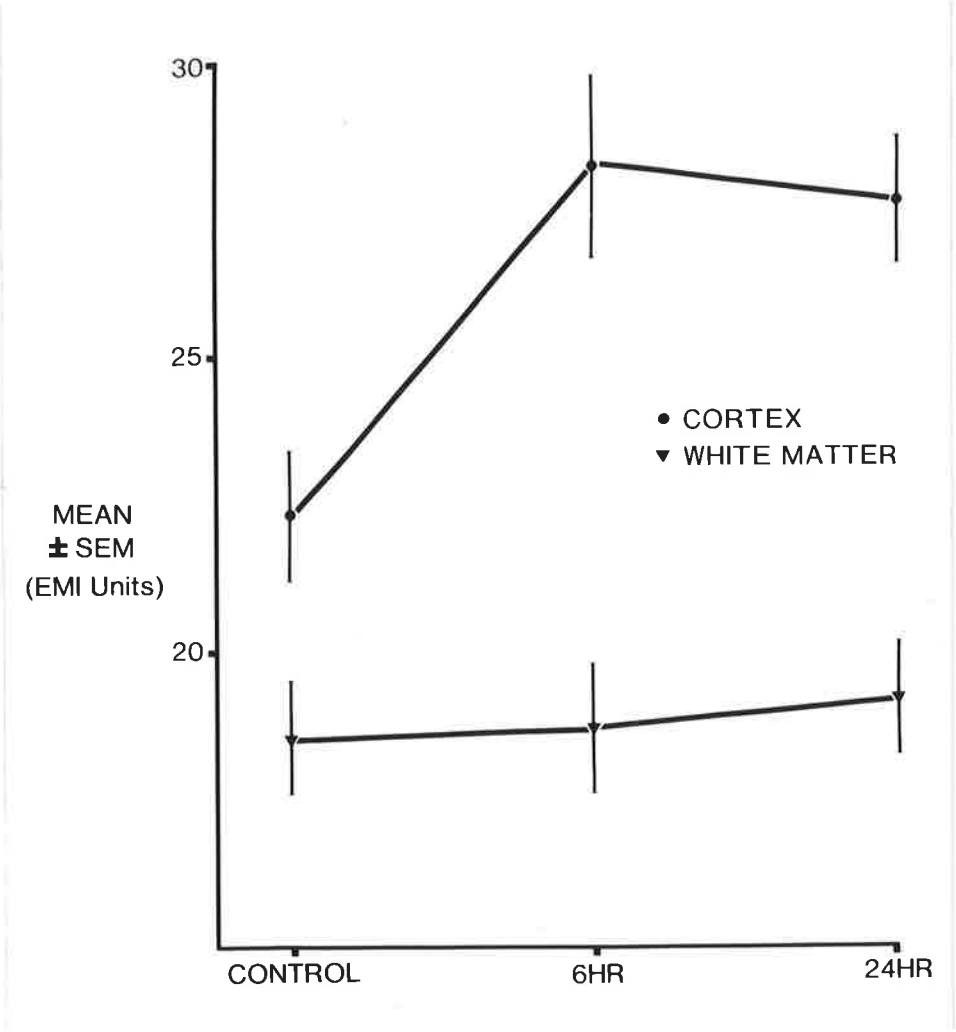


Figure 3.2.3.2. The mean of the mean CT attenuation values (ROI = 100 pixels) in the cortical grey matter and the white matter in patients 1-7. Brain penetration into the cortex is present at 6 hours and this persists without significant reduction in concentration at 24 hours. No significant alteration in the attenuation of the white matter is demonstrated at either 6 or 24 hours.

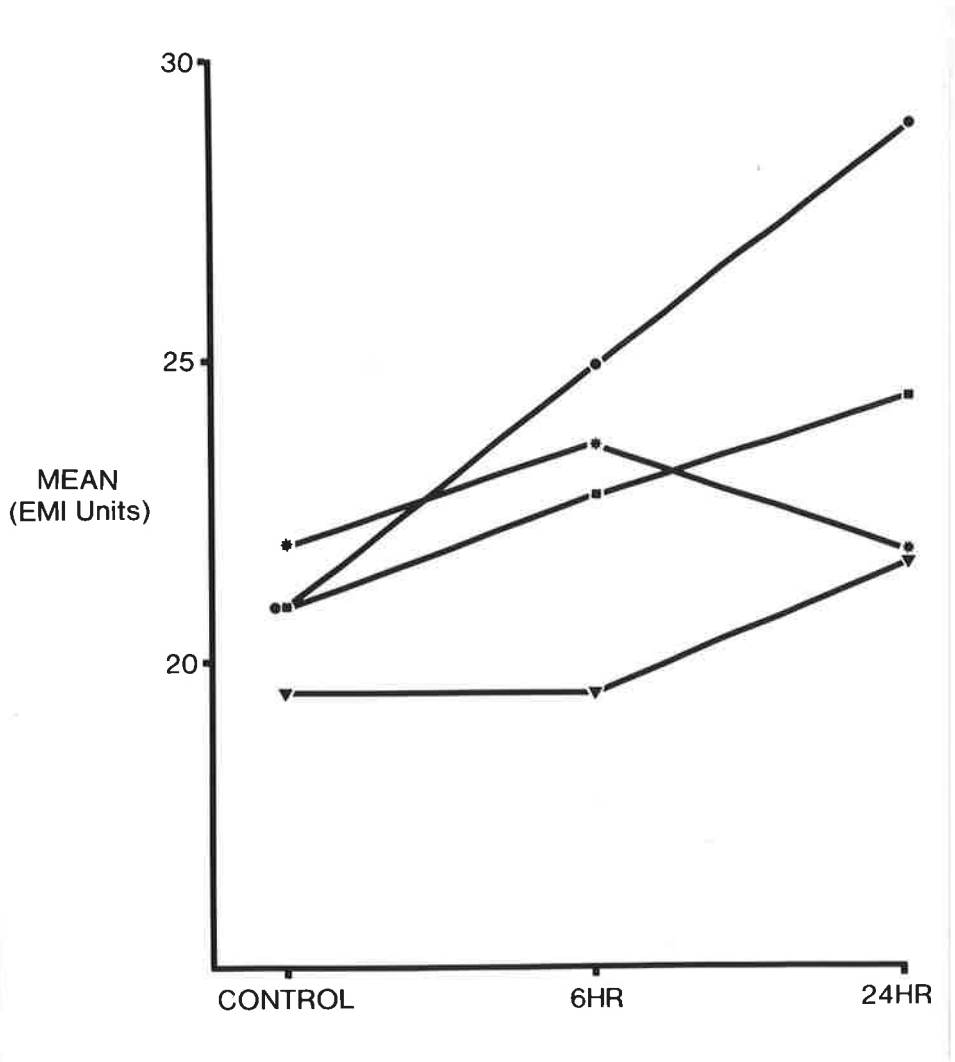


Figure 3.2.3.3a. Mean CT attenuation values (ROI = 100 pixels) in the cortical grey matter of patients 8-13 (■ = patients 8 & 9; ▼ = patients 10 & 11; ✸ = patient 12; ● = patient 13)

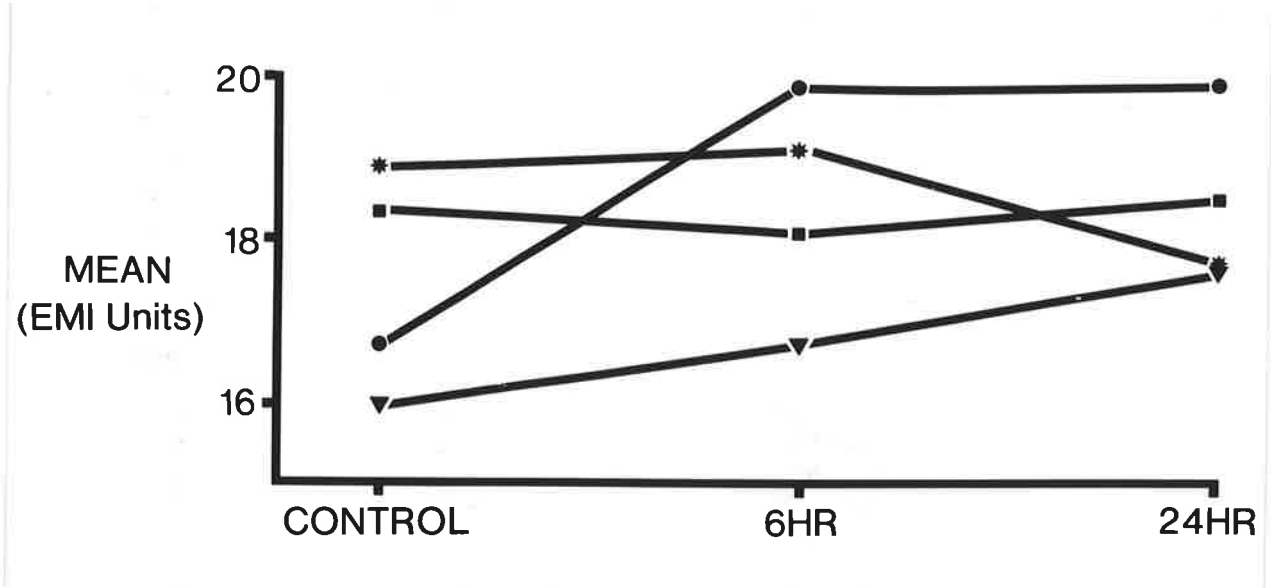


Figure 3.2.3.3b. Mean CT attenuation values (ROI = 100 pixels) in white matter adjacent to the anterior horns in patients 8 - 13 (■ = patients 8 & 9; ▼ = patients 10 & 11; ✸ = patient 12; ● = patient 13).

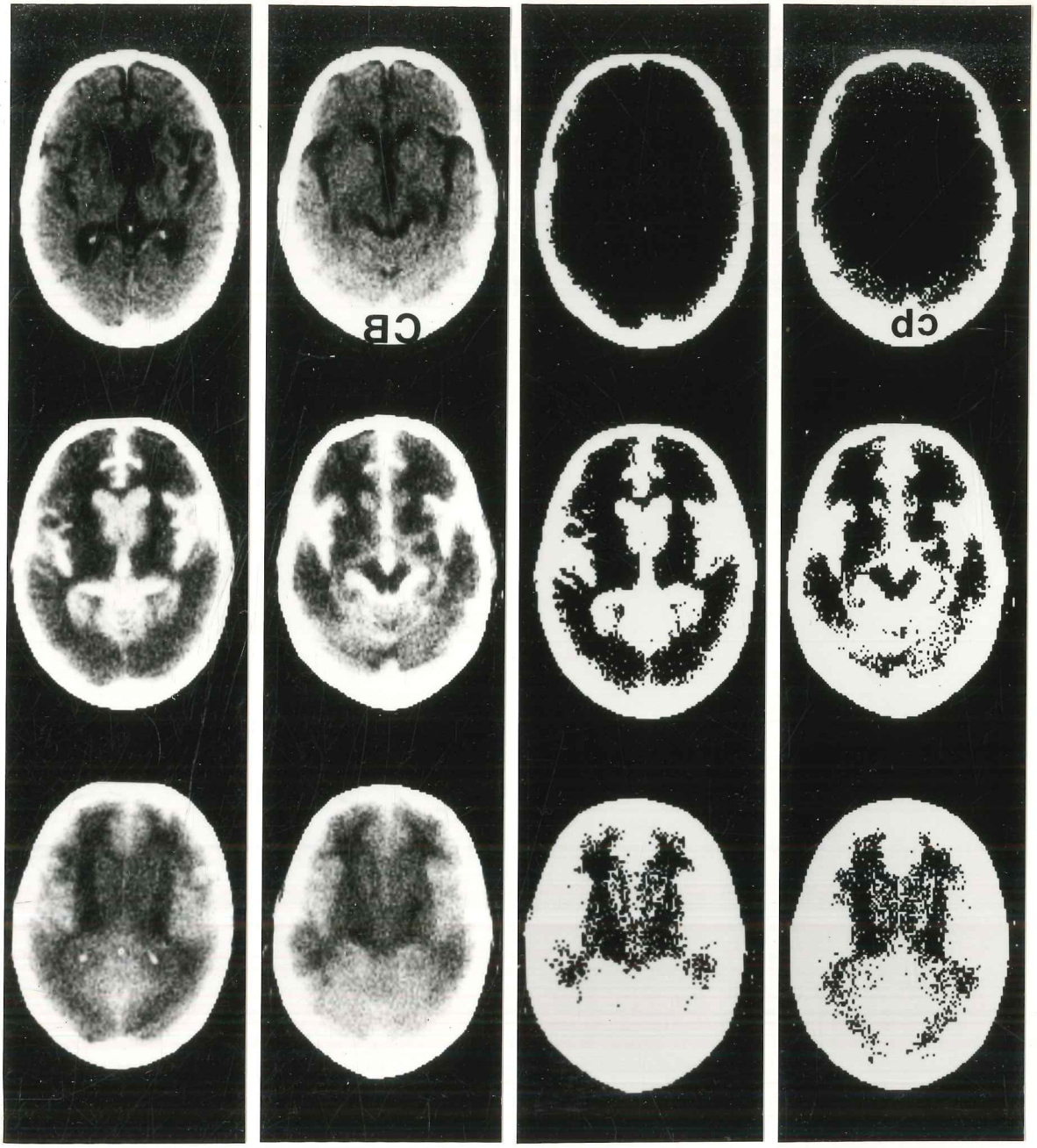


Figure 3.2.3.4. CT scans in patient 13 before and at 6 (middle) and 24 (bottom) hours following lumbar myelography. Gross atrophy is demonstrated and following intrathecal CM dense CM is noted at 6 hours in the basal cisterns, sylvian fissures and ventricular system. At 24 hours, dilute CM obscures the definition of the lateral ventricles and extensive CM penetration into the grey matter persists. Although less prominent, some penetration across the ependyma from the ventricles into the white matter has also occurred.

ventricles at 6 hours had a severe headache with associated vomiting but by 24 hours the severity of the headache was described as slight and the vomiting had stopped. Apart from this patient, only one other patient had a headache, described as mild at 24 hours. Patient number 13 was free from headache at both 6 and 24 hours.

No neurological change from the control examination was demonstrated in any of the thirteen patients at either 6 hours or 24 hours. The electroencephalogram before myelography was abnormal in five of the eight patients in which these examinations were performed. In three patients, the EEG showed a diffuse excess of slow activity, in one a focal excess of slow activity and in the other a diffuse excess of slow activity with episodic tendency. In two patients, the EEG after myelography showed a deterioration. Both patients were in the group of patients 1-6. One showed excess slow activity and an episodic tendency before myelography and more frequent episodic activity 6 hours after myelography. Unfortunately, in this patient the 24 hour EEG was not performed. The other patient had an abnormal EEG before myelography with excess slow activity and the 24 hour examination showed episodic activity. Both patients with deterioration in the EEG after myelography did not develop significant headache, nausea or vomiting.

None of the patients showed a marked increase in the density of the white matter at 6 hours or 24 hours apart from the patient (number 13) with dense intraventricular reflux at 6 hours (Table 3.2.3; Figure 3.2.3.2, 3.2.3.3 a & b). More particularly, there was no decrease in the density of the white matter or subjective change in ventricular size in any of the other twelve patients to suggest the development of cerebral oedema.

3.3 INTRAVENOUS STUDIES

3.3.1 Intravenous CM and CSF-Blood Interface

CSF iodine concentration result s are summarised in Table 3.3.1.1. "Student's t-test showed no significant difference in the mean iodine concentration between the 30 minute and 60 minute samples ($p>0.1$). Although plasma iodine concentrations did fall between the 30 minute and 60 minute collection periods (Table 3.3.1.1), the mean iodine concentration at each sample time was not found to be significantly different ($p>0.1$).

The ratio of CSF iodine concentration to plasma iodine concentration was higher for the 60 minute sample than at the 30 minute sample (Table 3.3.1.2), but this difference was not found to be significant ($p>0.1$).

3.3.2 Intravenous CM and the BBB

No Evans' Blue staining was demonstrated in either series of control studies or in the series involving sodium iothalamate. Results of the three series of experiments are given in Table 3.3.2. No significant difference between the three treatments was detected ($p>0.10$).

TABLE 3.3.1.1 Mean Plasma (mgI/ml) and CSF (ugI/ml) Iodine Concentrations ± SD for All Studies at Each Collection Time

TIME AFTER INJECTION	PLASMA [I] mgI/ml	CSF [I] ugI/ml
30 MINUTES	15.87 ± 1.20	17.30 ± 5.30
60 MINUTES	13.70 ± 2.29	19.25 ± 7.20

TABLE 3.3.1.2 A Comparison of the Mean CSF and Plasma Iodine Concentrations at Each Collection Time

$\frac{\text{CSF } [I]}{\text{PLASMA } [I]}\%$	
30 MINUTES	60 MINUTES
$\bar{\chi} \pm$ SD	$\bar{\chi} \pm$ SD
0.11 ± 0.03	0.15 ± 0.06

TABLE 3.3.2 Mean ratio of counts/gm brain to counts/ml blood ± SD (mls blood/gm brain) for each set of studies, N = 10 in each case. No statistical difference was detected between any of the different treatments ($p>0.1$)

INJECTED SOLUTION	(ml BLOOD/gm BRAIN ± SD)
CONTROL (NO IV INJECTION)	0.0237 ± 0.0046
ISOTONIC SALINE	0.0232 ± 0.0050
SODIUM IOTHALAMATE	0.0261 ± 0.0047

4 DISCUSSION

4.1 WATER-SOLUBLE CM AND THE BLOOD-BRAIN INTERFACE (BBB)

4.1.1 Distribution and Assessment of BBB Disruption Following Intra-arterial Injection of Hypertonic Solutions

Various hypertonic solutions, including water-soluble CM, have been shown to disrupt, or increase the permeability of, the BBB (Section 1.6.5.4). Increased permeability of the BBB following intracarotid injections of various ionic water-soluble CM has been documented in experimental animals (Sections 1.5, 1.6.5.4) and in humans (Sage et al 1981b). Since Broman and Olsson first used Trypan Blue in 1948, various techniques have been developed to demonstrate any increase in the permeability of cerebral capillaries. Evans' Blue has been used for qualitative assessment of BBB disruption for many years (Section 2.1.6). More recently enhancement demonstrated by CT has been shown to reflect alterations in the BBB (Section 1.6.1). CT has the advantage over Evans' Blue staining in that it allows potential in vivo assessment of BBB disruption and also a quantitative rather than a qualitative assessment.

In the studies in Section 2.1, hypertonic solutions of 25% mannitol, and the ionic water-soluble CM, methylglucamine iothalamate, were used to produce breakdown of the BBB. More

intense Evans' Blue staining was found to be associated with increased CT enhancement (Section 2.1.9; Figure 2.1.9). Given the accuracy which tissue contrast enhancement can be measured by CT (Wilson & Evill 1981) it seemed likely that CT enhancement would be a more sensitive measure of BBB permeability than subjective assessment of Evans' Blue staining. Indeed in two studies using mannitol in Section 3.1.1, a low level of CT enhancement was observed where no Evans' Blue staining was detected visually (Tables 3.1.1.1, 3.1.1.2).

Following the injection of hypertonic solutions into the common carotid artery in dogs, disruption of the BBB was not uniform in distribution (Section 2.1.8; Table 2.1.8). Regional disruption occurred in the distributions of the anterior, middle and posterior cerebral arteries. Despite individual variations between animals, the middle cerebral artery territory was involved in all cases where breakdown was demonstrated. Neuwelt et al (1979, 1980) also found variation in disruption following intra-arterial injection of hypertonic solutions in dogs although in several of their studies, the middle cerebral artery was not affected. It would appear that the area perfused by solutions injected into the carotid artery reflects the anatomical variability of the Circle of Willis (Miller et al 1964), therefore accounting for the distribution of the BBB disruption. This variability implies that if selective BBB disruption is contemplated for chemotherapy, as suggested by Neuwelt et al (1979, 1980, 1981), cerebral angiography and selective catheterisation will be required to ensure accurate regional delivery of the hypertonic solutions.

Studies by others (Neuwelt et al 1980), using hypertonic mannitol, suggest that reversible osmotic BBB disruption may increase markedly the delivery of chemotherapeutic agents to the cerebral parenchyma. The studies in Section 2.1.9 confirm that CT enhancement reflects the distribution of BBB disruption and therefore, in the clinical situation, CT enhancement could be used to monitor the effectiveness of BBB disruption produced by hypertonic solutions, such as mannitol, for therapeutic purposes. This would obviously not be possible with Evans' Blue. As a method for estimating BBB disruption, the CT scanner has the advantage of providing a sensitive, numerical measure of the anatomical distribution of BBB disruption, which can be carried out in vivo.

4.1.2 Intra-arterial CM and the BBB

There is not universal agreement about the mechanisms whereby hypertonic water-soluble CM disrupt or increase the permeability of the BBB (Gonsette 1982, Thevenin 1983). The hypertonicity of such CM is thought to cause shrinkage of the endothelial cells with subsequent opening of the tight junctions (Figure 1.2.2.1) while the chemotoxicity of the CM is mainly directed towards the endothelial cell membranes (Gonsette 1982, Thevenin 1983). While there is no clear-cut distinction between the relative specificity of hypertonic versus chemical toxicity, it is agreed that the osmolality of particular solutions appears to be a major factor in determining the disruption of the BBB (Rapoport et al 1971, 1972, 1973, 1974, 1976, 1978; Chieuh et al 1978; Grainger 1982).

Osmotic breakdown was confirmed by the studies using 25% mannitol and the hypertonic, ionic, water-soluble CM, methylglucamine iothalamate (Series I, Section 2.2.2). · Subsequent studies, comparing ionic methylglucamine iothalamate with the non-ionic CM, metrizamide, iopamidol, and iohexol (Series 2, Section 2.2.2) further suggested that the osmolality of particular water-soluble CM is a major factor in producing disruption of the BBB. Using similar iodine concentrations, methylglucamine iothalamate showed consistent disruption of the BBB as demonstrated by both Evans' Blue staining and CT enhancement (Tables 2.1.8, 3.1.1.2), while no evidence of disruption, as demonstrated by these markers, was shown with the non-ionic CM (Tables 3.1.2.1, 3.1.2.2, 3.1.2.3). Similar results were obtained with the non-ionic CM as demonstrated with isotonic saline and this lack of disruption of the BBB was thought to reflect the lower osmolality of these CM compared to a similar iodine concentration of methylglucamine iothalamate (Table 1.4.2.2). The effect of CM on the BBB is not related to the iodine content of the CM, as similar non-iodinated compounds have been shown by others to also produce BBB disruption (Brinker 1979, Bloor et al 1951).

The studies in Series I, (Section 2.2.2) indicated that while osmolality is important in determining BBB disruption, other factors must play a part. Methylglucamine iothalamate, while having a similar osmolality to 25% mannitol (1420 mO/kg and 1524 mO/kg respectively), produced a greater degree of BBB breakdown as reflected by both Evans' Blue staining and CT enhancement (Table 3.1.1.2). Others (Bassett et al 1953, Brinker 1979, Bloor et al 1951) also found that hypertonic glucose, and sodium chloride with

similar osmolalities to particular CM, produced similar but less pronounced effects, indicating that BBB disruption could not be explained by osmotic action alone.

The major effect of CM on the BBB presumably occurs during the initial passage through the cerebral circulation as dilution in the general blood pool would subsequently occur. Contrast media have a viscosity greater than blood and increase the blood viscosity, enhancing rouleaux formation (Bernstein et al 1964). Recent studies by Morris et al (1982) have indicated that viscous solutions, on reaching the micro circulation increase the resistance to flow and cause a transient in blood flow. CM with high viscosities would therefore be expected to increase the transit time of the CM through the micro circulation and hence, their contact time with the endothelium of the cerebral capillaries. In order to determine whether viscosity may be a factor in explaining the difference in the effect of 25% mannitol and methylglucamine iothalamate with a similar osmolality on the BBB, the viscosity of these solutions was measured using a viscometer (Table 2.2.2). The viscosity of 25% mannitol was found to be less than half of that of methylglucamine iothalamate, suggesting that the possible importance of viscosity of particular solutions in determining the effect on the BBB warrants further consideration.

The challenge dose of test solutions used in the studies in Section 2.2.2 was deliberately greater than the dose of CM used during human carotid angiography. During carotid angiography a dose of 8-10mls of CM is delivered in one second and the CM passes

through the cerebral circulation within 5-10 seconds (Sutton 1969, Hilal 1974). The contact time of the CM at its initial concentration with the cerebral capillaries is therefore very brief, lasting for 1-3 seconds. In the canine experimental model, initial observations noted that the pial vessels could be cleared by a test solution dose rate of 1.5mls/sec into the common carotid artery (Section 2.1.3) and this was maintained over 30 seconds. Therefore in the studies in Section 2.2, the CM was maintained in contact with the cerebral capillaries at a high concentration for 30 seconds. Despite this, the non-ionic CM, metrizamide, iopamidol and iohexol, like isotonic saline, failed to lead to any breakdown in the BBB whereas ionic methylglucamine iothalamate consistently led to breakdown. The studies indicate that during conventional carotid angiography, breakdown of the BBB by the new non-ionic CM would be extremely unlikely.

4.1.3 Intravenous CM and the BBB

After an intravenous bolus injection of CM, there is a rapid distribution of the CM throughout the vascular and extravascular spaces of the non-neural tissue (Kormano 1981). Peak blood levels are reached almost immediately after injection and there is a rapid fall during the next two minutes as the CM equilibrates between the plasma and the extracellular fluid (Section 1.4.3). of the non-neural tissue (Cattell et al 1967, Kormano 1981). The CM is rapidly and extensively distributed outside the blood vessels into volumes approaching that of the extracellular fluid (Section 1.4.3). In contrast to a direct intracarotid injection of CM, normal brain tissue is thought to be protected from

intravenous CM by the BBB because of the initial dilution in the blood pool. This assumption has led to the recommendations to use increasingly larger doses of intravenous CM for CT enhancement (Hayman et al 1979, Raininko et al 1982, Davis et al 1979, Hayman et al 1980). Although Paling (1979) concluded that the administration of doses larger than 25.2gm of iodine do not provide any useful diagnostic information during CT, doses of CM as high as 80gm of iodine have been recommended (Hayman et al 1980). Presumably, such authors assume that large intravenous doses of CM, while increasing the osmolality of blood, do not affect the normal BBB in the same way as intracarotid injections of the same solutions.

The normal slight attenuation of the cerebral parenchyma demonstrated by CT following intravenous CM is thought to represent the CM within the cerebral blood volume, with the CM remaining within the vascular bed, in the absence of pathology (Section 1.6.1). In 1978 Caille et al studied the attenuation of the cerebral parenchyma after intravenous injection of CM with different osmolalities. They concluded that iodine CM do not remain totally within the intravascular space and that leakage across the BBB is probably related to the lipo-solubility of the solutions rather than their osmolality. However, this same group subsequently agreed that the normal BBB does render cerebral capillaries impermeable to CM (Caille et al 1980).

Recently Zamani et al (1981) demonstrated increased permeability of the BBB in dogs using both qualitative Evans' Blue and quantitative CT assessment. Their studies showed that a dose

level of 1.13gI/kg appeared to break down the BBB in some dogs although there appeared to be considerable variation. In particular, breakdown was rather patchy and was demonstrated in only 3 of 10 animals. Limited electron microscopic studies showed no definite passage of horseradish peroxidase through tight junctions into the extravascular compartment but some swelling of astrocytic foot processes was demonstrated in one animal. Recently increased vesicular transport (pinocytosis) has been demonstrated in the cerebral capillaries following intravenous CM in other experimental animals (Burns et al 1980).

The studies in Section 2.7.2 were undertaken to assess the effect of a high dose of intravenous CM on the integrity of the BBB in rabbits. Despite a very large intravenous dose of ionic sodium iothalamate (3.36gI/kg), no obvious increase in the permeability of the BBB to Evans' Blue or 99mTc-pertechnetate was demonstrated. Minor morphological alterations in the normal cerebral capillaries could not be excluded, nevertheless the integrity of the BBB appeared to be maintained despite this large CM load. Although doses of CM approaching and even surpassing 1.23gI/kg body weight have been given for CT (Hayman 1980) , this dose was clearly exceeded in the rabbit studies without resulting in qualitative or quantitative evidence of BBB disruption.

A retrospective study (Pullicino & Kendall 1980, Kendall & Pullicino 1980) suggested that the prognosis of cerebral infarction is worse in patients who have undergone enhanced CT. It does suggest the possibility that the presence of water-soluble CM within the extracellular fluid of the brain may have a direct

toxic effect and hence the studies in Section 2.7.2 have been important to establish that the integrity of the normal BBB appears to be maintained in the presence of a large intravenous CM load.

4.1.4 Intrathecal CM and the BBB

Studies performed in Section 2.4, clearly demonstrated that both ionic and non-ionic water-soluble CM freely cross the CSF-brain interface following intrathecal injection. Presumably, such extracellular CM comes in contact with the brain side of the blood-brain interface (Figure 1.3.1.1). In these studies, Evans' Blue was injected intravenously to allow qualitative assessment of any gross alterations in the permeability of the normal BBB due to the presence of such CM within the extracellular fluid of the brain parenchyma. In none of the studies was there any evidence of Evans' Blue staining, even in the areas of obvious brain penetration demonstrated by CT. This would suggest that the presence of such CM does not in fact lead to gross disruption of the BBB, as assessed qualitatively by Evans' Blue.

4.2 WATER-SOLUBLE CM AND THE CSF-BRAIN INTERFACE

Unlike the BBB between cerebral capillaries and the brain parenchyma, no obvious barrier exists at the pial surfaces to the passage of small water-soluble molecules and evidence suggests that they enter the brain parenchyma by simple diffusion into the extracellular space (Section 1.3.1). In particular, brain penetration by the water-soluble, non-ionic CM, metrizamide, after intrathecal, injection has been previously documented experimentally and clinically after intrathecal injection (Section 1.7.2). It has been suggested that the complications following intrathecal metrizamide are directly related to such cerebral penetration.

The neurotoxicity of contrast media is thought to be related to both their chemical structure and osmolality (Section 1.5). Water-soluble ionic meglumine iothalamate (Conray 280) is known to be more neurotoxic than non-ionic metrizamide, at the same iodine concentration. Therefore studies were carried out to determine whether this was related to a difference in brain penetration. The studies in Section 2.4.3 confirmed that there is free passage of both methylglucamine iothalamate and metrizamide across the CSF-brain interface. CT measurements have been shown to be a valid method of quantitative comparison of CM density (Wilson & Evill 1981). The solutions of the two CM injected intrathecally had a similar iodine concentration and, after 60 minutes, a similar concentration of iodine, as measured by CT, was obtained with both within the brain parenchyma (Section 3.2.1; Tables 3.2.1.2, 3.2.2.1). These studies would therefore indicate that the increased toxicity of intrathecal ionic CM is not related simply to the iodine concentration reached

within the brain parenchyma as suggested by others (Section 1.7.2) but to molecular structure. The depth of brain penetration after one hour, as measured in the temporal region, was similar for both methylglucamine iothalamate and metrizamide (Section 3.2.1). Therefore, despite the significant difference in osmolality between the ionic and non-ionic CM (Table 1.4.2.2), a similar rate of diffusion across the CSF-brain interface into the extracellular brain tissue was demonstrated.

Studies were carried out to compare the degree and depth of brain penetration of intrathecal non-ionic iopamidol at 15 and 60 minutes, using an iodine concentration of 280mgI/ml (Section 2.4.4). After 60 minutes, the depth of penetration, and the iodine concentration within the brain parenchyma, of iopamidol was similar to that demonstrated with both methylglucamine iothalamate and metrizamide (Tables 3.2.1.2, 3.2.2.1). Although initial clinical trials suggest that intrathecal iopamidol is less neurotoxic than metrizamide (McAllister & Hall 1983, Drayer et al 1982) these results indicate that neurotoxicity is not related to a variable rate of brain penetration or brain concentration but to the molecular structure of the particular CM itself.

Marked brain penetration by intrathecal iopamidol was clearly demonstrated at 15 minutes (Section 3.2.2) indicating that once in contact with the brain surface, intrathecal CM rapidly crosses the CSF-brain interface. Although the maximum depth of penetration by iopamidol at 15 and 60 minutes was similar (Section 3.2.2), the distribution of CM at 15 minutes was very patchy, corresponding to the sites of the cortical sulci (Figures 2.4.1.1, 2.4.1.2). At 60

minutes, the distribution was more uniform and the iodine concentration was greater indicating that progressive penetration of CM had occurred up to 60 minutes following intrathecal injection (Table 3.2.2.1). The study shows that there is a rapid and progressive diffusion of water-soluble CM across the CSF-brain interface after intrathecal injection at least up to 60 minutes.

While brain penetration by metrizamide after intrathecal injection has been well documented clinically (Drayer & Rosenbaum 1977, Tamaki et al 1979, Hammer 1980, Manelfe & Chambers 1981, Hammer 1981), in most studies, deliberate positioning of the patients was used to encourage the passage of metrizamide into the basal cisterns to overcome the variability of CSF movement due to the absence of an active CSF circulation in the spinal subarachnoid space (Section 1.3.2). A limited clinical study was therefore carried out to confirm that brain penetration by metrizamide routinely occurs following lumbar myelography (Section 2.5). Patients were nursed with their head and trunk elevated and rapid passage of CM cranially was discouraged both during and after the procedure.

Despite this, CM was demonstrated in the basal cisterns in the majority of patients by 6 hours and penetration into the cortical grey matter was clearly demonstrated at this time (Figures 2.5.2, 3.2.3.2; Table 3.2.3). In the majority of patients, the CM was obvious within the basal cisterns, indicating a significant concentration, while in others it was more dilute or even absent. This variability was thought to reflect the lack of any active CSF circulation in the spinal subarachnoid space (Section 1.3.2). The penetration of CM into the brain parenchyma appeared to reflect the

concentration of the CM within the basal cisterns (Figures 3.2.3.2, 3.2.3.3). Patients with brain penetration at 6 hours showed a progressive penetration by CM into the grey matter during the next 18 hours (Figures 2.5.2, 3.2.3.1). At the same time, the concentration of CM in the grey matter did not change significantly from the 6 hour measurements (Table 3.2.3; Figure 3.2.3.2).

Despite obvious penetration by CM into the cortical grey matter at both 6 and 24 hours, no patient showed any obvious change in the density of white matter to suggest deep penetration by the CM (Table 3.2.3; Figure 3.2.3.2), apart from one patient with cerebral atrophy who showed dense reflux of CM into the lateral ventricles indicating an abnormal CSF circulation. In particular, there was no obvious decrease in the density of white matter in any of the patients to suggest that oedema had developed as a result of the brain penetration by CM.

It has been reported that the time sequence and nature of adverse reactions following intrathecal metrizamide correlates closely with CM penetration into the superficial brain substance (Drayer & Rosenbaum 1977, Drayer et al 1977, Caille et al 1980, Cala 1982). The number of patients included in the clinical study in Section 2.5 was limited and therefore it is difficult to draw firm conclusions about any association between morbidity and brain penetration by CM. However, although brain penetration by CM obviously occurs routinely following lumbar myelography, despite elevation of the head, the incidence of headache is variable and does not appear to be related specifically to such penetration. Although many neurological sequelae have been reported following intrathecal metrizamide (Picard

et al 1979, Rubin et al 1980, Schmidt 1980, Bertoni et al 1981), many of these followed deliberate rapid intracranial introduction of the CM. Although obvious brain penetration by CM was demonstrated following lumbar myelography (Section 2.5), no obvious neurological abnormalities were demonstrated.

Presumably, the dilution of the CM within the subarachnoid space is important in protecting the brain parenchyma, as once the CM is in contact with the CSF-brain interface, a rapid diffusion into the brain parenchyma itself occurs. The concentration within the brain parenchyma has been shown to reflect the concentration within the CSF and such a concentration persists at 24 hours with an increase in the depth of penetration. In particular, the incidence of headache does not appear to be related to such penetration. Changes in EEG findings have been demonstrated after metrizamide brain penetrance (Drayer & Rosenbaum 1977, Lundervold & Sortland 1977, Hammer 1981). However, several patients in the studies in Section 2.5 showed no obvious change from their pre-myelographic EEG findings, despite gross CM brain penetration.

4.3 WATER-SOLUBLE CM AND THE BLOOD-CSF INTERFACE

The studies in Section 2.7.1 were performed to determine the degree of transfer of water-soluble CM across the blood-CSF interface after intravenous injection. The results confirm the findings of McClennan and Becker (1971) that CM is demonstrated in the CSF after an intravenous bolus. However, only a very small amount of CM was demonstrated in the CSF at 30 minutes and 60 minutes despite the presence of high blood levels of CM (Tables 3.3.1.1, 3.3.1.2).

The interface between the blood and CSF occurs principally in the choroid plexus (Figure 1.2.4.1), being richly vascularised epithelial tissue that projects into each of the four major ventricles (Section 1.6.3). Unlike the cerebral capillaries, capillaries of the choroid plexus are fenestrated and therefore allow ultra-filtration of plasma (Potts et al 1977, Davson 1967) and the passage of other substances such as CM between the blood and the choroidal interstitium. The interstitium on the other hand is enclosed by a specialised epithelium of cuboidal cells resting upon a thin basement membrane and each cell is joined to its neighbour at the apical surface by tight junctions (Section 1.6.3; Figure 1.2.4.1). The epithelium forms an imperfect barrier between the CSF and the choroidal interstitium, restricting the passage of solutes, and of CM in and out of the CSF. The passage of substances from blood to CSF depends largely on their lipid-solubility (Almen & Golman 1979). Therefore, as water-soluble CM have very low lipid solubility, their CSF:blood ratio after intravenous injection would be expected to be low. This is confirmed by the studies in Section 2.7.1.

Another potential site of exchange between the CSF and blood is the arachnoid membrane (Figure 1.2.4.1) but the outermost layer of the arachnoid has capillaries with tight junctions that do not allow the free passage of solutes, including CM (Section 1.6.3). A secondary site of possible exchange between blood and CSF is via the brain tissue capillaries (Davson 1978). If materials actually escape across the BBB into the brain extracellular fluid they may diffuse freely across the ependyma or pia mater into the CSF (Section 1.6.3). However, the studies in Section 2.7.2 suggest that there is no increased permeability or disruption of the BBB despite a large

intravenous dose of CM.

McClennan and Becker (1971) demonstrated a CSF:blood ratio of 0.05 at one hour following intravenous ionic iothalamate in dogs. A ratio of 0.04 at one hour has been demonstrated by intravenous non-ionic metrizamide in rabbits (Golman & Dahl 1973). The results in Section 2.7.1 confirm these findings that there is only a minimal passage of water-soluble CM between blood and CSF and therefore the suggestion that intravenous CT myelography may be possible (Coin et al 1979) is incorrect.

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