

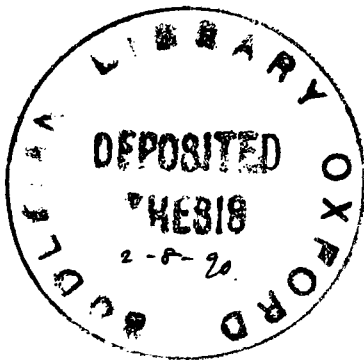
**Nervous system metabolism:
A magnetic resonance study.**

Submitted in partial fulfilment of the requirements for the
degree of Doctor of Philosophy

Thomas Anthony Daniel Cadoux-Hudson

New College

January 1990



To my parents who nurtured my insatiable curiosity,

To Helen who has sustained me

To Alexander and Daniel who have delightfully distracted

Nervous system metabolism: A magnetic resonance study.

Thomas A. D. Cadoux-Hudson, New College, January 1990.

The aim of this thesis was to investigate the cellular biochemistry and metabolism of the human brain *in vivo* using magnetic resonance as the basic technique. Magnetic resonance imaging (MRI) can provide images of human structure and has already proved to be diagnostically useful in Neurology. Magnetic resonance spectroscopy (MRS) has the potential for measuring the tissue metabolite concentrations and metabolic rates of intracellular reactions *in vivo*. The great advantage of MRS is that many elements already present in abundance can be used to follow these intracellular reactions; no alien compounds need to be injected into the subject in the hope that they will eventually enter the tissue under investigation. However there are still considerable problems in receiving signal from the region of interest. No single approach in MRS has proved to be suitable for all investigations.

An existing technique in MRS, phase modulated rotating frame imaging (PMRFI) was extended to measure absolute tissue concentration and enzyme flux rates of intracellular compounds containing phosphorus nuclei at above 1mmol/L tissue concentration in tissue volumes greater than 10ml. These technical limitations restricted the work of this thesis to the human cerebral hemispheres.

Adenosine triphosphate (ATP) and phosphocreatine (PCr) are essential cytoplasmic compounds, providing energy for transport and biosynthetic pathways within the cell. Phosphorus MRS can also measure intracellular pH (pHi), providing an insight into ion metabolism within the cell. Finally ^{31}P MRS can also measure the concentration of certain phospholipid groups and their precursors, phosphoethanolamine (PE) and phosphocholine (PC).

The initial work carried out involved the construction, testing and modification of a probe suitable for clinical work. Studies were performed on subjects to establish a normal range for absolute tissue concentrations and enzyme flux rates through creatine phosphokinase. Studies on patients with primary brain tumours, acromegaly, herpes simplex encephalitis, HIV infections and those recovering following severe head injury were studied. Consistent changes in pHi, high energy phosphate and phospholipid metabolism were found in these conditions. The probable mechanisms underlying these changes are discussed and further investigations suggested.

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Dr Peter Styles and Dr Martin Blackledge have developed PMRFI as a technique, which has proved to be sensitive enough for ^{31}P MRS *in vivo*, and robust enough for a fledgling surgeon to use as a clinical technique. Their patience, advice and guidance have, despite severe testing, been limitless.

Dr Bheeshmal Rajagopalan has provided invaluable support throughout this work, bridging the enormous gap between innocent ideas and pragmatic research. Dr Doris Taylor has also helped with many of the projects described in this thesis.

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CHAPTER 1

INTRODUCTION TO NERVOUS SYSTEM METABOLISM: A MAGNETIC RESONANCE STUDY.

Investigation into the structure, function and metabolism of the mammalian nervous system has proved to be a great challenge. A considerable body of knowledge exists describing the crude structure of the nervous system in all species, especially man (Williams and Warwick, 1980; Bo et al, 1980). Much attention has been paid to neurotransmission which directly links metabolism with function. Many eminent texts exist (Siesjo, 1979; Bradford, 1985; McIlwain and Bachelard, 1985) covering our current knowledge of mammalian neurochemistry. This study is primarily concerned with the intracellular metabolism of the human nervous system *in vivo*.

Central to intracellular metabolism is adenosine triphosphate (ATP) which transfers energy produced by oxidative phosphorylation to sites of energy utilization for transport and biosynthesis. The turn over of ATP in a mammalian cell is rapid, making analysis of cellular levels *in vivo* difficult by standard "wet" biochemical techniques assaying biopsy samples, since some of the ATP will have been consumed by the time of assay. Research into factors regulating ATP metabolism, as with many other aspects of intracellular metabolism, has been limited by lack of techniques which can measure levels of intracellular compounds *in vivo*.

Magnetic resonance spectroscopy (MRS) was developed in the late 1970's with the aim of eventually studying intracellular metabolism *in vivo*. The phosphorus atom (^{31}P) was initially selected because narrow peaks define individual intracellular compounds, similar to the use of radioactive labelled compounds for the study of metabolism *in vivo*. ^{31}P Phosphorus is a natural magnetic resonance label, representing

99% of all phosphorus in biological systems; no alien radioactive compounds need to be introduced into the subject. MRS has many advantages in studying human intracellular metabolism in organs where obtaining fresh tissue samples is difficult and accurate assay of certain compounds is impossible. However MRS requires large volumes of tissue (minimum 10cm³) containing compounds above 1mmol/L before a clear signal can be received with current technology, limiting the application of MRS to macroscopic studies of large organs such as brain, liver, and skeletal muscle (Radda, 1986). This thesis was aimed at extending an existing technique in MRS, phase modulated rotating frame imaging (PMRFI) (Blackledge et al, 1987), to measure absolute tissue concentrations and metabolism of ATP and other phosphorus containing compounds in normal human brain *in vivo*. The small size of peripheral nerves and spinal cord makethese structures difficult to study with MRS at present.

2.0 Human brain metabolism

2.1 Structure

A short overview of human nervous system structure is a useful precursor to a discussion of metabolism. Many extensive reviews exist (Williams and Warwick, 1980) describing gross anatomy and histology. The cells in the human brain can be crudely divided into two groups; neurons and supporting cells. The neuron has some characteristic tasks to carry out. The most fundamental task is the generation, modification and conduction of an electrical impulse. This function must be carried out reliably and in a controlled fashion.

The support cells in the human brain fall into two groups; glial cells and the rest. The adult human brain has twice as many glial cells as neurones (Tower and

Young, 1973), subdivided morphologically into astrocytes, oligodendrocytes and ependymal cells. The astrocytes predominate near neural cell bodies (cortex and deep nuclei of the cerebral hemispheres). Oligodendrocytes are concerned with maintenance of the neural axons and are found primarily in the white matter of the human brain. The increase in myelin lipids about the neural axons is thought to produce the white colour of "white matter" (Rumsby, 1978).

The "rest" of the cells forming the structure of the human brain comprise a variety of cell types from vascular endothelial to meningeal cells. These cells also play specific roles in human brain metabolism. The vascular endothelial cells form part of the blood brain barrier.

The gray and white matter have a similar water content, 80% in gray matter and 70% in white matter (Gore, 1982) with 15-25% extracellular space. In the grey matter (Bourke et al, 1965). The total number of cells per unit wet weight is similar between gray matter (128,000/mg) and white matter (135,000/mg) (Korey and Orchen, 1959), suggesting that the total intracellular volume is also similar between gray and white matter. However the cell populations between these two regions differ. The gray matter cells are; neurones (40%), astroglia (20%), oligodendrocytes (20%), and vascular endothelial cells (20%) (Siesjö, 1979). Whilst the cells of white matter are mainly oligodendrocytes (60%) with some astrocytes, neural axons (20%) and vascular endothelial cells (20%). These figures are crude, and are used to give a macroscopic picture of cell types in human brain.

³¹P MRS is a macroscopic technique in human studies requiring at least 10cc of tissue to achieve an interpretable signal, sampling a mixture of neuronal and glial

cells. The best compromise would be a technique which could discriminate macroscopic structures in the cerebral hemispheres such as gray from white matter in normal brain. Sampling from normal into pathological conditions may also be of use when trying to interpret metabolic changes due to altering cell types such as normal brain into tumour.

1.2.2 Metabolism

Intracellular metabolism is driven by the energy contained in adenosine triphosphate (ATP), used for transport and biosynthetic pathways (Figure 1.1). The brain utilises glucose for the majority of its energy supply taken up from circulating blood, as demonstrated by experiments using brain slices (Cox et al, 1983), arteriovenous difference (Himwich and Himwich, 1946) and positron emission tomography (Reivich et al, 1979) studies in man. Oxidative phosphorylation releases the energy available in the glucose molecule which is converted into adenosine triphosphate (ATP) via the glycolytic and mitochondrial systems.

The energy in ATP drives transport and biosynthetic pathways in neurones and glial cells. Transport pathways are present in the cytoplasm and its membrane. Membrane transport systems have been widely studied, with the sodium/potassium ATPase (Na^+/K^+ ATPase) creating the electrochemical difference used to conduct impulses across all mammalian cells, including neurones and glial cells. The Na^+/K^+ ATPase may consume approximately 30% of the ATP consumption of human brain (Whittam, 1962). The negative charge inside the cell (-60 to -80mv) will attract in to the cell extracellular cations (Na^+ , H^+), generating a theoretical difference of 1pH unit difference between intracellular (6.3pH units) and extracellular pH (7.3-7.4pH units).

Figure 1.1

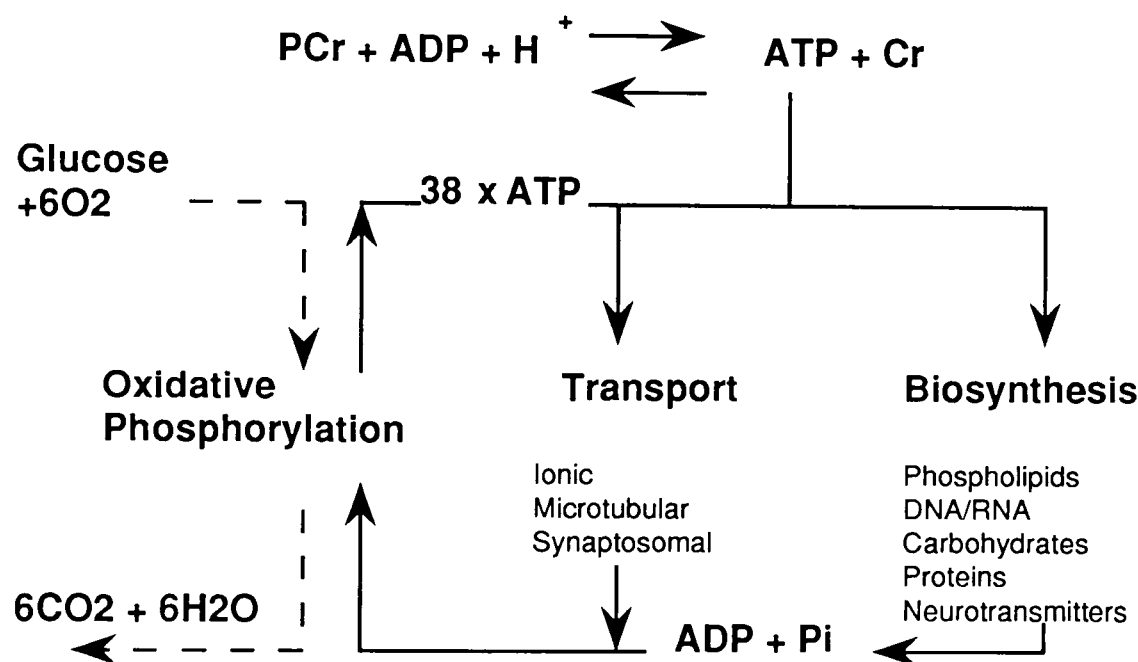


Figure 1.1 Oxidative phosphorylation transfers energy to adenosine triphosphate (38 ATP for each glucose molecule). Transport and biosynthetic pathways are driven by the energy released from the breakdown of ATP to adenosine diphosphate (ADP) and inorganic phosphate (Pi).

The observed intracellular pH *in vivo* has been recorded by various techniques at between 6.99-7.05pH units (Brooks et al, 1986; Taylor et al, 1983; Siesjö, 1979). To maintain a negative intracellular charge and a pHi of 7.0 the cell must consume energy to remove hydrogen ions. The Na⁺/H⁺ antiport exchanger has been identified on both neurones (Jean et al, 1985) and glial cells (Kimelberg et al, 1979) and probably serves to maintain pHi at 7.05pH units. In addition there are both active and passive intracellular buffering mechanisms which have been extensively reviewed (Roos and Boron, 1981; Lowe and Lampert, 1983; Mahnensmith and Aronson, 1985). All these mechanisms are designed to maintain a constant intracellular pH (pHi).

³¹P MRS can measure intracellular pH (Taylor et al, 1983), since the chemical shift of inorganic phosphate (Pi) titrates with pH, but phosphocreatine does not within a physiological pH range (6-7.6pH units). This approach assumes that all the Pi is within the intracellular compartment, and that the extracellular space is small. The extracellular space in the human brain is small (10-20% of total volume) as measured by physiological techniques (Bourke et al, 1965; Levin et al, 1970), contributing an insignificant Pi signal in ³¹P MRS.

Classical theories on tumour metabolism (Warburg, 1931; Kahler and Robertson, 1943) predicted an acidic tumour pHi due to large lactate production. These views have dominated clinical approaches to tumour therapy for the last 40 years. However recent reports on primary brain tumour metabolism using PET (Brooks et al, 1985) and ³¹P MRS (Oberhaesli et al, 1986) have shown that the pHi was alkaline (7.1pH units) or normal (7.03pH units).

Many aspects of cellular metabolism such as amino acid and carbohydrate metabolism have not been covered in this short summary, which has been biased towards the narrow biochemical window permitted by ^{31}P MRS. Despite this narrow biochemical window ^{31}P MRS has the proven potential of providing a unique insight into human cellular metabolism.

1.3.0 **Magnetic resonance**

Advances in our understanding of atomic structure were made in the years between the Great World Wars. Experiments into the electromagnetic properties of matter were being performed in the late 1930's (Kellogg et al, 1939). After the Second World War two letters appeared describing observations on the ability of water to absorb electromagnetic energy at a specific frequency (Bloch et al, 1946; Purcell et al, 1946). Different methods can be used to describe these findings; quantum mechanics or a classical description using Newtonian physics. The quantum mechanical description provides the most accurate analysis of nuclear physics (Abragham, 1961), although the classical description will be used extensively in this text.

Magnetic resonance was used by analytical chemists for spectroscopy in the late 1960's (Becker, 1969). Biochemists started applying magnetic resonance spectroscopy (MRS) to biology systems in the 1970's (James, 1975). The first imaging experiments were performed (Lauterbur, 1973) at this time, quickly followed by early human studies (Mansfield and Maudsley, 1977). The 1980's have seen an explosion in both structural and metabolic imaging of man.

The principles of magnetic resonance are similar for magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI). Furthermore the localizing techniques in magnetic resonance also have similar principles. A short summary will be used to introduce these principles.

Magnetic resonance depends on the presence of two nuclear properties; charge and spin. The combination of these two properties gives the atomic nucleus magnetization. The Larmour equation defines the frequency of spin;

$$\omega = \gamma \cdot B_0 \quad (a)$$

where ω is the precessional (Larmour) frequency

γ is the gyromagnetic ratio

B_0 is the magnetic field strength.

The precessional frequency is directly related to magnetic field strength, since the gyromagnetic ratio ("magnetism" of the nucleus) remains constant. Localization in MRI and chemical information in MRS are derived from the direct relationship between precessional frequency and magnetic field strength.

All the nuclei in the sample will align in the magnetic field (B_0), some in the direction of the B_0 field (low energy) and some in the opposite direction (high energy) (Figure 1.2). The number of nuclei recruited to align in the direction of the B_0 field is determined by the Boltzman equation;

$$\frac{\text{High energy}}{\text{Low energy}} = \exp(-\gamma \cdot h \cdot B_0 / kT) \quad (b)$$

where h is Planck's constant

k is Boltzman's constant

T is absolute temperature

The greater the magnetic field strength, or lower the temperature, the more nuclei align in the magnetic field (low energy), increasing the signal acquired in the magnetic resonance experiment.

The aligned nucleus must be perturbed so that energy is released whilst the nucleus relaxes back to the axis of the magnetic field. A radio frequency (r.f.) pulse oscillating sinusoidally with the precessional (Larmour) frequency is used to perturb the aligned nucleus. The nucleus now precesses at an angle to the B_0 field, creating a weak electromagnetic force which can be detected as small oscillating current in an loop of copper wire, called a free induction decay (f.i.d.) (Figure 1.2). The amplitude is determined by the number of relaxing nuclei and the angle to which they were tipped by the perturbing r.f. pulse.

The time taken for the nucleus to relax back fully (Figure 1.3) into the original low energy alignment (T , relaxation time) can vary for two reasons; energy lost to the surrounding sample (spin-lattice or T_1 relaxation time) or energy lost to other similar nuclei at slightly different frequencies (spin-spin or T_2 relaxation time). The two relaxation times (T_1 and T_2) are determined by properties of the nucleus and its surrounding physical environment. Changes in relaxation times of the proton of water are the basis for contrasting differences in normal and pathological structure in human tissues *in vivo* (Figure 1.4), making MRI a useful diagnostic procedure.

Figure 1.2

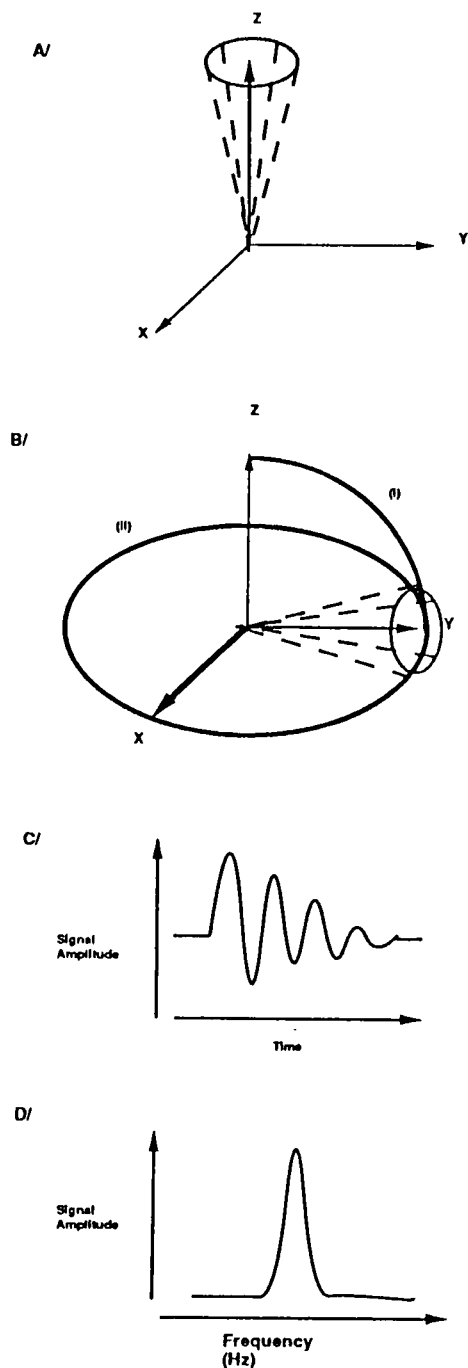


Figure 1.2 A number of nuclei precess about the Z axis of the magnetic field (A). Application of a radio frequency pulse along the X axis (B(i)) will cause the nuclei to tip into the ZY plane (B(ii)). After the excitation pulse has finished, the nuclear magnetization induces an exponentially decaying sinusoidal pulse (free induction decay, F.I.D.) in a nearby receiver coil (C). A Fourier transform can be used to convert the F.I.D. into a spectrum (D).

Figure 1.3

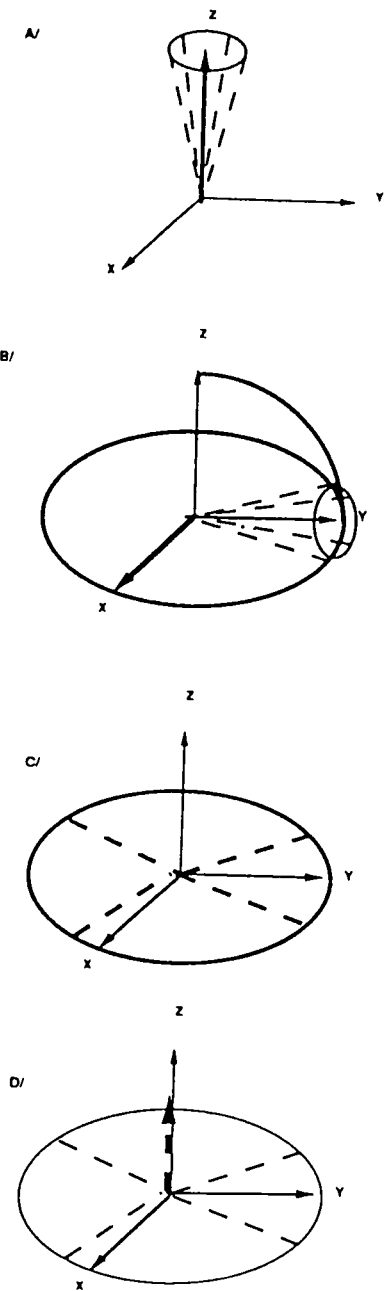
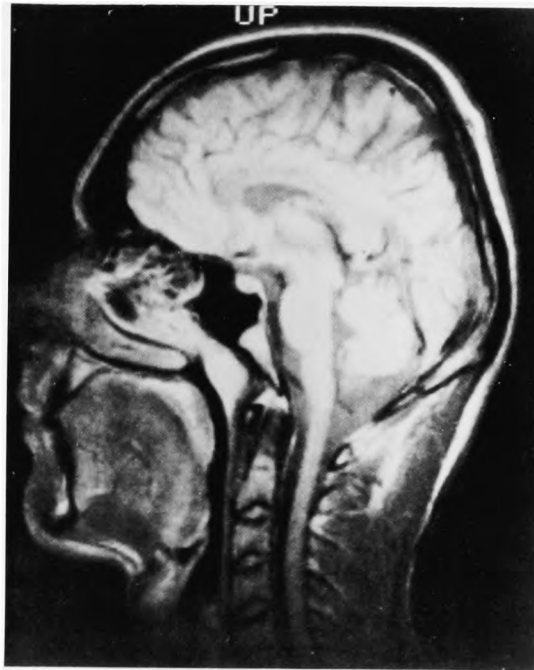


Figure 1.3 Nuclei precess in the Z axis without coherence (A). Following a radio frequency pulse along the X axis (B), the nuclei precess with coherence which is lost (C) with T_2 relaxation, whereas with T_1 relaxation (D) nuclei return to the Z axis.

Figure 1.4

A



B



Figure 1.4 Magnetic resonance imaging (MRI) can be used to image the normal human brain (A), and pathological lesions such as primary brain tumours (B).

(from General Electric Co, USA)

1.3.1 Magnetic resonance spectroscopy

Magnetic resonance spectroscopy is based on the principle that the chemical bonds formed by different compounds will alter the precessional frequency of the nucleus. The surrounding electron cloud of chemical bonds will alter the static magnetic field strength experienced by the nucleus (A) (Figure 1.5), altering the precessional frequency. For example the different compounds containing the phosphorus nucleus in skeletal muscle will produce five (major) precessional frequencies, each "labelling" intracellular molecules; ATP (α , β and γ phosphate groups), PCr and Pi.

The study of internal organs such as brain, liver and kidney requires localization of the chemical shift information from the region of interest (whole or part of the organ/pathological lesion). Various strategies have been developed to solve this problem (Aue, 1986), but no satisfactory solution has been found. Different localizing techniques have to be selected for different nuclei and investigations. Localization in MRS can be divided into two broad groups, depending on whether static (B_0) or radio frequency (B_1) gradients are used; B_0 or B_1 localization. Each group can be further subdivided into single and multiple point techniques. Single point techniques provide one spectrum from one region of the subject. Whilst multiple point localization techniques provide several spectra from one region of interest. Each approach has to be selected for a particular task, and no single technique has been universally accepted.

Figure 1.5

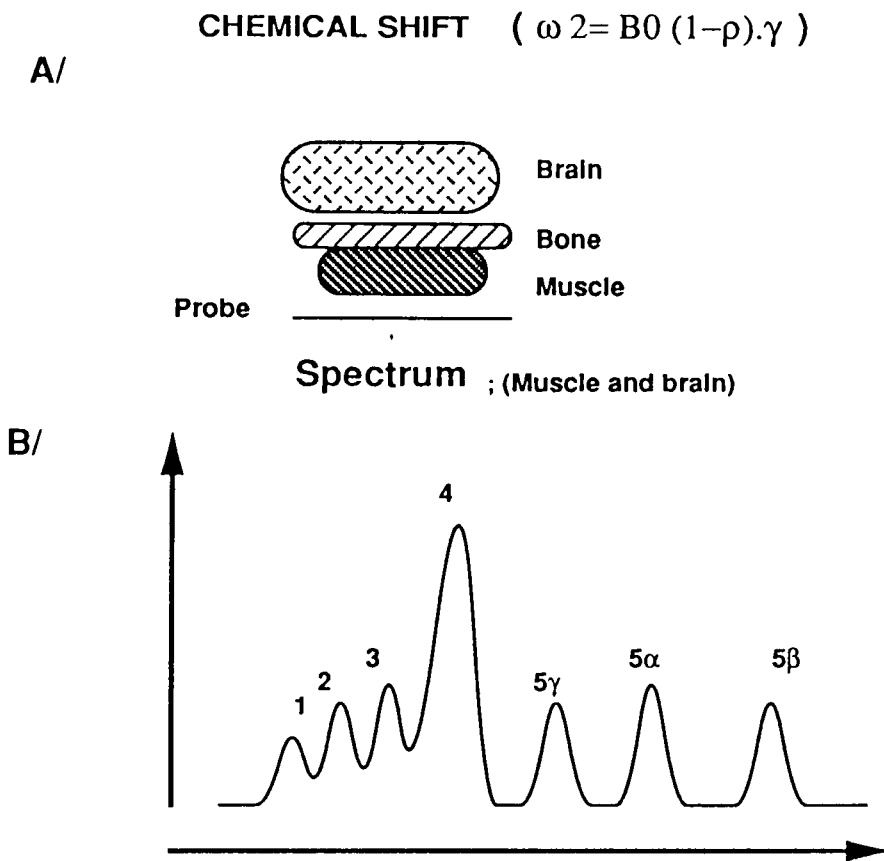


Figure 1.5 Chemical shift is due to alteration of the B_0 field by the shielding effects (ρ) of nearby electron cloud of the phosphorus containing compound (A). The human brain has a covering layer of scalp, with some muscle (eg Temporalis), and bone. A diagrammatic spectrum (B) is shown of chemical shift information from a mixture of muscle and brain. The three phosphate resonances of adenosine triphosphate (5), phosphocreatine (4), phosphodiester compounds (3), inorganic phosphate (2) and phosphomonoester compounds (1) can be resolved.

Figure 1.6 **Chemical shift and Imaging**

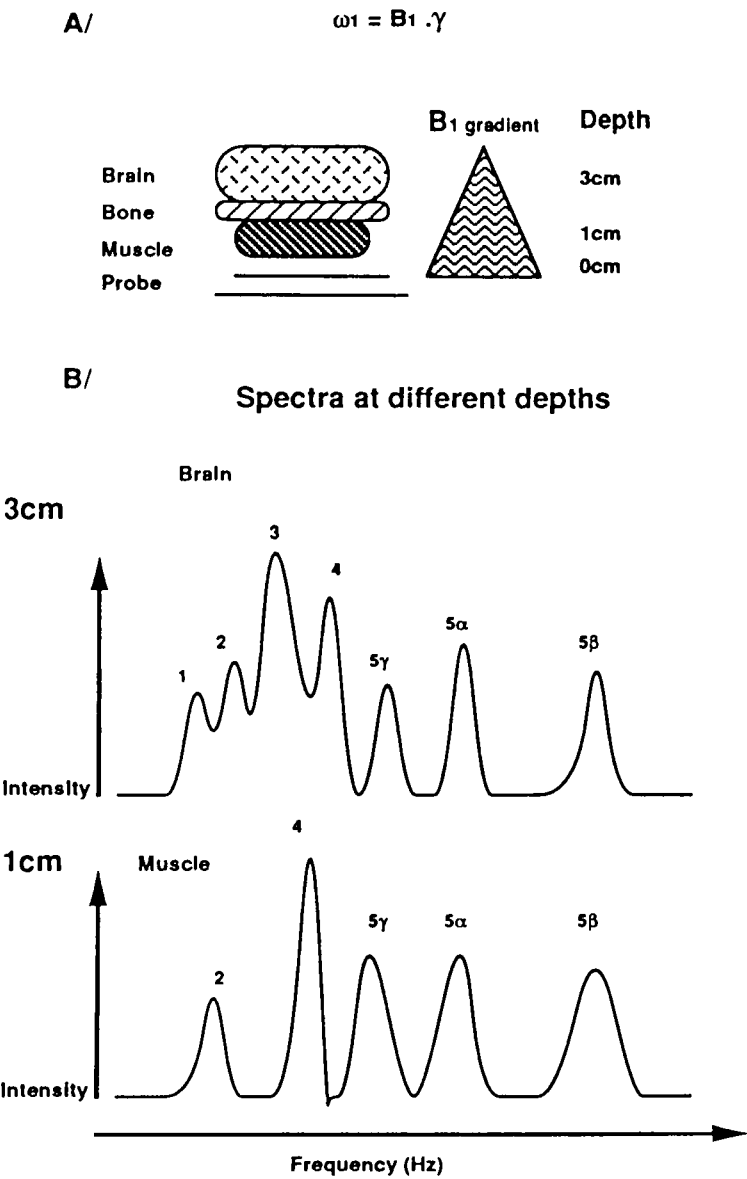


Figure 1.6 In a similar way to the use of magnetic gradients in MRI, a B₁ radio frequency gradient can be used to create a one dimensional image across a sample (A), using a surface probe. Spectra at 1cm (B) are from superficial muscle, are different from spectra at 3cm, mainly brain. Skeletal muscle has greater intracellular concentrations of phosphocreatine (4) and ATP (5), but small amounts of phosphomonoester (1), such as phospholipid precursors phosphoethanolamine (PE) and phosphocholine, and phosphodiester (3) compounds, such as phospholipids and their breakdown products.

The phase modulated rotating frame imaging (PMRFI) technique, a B_1 multiple point localization technique for ^{31}P MRS (Figure 1.6), was chosen for the study of phosphorus compounds in human cerebral metabolism for three specific reasons;

1/ PMRFI is a multiple point localization technique.

2/ Fast switching gradients, as required for multiple point B_0 localization techniques, are not required to achieve localization of the signal from the region of interest.

3/ The data collection is time efficient (investigations times from 20-45 minutes) and the signal is not significantly affected by the fast (T_2) relaxation of phosphorus nuclei.

However certain disadvantages exist.

1/ The image is one dimensional, with depth from the surface probe into the sample.

2/ Spatial comparison between a conventional proton image and phosphorus PMRFI can not be made directly since different magnetic fields are used in each technique.

3/ The width of the volume imaged by the phase modulated rotating frame is determined by probe geometry.

Points 2&3 were further investigated in the work described in chapter 2&3.

1.3.2 Magnetic resonance imaging

The techniques employed in magnetic resonance imaging (MRI) have been extensively reviewed (Morris, 1986). The principles involved are similar to MRS,

except that only one nucleus is studied; the proton of water. A combination of static magnetic gradients across the B_0 field and radio-frequency pulses are used to define the position of small volumes of water (voxels, typically 1mmx1mmx1cm) within a sample. A linear static magnetic gradient is created along the long axis of the magnet (Z axis) generating a wide range of proton precessional frequencies directly related to distance along the bore of the magnet. A slice across this axis is selected by transmitting the radio-frequency pulse at a slice specific precessional frequency. Protons either side of this slice will be at different precessional frequencies and will not be perturbed.

Distance in the vertical (Y) and horizontal (X) axis have to be encoded. After switching off the Z static gradient and radio-frequency pulse, a static gradient is applied along the X axis during acquisition. The precessional frequency now encodes for distance along the X axis of the sample. Distance along the Y axis of the sample is phase encoded. Spin-echo (T_2 relaxation) and inversion recovery (T_1 relaxation) techniques have been combined with imaging sequences to exploit differences in relaxation rates, contrasting regions within normal and pathological tissues.

Magnetic resonance imaging is now widely used for diagnostic purposes. In this work MRI was used to provide structural information on the region from which the MRS signal was collected.

1.4.0 Summary

The aim of this work was to investigate human brain metabolism *in vivo*, using an existing MRS technique, phase modulated rotating frame imaging (PMRFI).

PMRFI was chosen for three specific reasons; multiple point localization, short investigation time (45 minutes) and possibility of measuring absolute tissue concentrations and imaging enzyme flux rates within the human brain *in vivo*. The PMRFI experiment is limited to studying phosphorus compounds at or greater than 1mmol/L tissue concentrations to a depth of 6cm from the surface of the skull into the brain. Deeper regions of the human brain such as basal ganglia, pituitary and infra-tentorial regions can not be examined with PMRFI. Therefore anatomically this work was restricted to examining the intracellular metabolism of superficial gray and white matter in the cerebral hemispheres *in vivo* to 6cm depth.

The study of two specific areas of human brain metabolism were planned; high energy phosphates (ATP and PCr) and intracellular pH (pHi). This work was planned in three stages; application of the PMRFI technique and probe design to the study of normal brain metabolism, designing physiological experiments on normal brain and the study of selected brain pathologies. A considerable amount of the work on PMRFI theory and probe design had already been carried out by Hoult (Hoult, 1979), Styles (Styles et al, 1985) and Blackledge (Blackledge et al, 1987). However the potential for measuring absolute tissue concentrations and therefore imaging of enzyme flux rates with depth *in vivo* had not been developed at this stage.

Normal healthy subjects were studied prior to clinical studies. Two groups of human brain pathologies were investigated; tumours and neurodegenerative conditions affecting the cerebral hemispheres. The intracellular metabolism of neurodegeneration due to multiple sclerosis, viral encephalitis and non-penetrating head-injury were also studied.

CHAPTER 2

LOCALIZED ^{31}P PHOSPHORUS SPECTROSCOPY *IN VIVO*: PHASE MODULATED ROTATING FRAME IMAGING

This chapter briefly describes the theory, development, imperfections and probe construction for the phase modulated rotating frame imaging (PMRFI) technique for the study of human brain metabolism *in vivo*. Although a full description of the nuclear magnetic resonance phenomenon requires the concepts of quantum mechanics (Abragham, 1961), the classical description will be used to describe phase modulated rotating frame imaging (PMRFI) in this text. The classical description of magnetic resonance considers the atomic nucleus behaving like a gyroscope, possessing spin and charge.

The ability of magnetic resonance to identify the position of nuclei in space was first proposed by Lauterbur (Lauterbur, 1973). This experiment creates a linear gradient across the static magnetic field causing a predictable linear variation in the precessional (Larmour) frequency. The precessional frequency now identifies the spatial position of the proton nucleus of water in a given sample. Hoult (Hoult, 1979) first proposed the use of a radio frequency field gradient (B_1) gradient to define spatial position (rotating frame imaging). Cox and Styles (Cox and Styles, 1980) demonstrated that the rotating frame imaging approach could be used to spatially locate different chemical species in one dimension. The amplitude modulated rotating frame method (Garwood et al, 1984) has also been applied to the study of human cellular biochemistry *in vivo*, but does not acquire all the available magnetic resonance signal. The phase modulated rotating frame imaging (PMRFI) technique has been developed (Blackledge et al, 1987) to acquire all the available signal from the region of interest.

2.2.0 A description of phase modulated rotating frame imaging.

2.2.1 Theory

The rotating frame

Classical description of magnetic resonance considers the nucleus to behave like a gyroscope, possessing spin at a precessional frequency and charge resulting in magnetization. Motion about any restorative force such as a static (B_0) or an applied radio frequency magnetic field (B_1) will be at right angles to the restoring force ie precessional. The effect of a restorative force on a gyroscope can be tested by spinning a bicycle wheel. The spinning wheel is held at the axel pin and any movement applied to the axle pin will be resisted. The applied force will be deflected at right angles.

To visualise the angle of precession about the restorative force the precessional element is removed by using the concept of a rotating frame (Figure 2.1). The observer rotates at the precessional (Larmour) frequency leaving only the angle of precession. An analogy can be made, in two dimensions, with a fly walking across a revolving record on a record player. If the observer is stationary the fly will appear to spiral as it walks in a straight line to the edge. If the observer now rotates at the speed of the record, the fly will appear to move in a straight line.

In the rotating frame the magnetization is aligned along the Z' axis (B_0 magnetic field). A radio frequency pulse, at the precessional frequency, is applied along the X' axis, causing the nuclei to tip into the $Z'Y'$ plane.

Figure 2.1

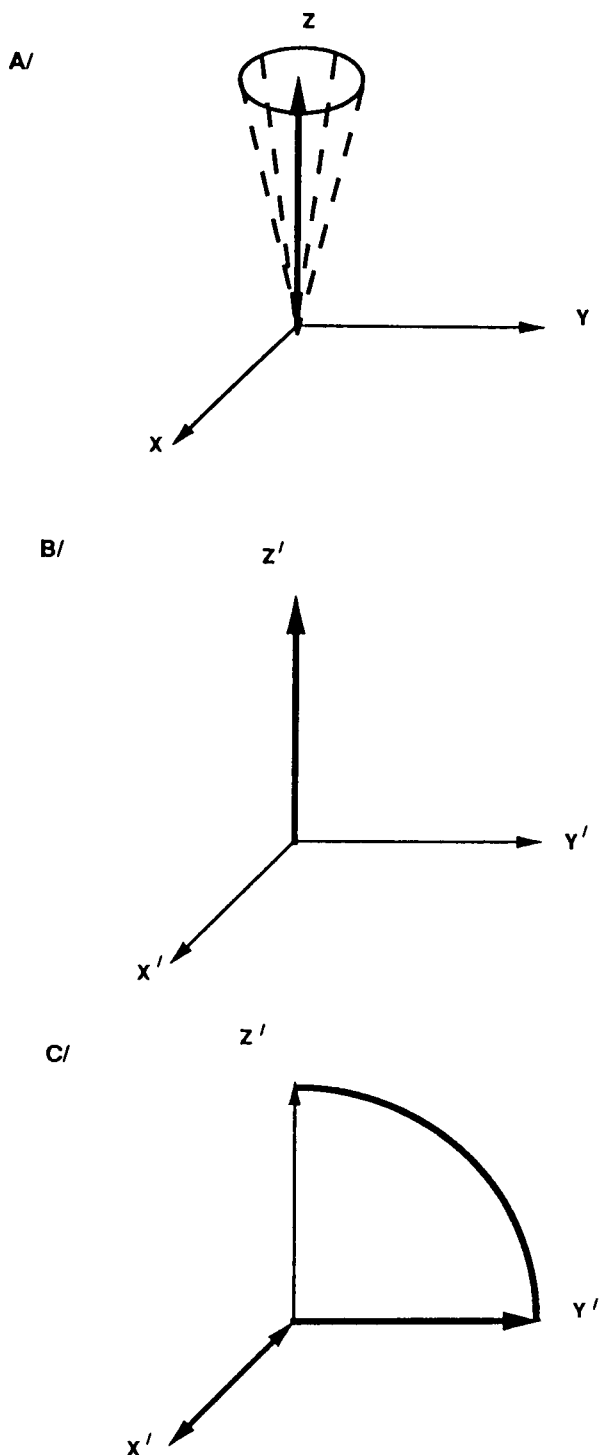


Figure 2.1 The rotating frame removes the movement of precession (a) leaving only the angle of precession (b). When a radio frequency pulse is applied, at the precessional frequency, along the X' axis (c) the nuclei are tipped into the $Z'Y'$ plane.

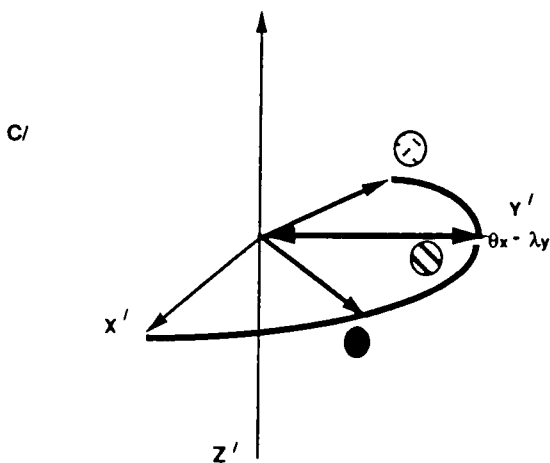
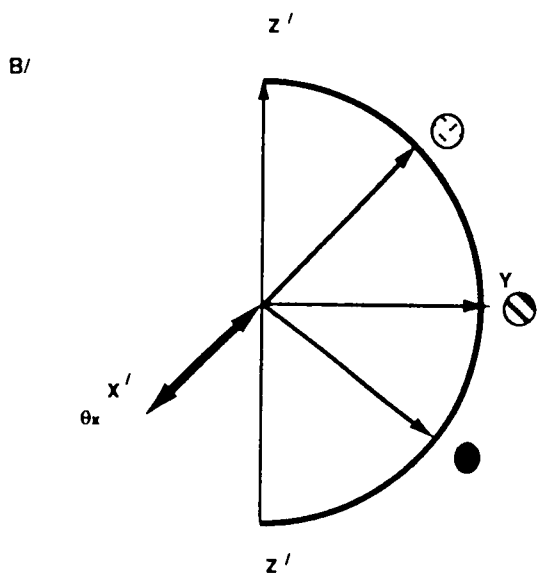
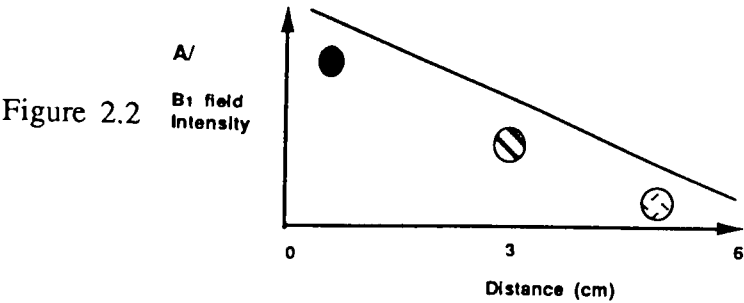


Figure 2.2 A B_1 radio frequency field is created by a transmitter coil at 0cm (A). The intensity of the B_1 radio frequency field decreases with depth. A radio frequency pulse (θ_x) is applied along the X' axis (B), tipping nuclei into the $Z'Y'$ plane according to position in the B_1 field. A further pulse along the Y' axis (λ_y) will rotate the nuclei into the $X'Y'$ plane (C).

The tip angle (θ) is determined by the intensity (B_1) and pulse length (t_p) of the radio frequency pulse;

$$\theta = B_1 \cdot (t_p) \cdot \gamma \quad (b)$$

where (t_p) is the pulse length.

γ is the gyromagnetic ratio

B_1 is the absolute intensity of the B_1 field.

Nuclei precessing in the $X'Y'$ plane will create a weak oscillating current (signal) in a receiver coil placed (loop of copper wire) near to the sample. Signal in the Z' plane can not be detected, so the signal can be described as;

$$\text{signal} \propto \sin(B_1 \cdot t_p \cdot \gamma) \quad (c)$$

B_1 field

In the same way as a static B_0 gradient is used to spatially encode distance in MRI, the B_1 field gradient is used to define distance in the phase modulated rotating frame imaging experiment, by recording the different tip angles created when applying a radio frequency pulse. The intensity of radio frequency pulse (B_1 field gradient) will decrease with distance from the source; in this case a coil of copper called a transmitter coil (Figure 2.2). Nuclei close to the transmitter coil will experience a greater tip angle than those further away. If the B_1 field gradient is linear from the r.f. source (transmitter coil) in to the sample, distance into the sample can be accurately encoded. The rate of rotation of the nuclei, into the Z'/X' plane, in response to a radio frequency pulse is called the nutational frequency and, in the PMRFI experiment, is determined by the intensity of the B_1 field gradient.

A series of incremental radio frequency pulses ($n\theta$) can be used to investigate the nutational frequency across a sample (Figure 2.3). The simplest version of this approach is the amplitude modulated experiment (Garwood et al, 1986). This experiment is inherently inefficient because signal is not created by the Z' component of precession. This signal deficit can be corrected by applying a further radio frequency pulse along the Y' axis of the rotating frame (λ_y), tipping the nuclei from the Z'/Y' into the $X'Y'$ plane (Figure 2.3), as used in the phase modulated rotating frame imaging experiment (Blackledge et al, 1987).

Phase modulation

Nuclei aligned along the Z' will precess at their precessional frequency, but at random phase with respect to each other; without coherence. When a radio frequency pulse in the X' plane is applied, the nuclei precess at a greater tip angle in the Z'/Y' plane, with coherence, and phased at 90° to the radio frequency pulse. A further radio frequency pulse along the Y' axis will now tip the Z'/Y' magnetization into the $X'Y'$ plane where all the available signal can be received and is phase modulated. The pulse sequence in the PMRFI experiment can be described as;

$$\theta_x - \lambda_y \text{ acquire}$$

$$\text{where } \theta_x = X' \text{ r.f. pulse}$$

$$\lambda_y = Y' \text{ r.f. pulse.}$$

The phase twist inherent in the two dimensional Fourier transform has been described elsewhere (Blackledge et al, 1988a), and removed by the addition of two experiments (θ_x and θ_{-x}).

Figure 2.3

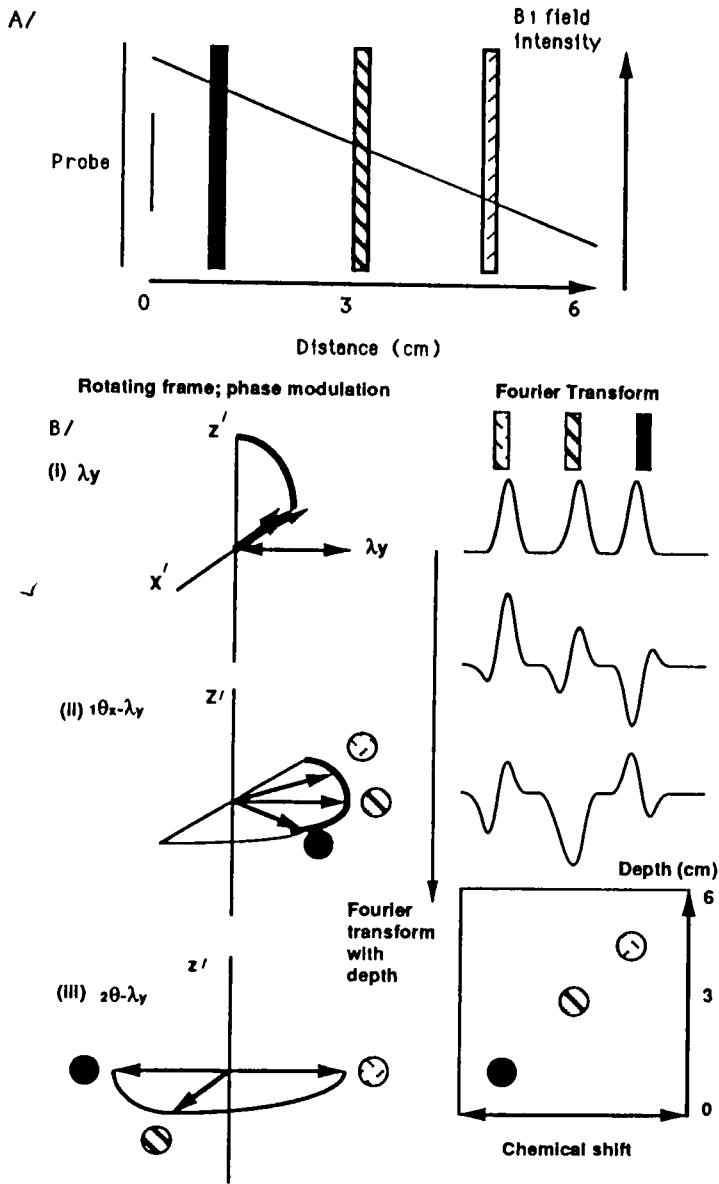


Figure 2.3 The B_1 radio frequency field is created by a transmitter coil and three samples (with three chemical shifts) at three different depths are considered (A). Following a radio frequency pulse along the Y' axis the nuclei tip into the $Z'X'$ plane (B(i)), signal acquired and Fourier transform produces a spectrum from which the phase is set. A pulse $(\theta_x - \lambda_y)$ (B(ii)) is applied and the phase is seen to modulate, most rapidly in nuclei close to the coil (black) and least in nuclei furthest away (dashed) (B(iii)).

Figure 2.4

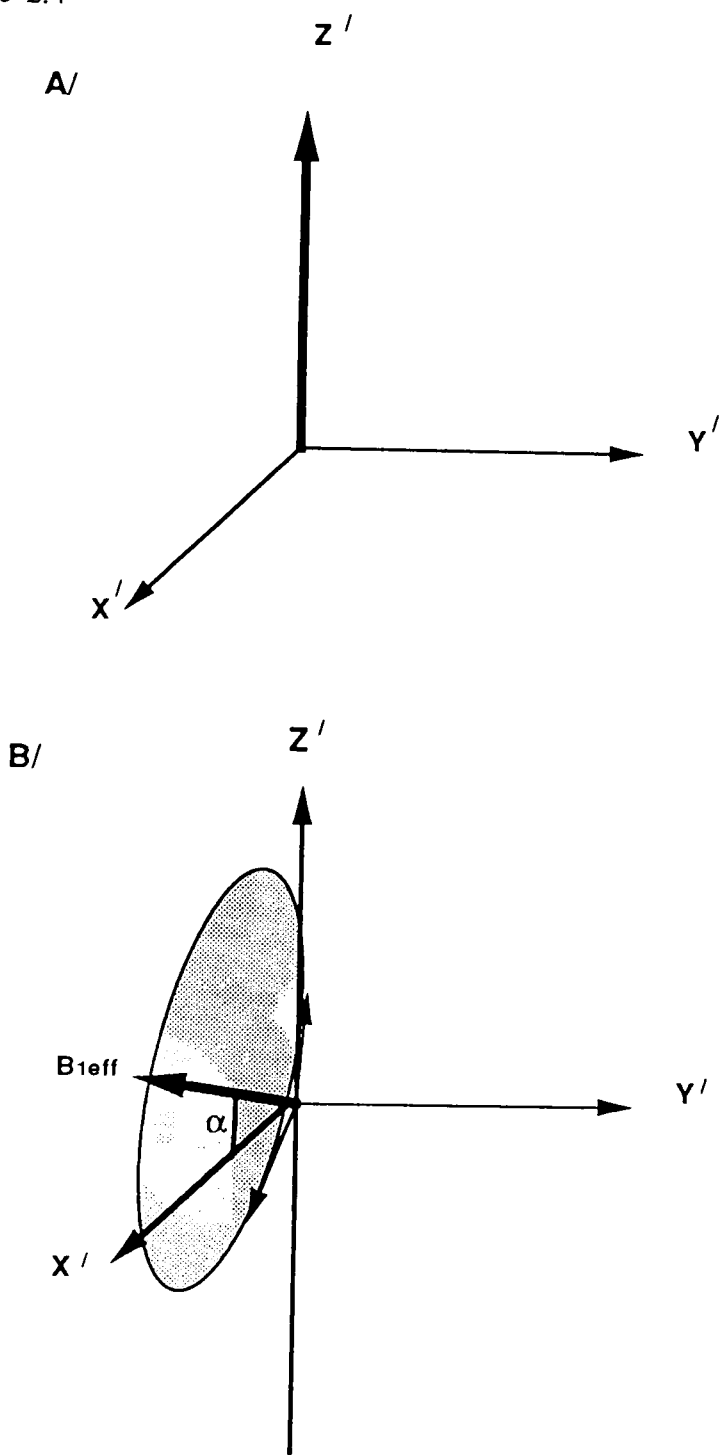


Figure 2.4 The off resonance nucleus (**B**) experiences a different B_1 field (B_{1eff}) than the on resonance nuclei (**A**), creating an angle (α). The off resonance nuclei nutate at a higher frequency for a given B_1 field strength.

2.2.2 Imperfections

The phase modulated rotating frame imaging technique has two potential imperfections which have been fully discussed by Blackledge (Blackledge Thesis, 1988b). Two imperfections, signal intensity variation in the depth dimension and off resonance in the chemical shift dimension, need to be considered prior to explaining the method for measuring absolute tissue concentration of phosphorus metabolites.

The off resonance effect (Figure 2.4) describes the result of applying a radio frequency pulse (B_1 field) which is not at the precessional frequency of the nucleus, which will occur when a series of phosphorus compounds at different frequencies are perturbed by one radio frequency pulse (Blackledge and Styles, 1988c). and more so with β ATP. For example when PCr is on resonance Pi and α ATP will be off resonance. The off resonance effect will be considered with respect to the applied B_1 field. An additional B_1 field (B_{1eff}) is created for the off resonance nucleus;

$$B_{1eff} = \sqrt{B_1^2 + \frac{(\omega_0 - \omega')^2}{\gamma^2}} \quad (c)$$

where ω_0 is the frequency of the r f pulse

ω' is the frequency of off resonant compound

γ is the gyromagnetic ratio

B_{1eff} will introduce an X' component into the magnetization in the Z'/Y' plane following the θ_{tx} pulse. The off resonance nucleus will describe a cone shaped precession at a greater nutational frequency than nuclei on resonance. In the one dimensional image the off resonance nuclei will appear closer to the surface coil than those on resonance producing a characteristic bowing (Figure 3.2).

As described in (c) the off resonance is due to the difference in frequency between the radio frequency pulse and frequency of the ^{31}P compound and is therefore fixed irrespective of pulse length. However the angle (α), created by the difference of B_1 and $B_{1\text{eff}}$, can be minimised by making the intensity of B_1 as great as possible.

The signal intensity variation in the spatial dimension (ω_1) is due to three possible factors; loss of signal due to decreasing receiver sensitivity with depth, variation in sampled volume and signal loss due to the λ_y pulse. Experiments were constructed to investigate the size of the volume sampled (Chapter 3), defined by overlapping flux lines between the transmitter and receiver coils. The λ_y pulse is 100% effective at tipping the nuclei precession from the Z'/Y' plane into the X'/Y' plane at the middle of the sample, but less effective in other parts of the B_1 field linear gradient. The signal variation is predictable and varies accordingly;

$$\text{Signal intensity variation } (\lambda_y) = \frac{1 + \sin\theta}{2}$$

The signal intensity in the image was corrected for the variation due to the λ_y pulse.

2.3.0 PMRFI experiments

2.3.1 The imaging experiment

The PMRFI experiment uses a simple 2 pulse sequence to localize a range of different phosphorus containing compounds at different depths into a sample (Blackledge et al, 1988b). Sixteen transients, with a 3 second repetition rate, were summated at each increment of $\theta_{\text{tx}} - \lambda_y$. In the human brain studies 16 increments were used. The spectrometer was programmed to carry out these commands

automatically using a programme named PHAXMOD. The total acquisition time was about 27 minutes for the PMRFI sequence. A 2DFT was performed in the manner described previously to produce an image (Figure 3.2).

2.3.2 From images to concentrations

Spectra from selected depths in the PMRFI image can be used to estimate metabolite ratios. Before absolute tissue concentrations can be estimated, the intensity of the signal in PMRFI experiment has to be calibrated against a standard phosphate solution. Three steps are required; Calibration of the B_1 field with depth, definition of the sample volume, and calibration of a standard against a known concentration of phosphate with similar relaxation times (T_1) to human brain.

Depth into the region of interest can be calculated if the position of the receiver coil is identified in the phosphorus image, since the B_1 field gradient is linear from the receiver coil. A small vial (Receiver coil phantom containing diphenyl phosphate 200mmol/L in chloroform) was placed in the centre of the perspex holding the receiver coil. In the image the receiver coil phantom produced a peak corresponding to the receiver coil depth (0cm). The multislice phantom (Figure 2.5) was made up with a series of "shelves" 1cm apart containing different phosphates so that peaks corresponding to each shelf appear 1cm apart in the image.

Width of the sampled volume was investigated by using a concentric phantom (Figure 2.6). Different phosphates were placed in each beaker. The width of the sampled volume was found to increase with depth (8.5cm at 6cm depth).

Figure 2.5

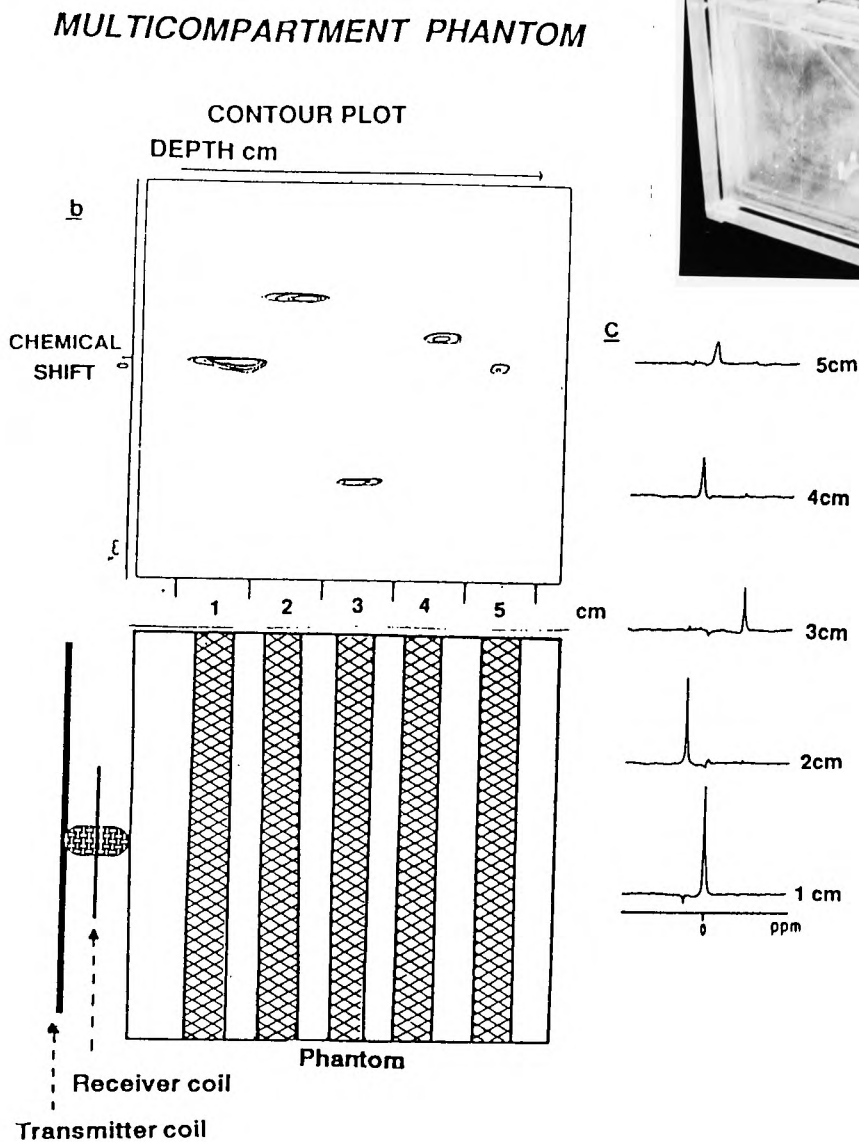


Figure 2.5 A five compartment phantom with each cavity 0.5cm deep, 10cm³, separated by 0.5cm thick glass containing 100mmol/L phosphate solutions, was used to define the resolution of PMRFI technique. The first compartment (at 1cm from the receiver coil) contained a solution of NaH₂PO₄ at pH4; the second (at 2cm) contained NaH₂PO₄ at pH12; the third (at 3cm) contained pyrophosphate at pH 10; the fourth (at 4cm) contained NaH₂PO₄ at pH7; and the final compartment (at 5cm) contained NaH₂PO₄ at pH4, as shown in a. The 2DFT matrix is presented as a contour plot (b) with intensity of signal plotted against chemical shift (y axis) and distance (x axis), from which selected spectra (c) are taken.

Figure 2.6

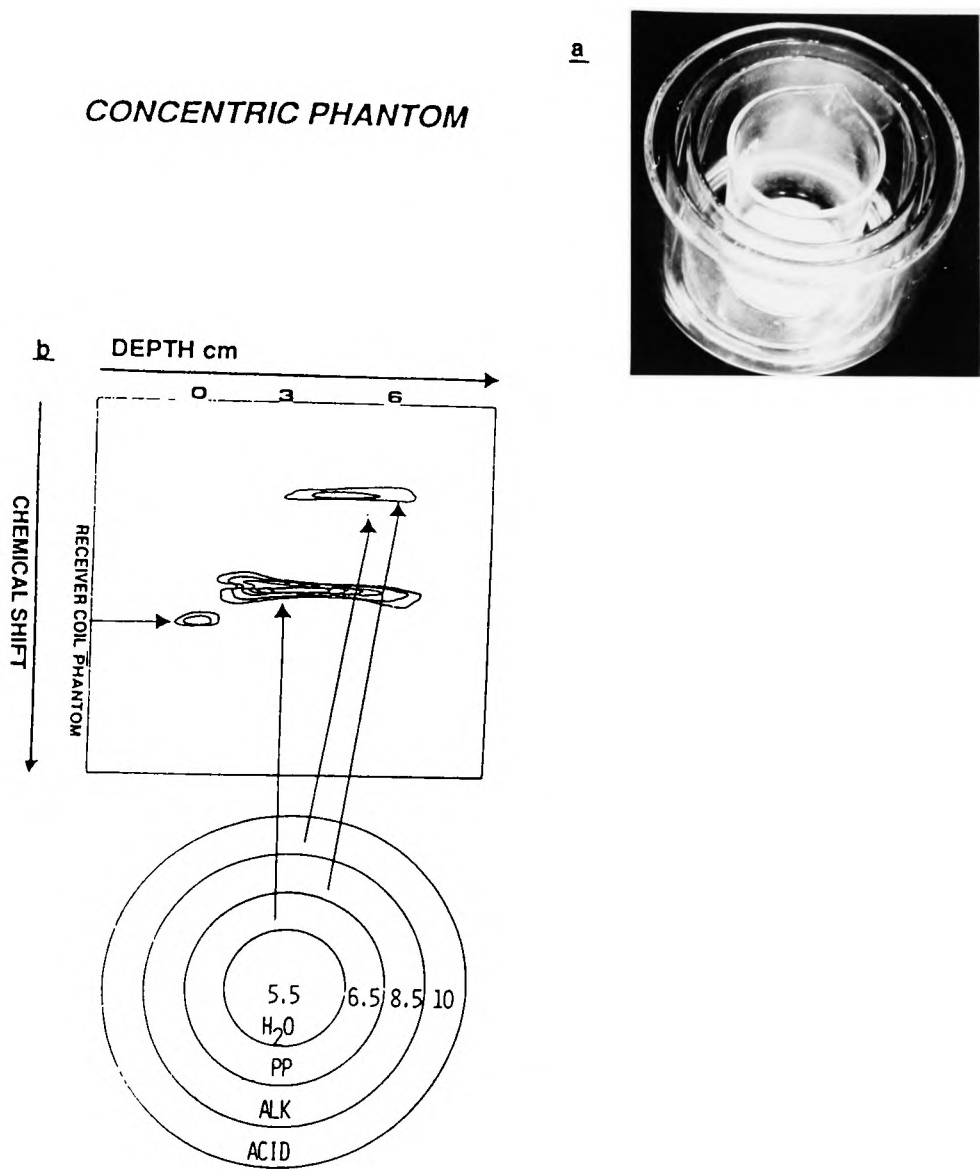


Figure 2.6 The concentric phantom (a) has four cylindrical compartments placed one within the other, each filled with a different phosphate solution. The central compartment (0-5.5cm diameter) contained water; the second (mean diameter 6.5cm) contained pyrophosphate; the third (mean diameter 8.5cm) contained NaH_2PO_4 at pH12; and the fourth (mean diameter 10cm) contained NaH_2PO_4 at pH4. Signal was received from the 2nd compartment from 1cm to 6cm, and from the third compartment from 3cm to 6cm (b), with an insignificant contribution from the fourth compartment (10cm).

The observed receiver sensitivity can be investigated by imaging a large container (diameter=10cm, height=10cm) filled with 5mmol/L phosphate dissolved in water, and dysprosium polyphosphate added to shorten the T_1 relaxation time to less than 1 second, similar to human brain. A ratio of signal intensity representing 5mmol/L phosphate was divided by the signal intensity for the receiver coil phantom, producing a normalization constant. The intensity of the human metabolite signal can be normalized via the receiver coil phantom to give absolute tissue concentrations (Figure 2.7) as follows;

$$[\text{Metabolite}]_x = \frac{A_M/A_{PM}}{A_S/A_{PS}} \times 5\text{mmol/L}$$

where $[\text{Metabolite}]_x$ = Metabolite concentration (mmol/L tissue) at xcm from receiver coil

- A_M = Area of metabolite peak at xcm
- A_{PM} = Area of receiver coil phantom peak measured in human studies
- A_{PS} = Area of receiver coil phantom peak measured in standard study
- A_S = Area of standard solution peak at xcm (5mmol/L)

All metabolite peak areas have to be corrected for the signal intensity variation due to the λ_y pulse. Corrections also have to be made for T_1 relaxation time differences of the metabolite. Experiments have been conducted to investigate T_1 relaxation times of metabolites with depth (Blackledge and Styles, 1989).

Figure 2.7

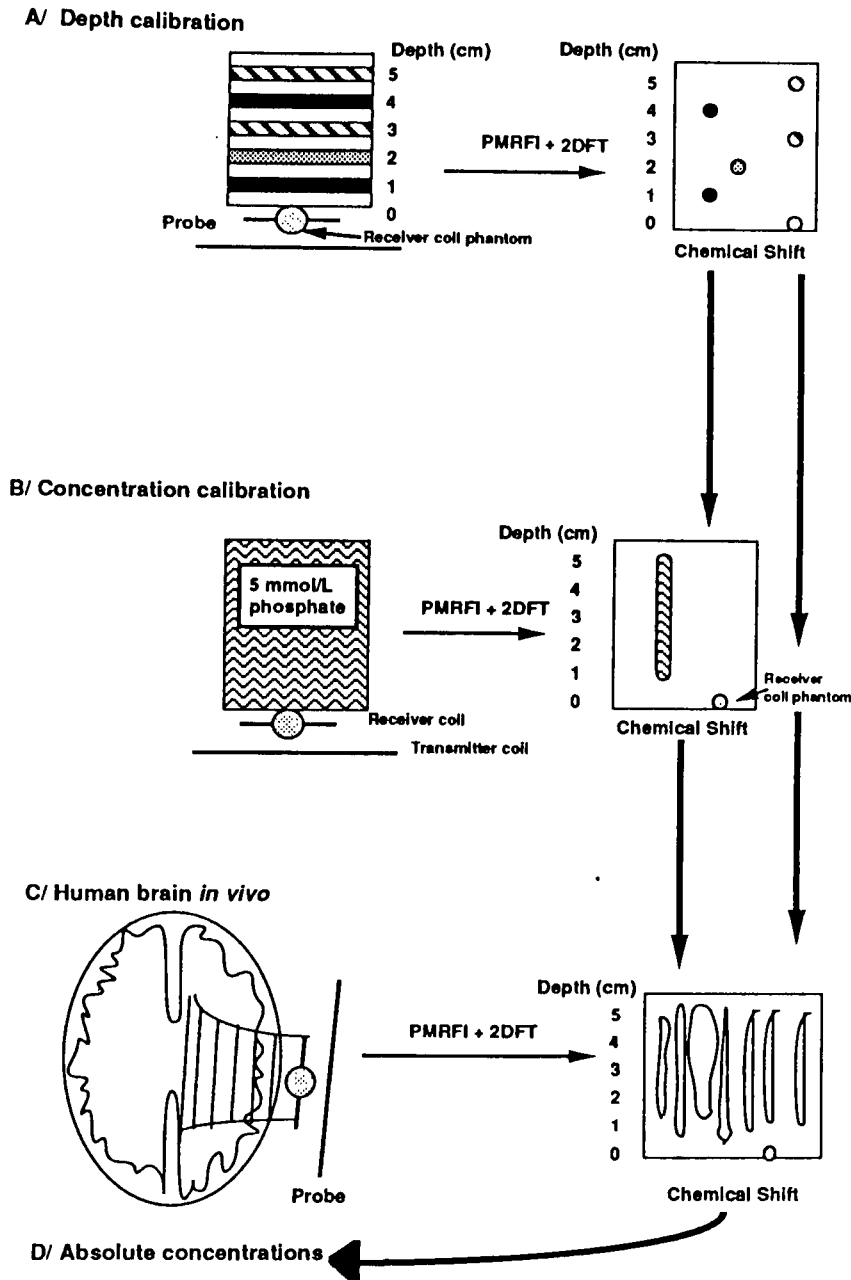
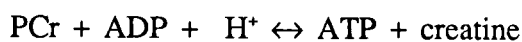


Figure 2.7 The flow diagram summarises the steps involved in measuring absolute concentrations *in vivo*. Depth is calibrated (A) against a multislice phantom. The receiver coil phantom is calibrated against a known solution of phosphate (B) with similar relaxation rates to living systems (ATP T_1 less than 1 second). The same receiver coil phantom is used in experiments *in vivo* (C) to measure absolute concentrations.

2.2.4 Enzyme flux rates

The possibility of measuring the rate of flux through an enzyme of one chemical species to another is also of great interest, since flux rate changes may occur before significant changes in absolute concentration. The theory behind saturation transfer techniques has been extensively reviewed (Brindle, 1988). The technique involves the selective irradiation of the exchanging partner (Figure 2.9) in a chemical reaction such as the creatine kinase (C K) catalysed reaction between phosphocreatine and ATP:



The steady state magnetization of PCr (M_z) is measured following the selective irradiation of its exchanging partner γATP , determined by the following equation:

$$M_z / M_0 = 1 + (T_{\text{INT}})^{-1} / K_F$$

where T_{INT} = Intrinsic spin lattice time of PCr.

K_F = Forward rate constant of PCr-ATP reaction.

M_z relaxes with an apparent relaxation time (T_{IAPP}), given by :

$$T_{\text{IAPP}} = ((T_{\text{INT}})^{-1} + K_F)^{-1}$$

which was measured in a separate experiment by performing an inversion recovery PMRFI experiment (Blackledge and Styles, 1989) in the presence of saturation of the γATP . The ratio of M_z/M_0 is then combined with this measurement to derive K_F by the derived equation:

$$K_F = (T_{\text{IAPP}}^{-1}) \times (1 - M_z/M_0)$$

M_z/M_0 is measured by irradiating the γATP under fully relaxed conditions. The forward flux rate for PCr is given by the product $[\text{PCr}] \cdot K_F$.

Figure 2.8

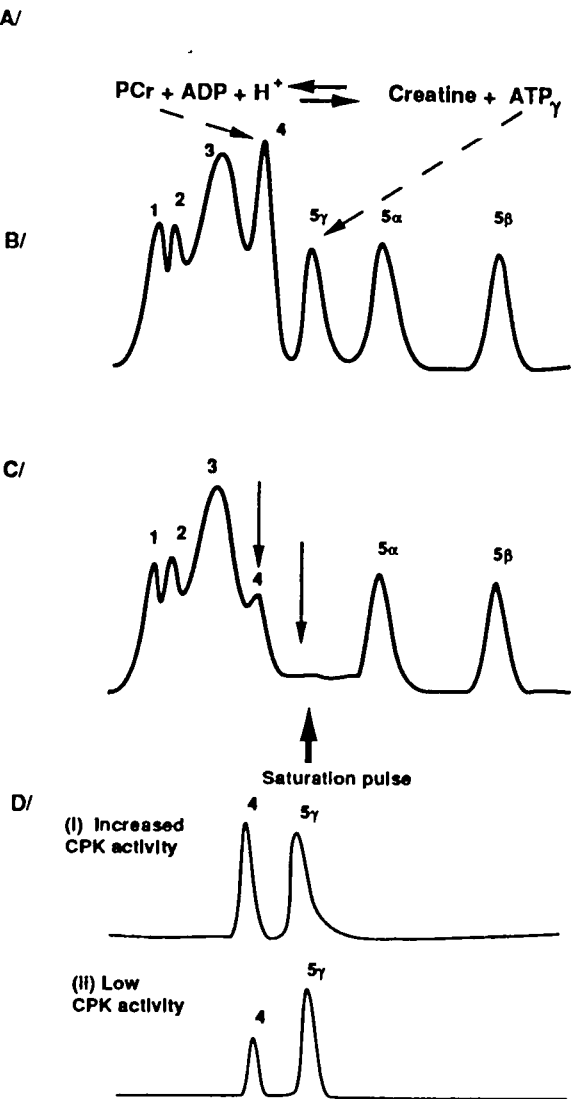


Figure 2.8 PCr and the γ phosphate of ATP are exchanging partners in the reaction (A), catalysed by creatine kinase. Both PCr and γ ATP are delineated in a phosphorus spectrum of the human brain *in vivo* (B). Saturation of the γ ATP will reduce the intensity of the PCr peak (C) at a rate determined by the forward flux (PCr $\rightarrow$ ATP). Rapid flux will produce a greater reduction in the PCr peak (D) than a low flux. (D are difference spectra B-C))

Separate f_1 and f_2 channels were used for high power pulse (150W) and low power irradiation (1W net) respectively. The inter-pulse delay was set at 15 seconds to ensure full relaxation of the magnetization prior to each transient. As described before, two experiments were performed for each image in order to achieve optimal sensitivity and pure phase presentation of spectra. The collection of two images were interleaved, the first with irradiation set on γ ATP, and the second with irradiation at an equidistant frequency on the other side of the PCr peak. This provides the control data with which to compare the saturated image. Four transients were averaged per increment. Ten increments were collected for each image with a total acquisition time of 40 minutes.

A phantom experiment was performed to test for any non-specific chemical shift or distance dependent artifacts of the saturation pulse. Identical parameters were used as in the investigation *in vivo* so that the available spatial resolution could be characterised, as previously described. In these tests of the techniques three images were collected, with f_2 irradiation of either peak, and one with the irradiation frequency set between the two resonances.

The ear phone coil (Figure 2.9) was attached to the first probe built for the saturation experiments. This untuned coil was used for saturation experiments, both for creatine kinase investigations, and phosphodiester off resonance saturation experiments. A piece of perspex was bent in a vice with an electrical blow torch to soften the plastic (75cmx25cm). Thin copper plate was cut and glued onto the perspex. This simple, untuned coil was considered to be a safe design, using 15 Watts transmitted and reflecting 14 Watts (Net power deposition = 1 Watt).

Figure 2.9

(A)



(B)

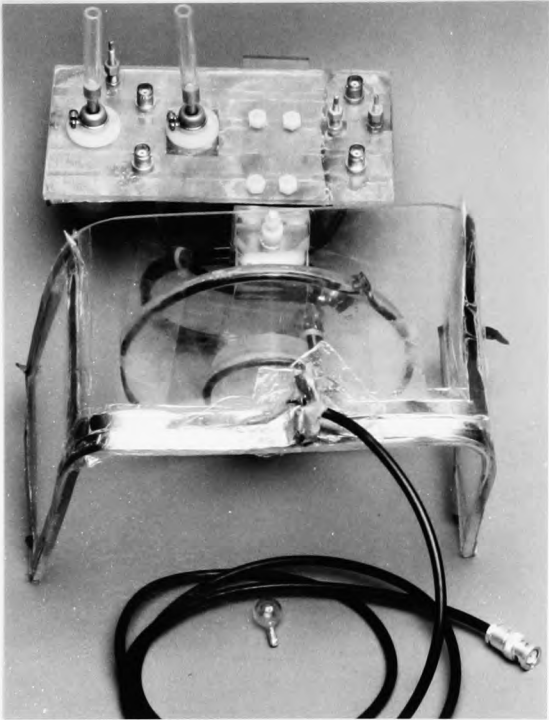


Figure 2.9 The first probe (A) was constructed by the author, to which was attached a third "ear phone" coil (B) for magnetization transfer experiments.

2.4.0

The probe

The double surface coil used has been designed and described by Styles (Styles, 1988). This geometric arrangement was developed so that the receiver coil could interrogate a region in which the flux patterns were parallel to the transmitter coil and B_1 gradient linear. Electronically the two coils must be isolated. A brief description of the problems that arose in applying to human brain investigations *in vivo* follows.

2.4.1

Construction

The double concentric surface coil was made according to Styles (Styles, 1988). The main problem encountered when operating the coil at the high power settings (150-500 Watts), required to generate sufficient B_1 field, was flashing in the match capacitor in transmitter circuit (Figure 2.9). This flashing destroyed the B_1 field. This problem was not identified for a few months until the experiment was run at night in the dark (after an outing on the river!). The experiment was run "unloaded" on a phantom, developing maximum current in the transmitter circuit. The solution was to use vacuum sealed capacitors capable of 15KV peak (English Electric Valve Company Ltd, Chelmsford, Essex). These capacitors dramatically improved the spectral quality of the image and proved extremely reliable.

Further developments by Styles (Styles et al, 1989) improved the probe performance further and the addition of a "butterfly" proton tuned coil for MRI of the sampled volume (Figure 2.10).

Figure 2.10

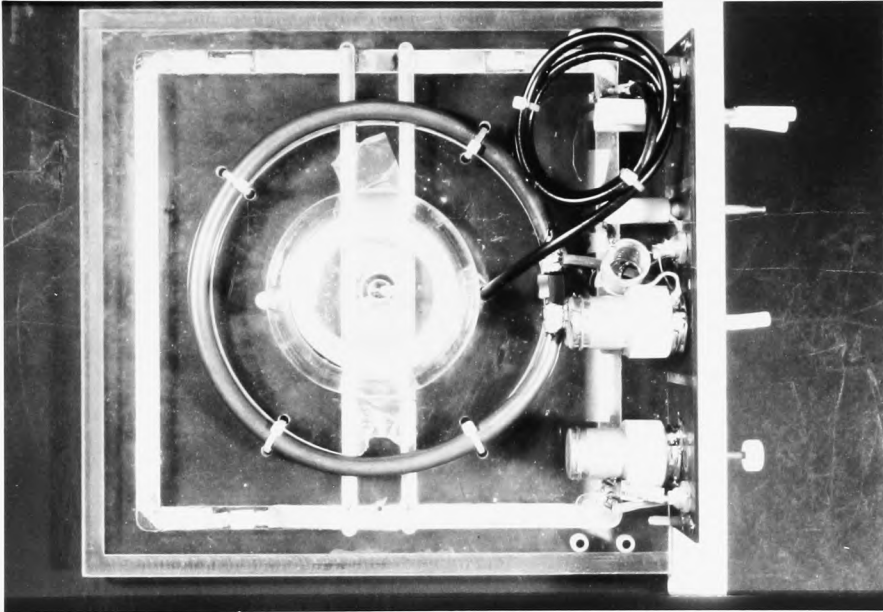


Figure 2.10 The second probe was designed and constructed by Dr.P.Styles, incorporating the double surface coil circuit for phosphorus spectroscopy and a "butterfly coil" for proton imaging. Proton imaging (MRI) was used to locate pathological lesions within the brain to target PMRFI. Two rings of agarose identify the phosphorus receiver coil in the proton image.

2.4.2 Tuning procedure

Tuning of the probe has been described elsewhere (Styles, 1988) and must be carried out before each investigation.

In the first version of the probe, constructed by the author (Figure 2.9) B_0 magnetic homogeneity was optimised in the region of interest by collecting proton signal ("shimming" to the narrowest line width) via the receiver coil. In this configuration the cross diodes were removed and the tuning apparatus plugged into the receiver coil using a system proposed by Gordon and Timms (Gordon and Timms, 1982). In the later model, constructed by Styles (Figure 2.10), the crossed diodes were incorporated in the receiver coil circuit, and the large "butterfly" coil used for shimming and imaging at the proton frequency (80.783MHz).

After solving the transmitter match capacitor problem, the probe proved to be reliable and robust. The six failed investigations were due to crossed diode failure (4) and soldered joints failing (2), out of several hundred experiments. The tuning procedure requires practice on phantoms (and friends) before studying a patient. In practised hands the whole tuning procedure can be completed in less than a minute.

2.5.0 Operational details

2.5.1 PMRFI

Prior to each investigation the subjects were checked for metal objects. In the magnet the head was supported on a "bean bag". When the subject was positioned correctly, with the probe over the region of interest, the bean bag was connected to a suction line, creating a firm but comfortable head rest. The probe was tuned and the B_0 field was optimized by shimming to the narrowest water

(proton) line width possible (10-20Hz), using suitable proton frequency parameters. Proton imaging was carried out at this point.

The spectrometer was now set to phosphorus frequency (32.705MHz) and a simple pulse and collect spectrum collected. The PCr frequency was identified and transmitter frequency offset adjusted to this frequency. The PMRFI programme was recalled, parameters checked, and run. A pulse and collect was repeated at the end of the investigation to check for any changes in spectral quality, such as subject moving.

2.5.2 Proton imaging

Proton imaging spin echo and inversion recovery sequences were programmed by Mr. G. Hogan. Two circular phantoms^{were} placed above the phosphorus receiver coil so that position of the phosphorus probe could be identified in the proton image. The subjects were repositioned if the probe was not over the region of interest.

2.5.3 Magnetization transfer

The magnetization transfer sequence requires the additional recording of the γ ATP and equidistant control frequency from the pulse and collect prior to running the programme, so that the saturation pulse can be applied to the correct chemical species. This experiment requires a stable B_0 field throughout (Less than 3Hz shift per hour) and any B_0 field changes will ruin the accuracy of the saturation pulse, and no result obtained.

2.6.0

Summary

This chapter gives a shortened version of the history, theory and practical aspect of PMRFI and the double surface coil. Understanding the theory helped in solving some of the problems encountered in applying this technique to human studies. Some problems were solved, such as the flashing capacitors in the transmitter coil circuit. In other problems a compromise was reached, such as the level of radio frequency power and off resonance effects on β ATP. PMRFI is a multiple point localization technique providing biochemical information at the point interest and in nearby tissue, which single point localization techniques can not achieve within a reasonable investigation time.

The advantages and disadvantages of the various localizing techniques in MRS must be carefully weighed. This work was aimed at investigating biochemical heterogeneity in the normal and pathological human brain *in vivo*. As the following chapters will show, PMRFI proved to be a reliable method for measuring intracellular pH (pHi), absolute tissue metabolite concentrations and enzyme flux rates.

CHAPTER 3

NORMAL HUMAN BRAIN METABOLISM *IN VIVO*

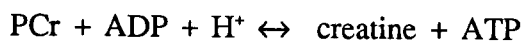
3.1.0. Introduction

Previous magnetic resonance spectroscopy (MRS) studies of the human brain have received signal from large volumes, ^{having to accept} biochemical and metabolic homogeneity. Positron emission tomography (PET) (Reivich et al, 1979) studies have shown differences between gray and white matter in different regions of normal human brain suggesting that significant metabolic heterogeneity does exist.

The aim of the following series of ^{31}P MRS studies of the normal human brain was to investigate the possibility of biochemical and metabolic differences between separate lobes of the cerebral hemispheres and between gray and white matter within these regions.

^{31}P MRS data have been presented as ratios, comparing the signal intensity of one metabolite with another, and recording changes in these ratios. Yet the intensity of the MRS signal is directly related to the concentration of the metabolite (Tofts and Wray, 1988). However estimating the volume from which the signal was derived has been difficult with single surface coils. The PMRFI experiment has the potential for receiving all the available signal from a volume defined by the geometry of the double surface coil. The volume from which the signal was received was investigated. The intensity of signal was then calibrated against a solution of known concentration via a phantom in the receiver coil. With the same receiver coil phantom in the human studies absolute tissue metabolite concentrations were measured.

Metabolic differences between gray and white matter were investigated using magnetization transfer experiments (Brindle, 1988). The forward flux of high energy phosphate transfer from phosphocreatine to ATP, catalysed by creatine kinase (C K) isoenzyme BB in the brain was measured;



Saturation of the γ ATP will reduce the intensity of the PCr peak, since the phosphate is transferred in the creatine kinase catalysed reaction. Magnetization transfer was combined with PMRFI experiment to produce a one dimensional image of CPK activity with depth into the human brain.

Magnetization transfer was also used to study phospholipid mobility differences between gray and white matter. Investigations of the compounds comprising the liver phosphodiester (PDE) peak (Murphy et al, 1989) have shown that this peak is made up of resonances from both mobile and relatively immobile phospholipid molecules. The mobile phospholipids were found to be in the endoplasmic reticulum of liver; no comparable studies have been made of brain. The immobile phospholipid signal was derived from other membrane structures such as the cytoplasmic membrane. Myelin has a high concentration of phospholipid (Rumsby, 1978). Experiments were performed to investigate possible differences between gray and white matter PDE signal.

3.2.0. Calibration

Two separate phantoms containing 5 and 10 millimolar solutions of inorganic phosphate in volumes similar to that of the human brain (1.3 litres) were used to standardise the receiver coil phantom. Dysprosium polyphosphate was added to these solutions to shorten the T_1 to 0.5 seconds.

Table 3.1 **CALIBRATION OF RECEIVER COIL PHANTOM AGAINST
STANDARD PHOSPHATE SOLUTIONS**

(A)	Depth (cm)	EXPERIMENT						<u>Mean</u> ±SD	
		1	2	3	4	5	6		
	1	0.90	0.74	0.65	0.70	0.85	0.83	<u>0.78</u>	0.09
	2	0.77	0.72	0.75	0.78	0.71	0.77	<u>0.75</u>	0.05
	3	0.64	0.66	0.72	0.69	0.63	0.65	<u>0.67</u>	0.04
	4	0.58	0.58	0.62	0.64	0.59	0.57	<u>0.57</u>	0.03
	5	0.49	0.47	0.60	0.55	0.63	0.53	<u>0.54</u>	0.06
	6	0.33	0.33	0.45	0.49	0.34	0.23	<u>0.36</u>	0.09

(B)

Depth(cm)	Experiment Number (PO4 peak area/Standard area)								Mean SD	
	1	2	3	4	5	6	7			
1.5	1.6	1.8	1.4	1.4	1.5	1.4	1.5	1.5	±0.2	
2.0	1.6	1.6	1.6	1.7	1.5	1.5	1.7	1.6	±0.1	
2.5	1.4	1.3	1.4	1.6	1.3	1.3	1.5	1.4	±0.1	
3.0	1.3	1.4	1.3	1.3	1.1	1.2	1.3	1.3	±0.1	
3.5	1.0	1.1	1.2	1.2	1.0	1.0	1.2	1.1	±0.1	
4.0	1.0	1.0	1.0	1.0	0.9	0.9	1.1	1.0	±0.1	
4.5	1.0	1.1	0.9	0.9	0.9	0.9	1.0	0.9	±0.1	
5.0	0.9	0.7	1.0	0.8	0.9	0.9	0.9	0.9	±0.1	
5.5	0.8	0.8	0.9	0.8	0.9	0.8	0.8	0.8	±0.05	
6.0	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	±0.05	

The Normalization constant is the ratio of peak area of phosphate standard divided by the receiver coil phantom peak area after correction for λ_y pulse. The normalization constant is calculated with depth and includes variation due to receiver sensitivity and sampled volume. Two standard phosphate solutions were used; 5mmol/L (A) and 10mmol/L (B). Several calibration experiments were run on each standard to estimate the range of variation (Standard deviation from the mean; SD).

polyphosphate, to mimic the magnetic resonance characteristic of biological solutions. The signal intensity at increasing depth (1, 2, 3, 4, 5, 6cm) was measured, representing 5mmol/L at these depths. The observed peak area for 5mmol/L was divided by the receiver coil phantom peak area, giving a normalization constant with increasing depth. Absolute tissue concentrations were calculated by normalizing the peak area of the phosphorus metabolites *in vivo* with the same receiver coil phantom. The normalization constant coefficient of variation (Table 3.1) with depth was found to be small (5% at 3cm) in the superficial regions but increased with depth (10% at 6cm).

3.3.0. Regional studies of ^{31}P Metabolites

3.3.1 Temporoparietal image

The temporoparietal image was selected because of the parallel tissue planes both histologically and biochemically; namely temporalis muscle, gray then white matter. However skeletal muscle, in overlying temporalis superficialis, has approximately four times as much phosphocreatine (Harris et al, 1974) as brain by wet weight (Veech et al, 1973) and can "contaminate" the brain spectrum. The distance between muscle and brain is relatively short, separated by a thin layer of squamous temporal bone (approximately 0.5cm). The spatial resolution needs to be less than 1cm so that superficial brain is not contaminated by muscle signal.

The white matter is extensive as many axons converge on the internal capsule, as well as the optic radiation sweeping around to the occipital lobe. To confirm this, a human brain at autopsy was sliced in the sagittal plane with

Figure 3.1

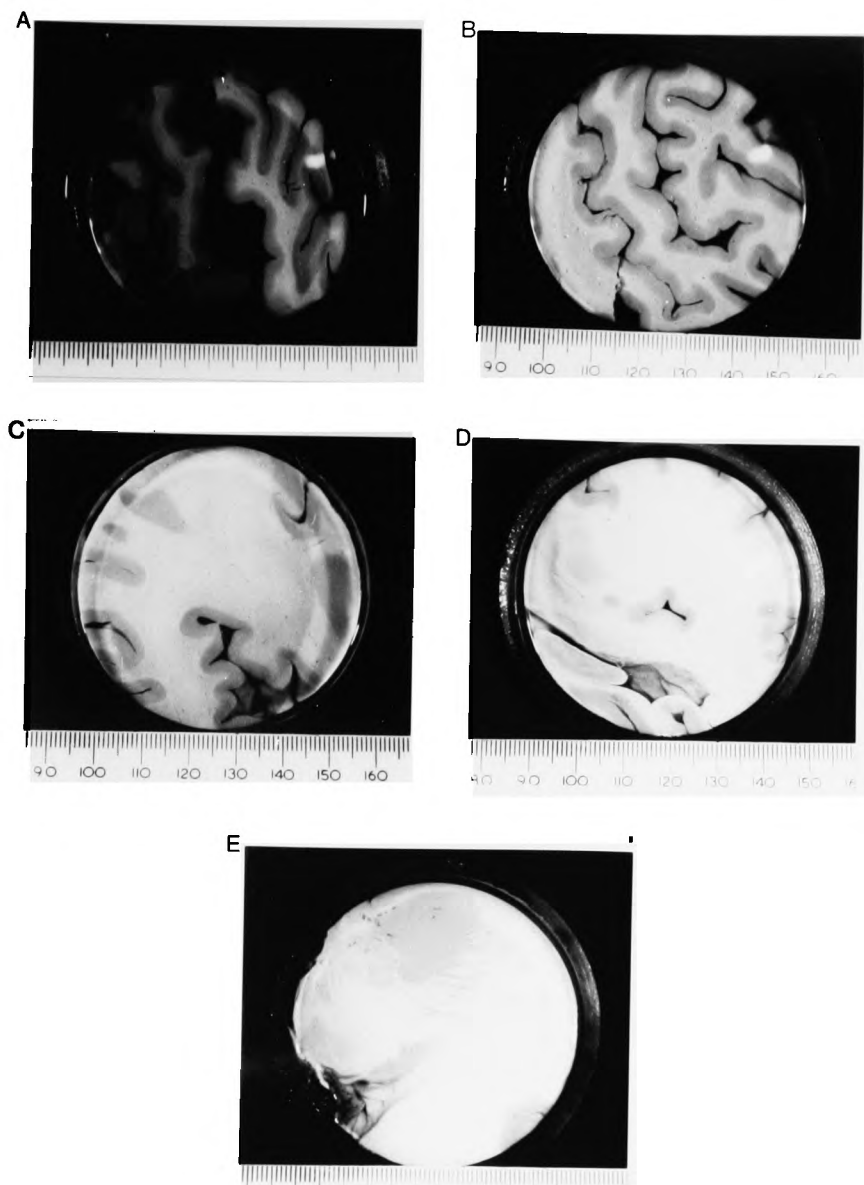


Figure 3.1 A human brain was sliced at autopsy into 1cm thick sagittal sections through the temporoparietal region from superficial (A, approximately 2cm from the receiver coil) to deep (E) brain (Dr.M. Esiri, Dept. of Neuropathology, Radcliffe Infirmary). Note the predominance of gray matter in the superficial and white matter in the deeper slices.

Figure 3.1b

TABLE SHOWS THE RELATIVE AREAS OF GREY AND WHITE MATTER WITH DEPTH INTO THE TEMPOROPARIETAL AREA OF THE HUMAN BRAIN.

Depth (cm)	Grey		White	
	Weight (g)	%	Weight (g)	%
1	1.32	85.2	0.23	15
2	0.96	51	0.92	48
3	0.62	31	1.4	69
4	0.34	17	1.62	83
5	0.2	9	1.84	91

Figure 3.1b The grey and white matter sections of photographs of figure 3.1a were "cut and weighed" to estimate the relative fractions occupied by these tissues.

Figure 3.2

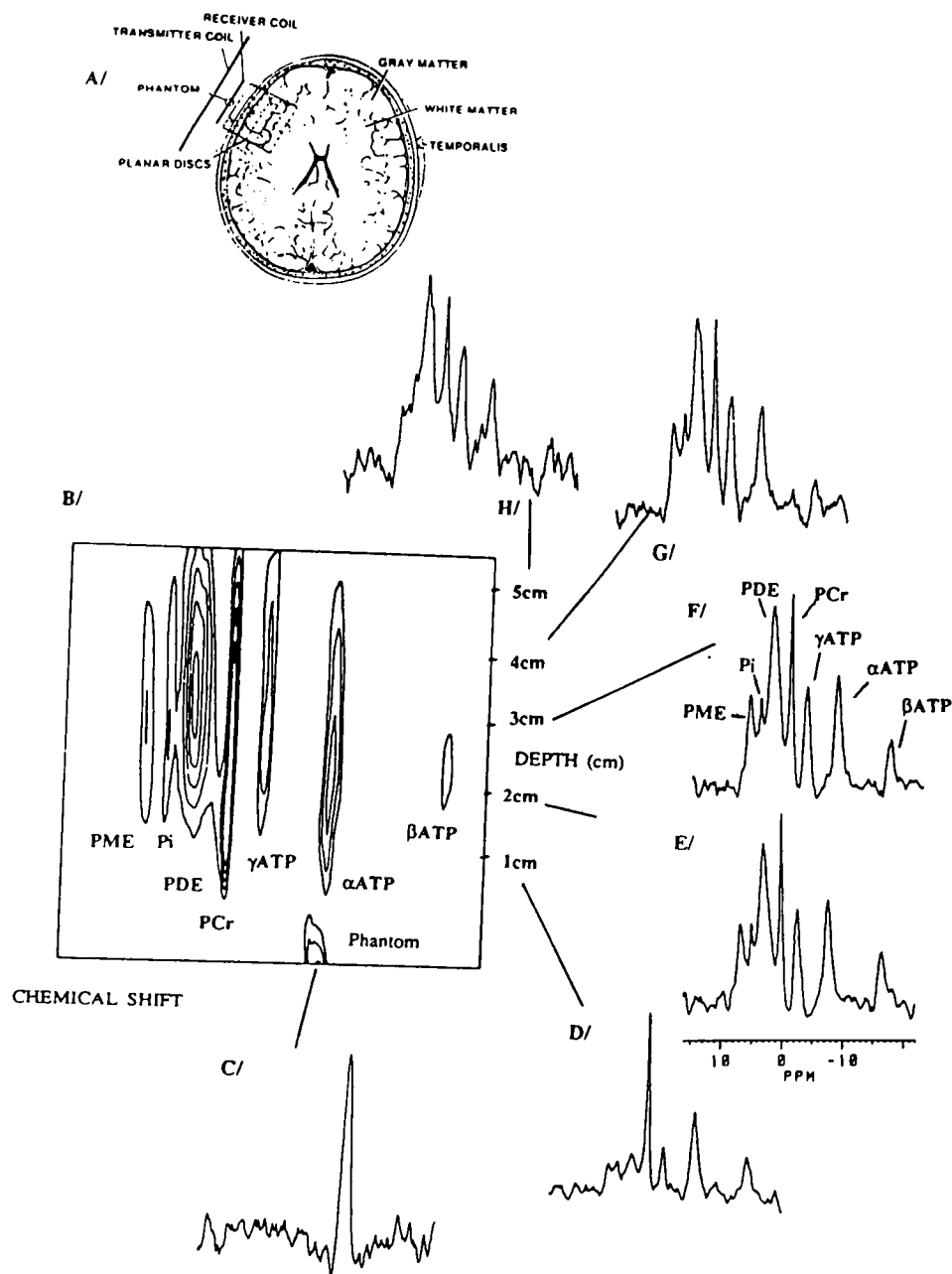


Figure 3.2 The diagram (A) describes the anatomical regions from which the phosphorus signal is received. The contour plot and selected spectra (B) from a normal subject show signal from the receiver coil phantom at 0cm (C), temporalis muscle at 1cm (D) and superficial (E) and deep (F-H) brain.

(Figure 3.1). At 4-5cm the field of view is entirely white matter, whilst the superficial slices are a mixture of both. Surface anatomy was used to identify a point *in vivo* over which the centre of the receiver coil was placed. A point 3cm along the zygoma from the tragus of the ear was marked. The centre of the receiver coil was placed 4cm above the point on the zygoma.

Two groups of healthy subjects were studied; 10 with the first probe with ratios reported and 24 with the second probe measuring absolute concentrations. Results, with pHi measurements, are shown (Tables 3.2, 3.3, 3.4; Figure 3.2). The temporoparietal images show intense superficial PCr signal corresponding to the overlying temporalis muscles. The PCr/ATP ratio falls from muscle into normal brain (muscle(1cm)= 4.2 ± 1.07 to brain(2cm)= 1.47 ± 0.37). The Pi/ATP ratio (0.84 ± 0.31 SD) is also greater in temporalis muscle than underlying brain (0.26 ± 0.1). The pHi may be more alkaline in overlying muscle (7.06 ± 0.04) than brain (7.03 ± 0.02). There is no pHi difference between superficial (mainly gray matter, 2cm= 7.03 ± 0.03) and deep brain (mainly white matter, 5cm= 7.02 ± 0.03). The PME/ATP ratio remains constant with depth (0.65 ± 0.16 to 0.70 ± 0.11). The PCr/ATP ratio decreases slightly with depth into brain (2cm= 1.47 ± 1.05 to 5cm= 1.05 ± 0.27). The PDE/ATP ratio increases with depth (2.32 ± 0.58 to 3.4 ± 1.45). There is also an increase in the coefficient of variation of PDE (25% to 43%).

The "pulse and collect" (P+C) data is a mixture of muscle and brain, with a raised PCr/ATP (1.63 ± 0.46), and Pi/ATP (0.34 ± 0.08). The β ATP/ γ ATP being similar (1.21 ± 0.27), suggesting that the use of γ ATP for comparison was reasonable. Areas of the overlapping peaks were estimated using a Lorentzian line fitting routine (Glinfit, Bruker). The frequency difference between the Pi and PCr peaks (ppm) was measured by the spectrometer.

3.3.2 Occipital image

The occipitoparietal region was imaged in four healthy subjects from lateral to medial to maximise gray/white differences. This approach to the occipital lobe introduced some parietal lobe into the image. Centre of the receiver coil was placed over a point 5cm above the mastoid process, behind the ear. There was minimal superficial skeletal muscle, but the skull was relatively thick (1-2cm). Narrow proton line widths were found in this region (10-15Hz).

This image (Table 3.5) did not differ from the temporoparietal brain data. The pHi (2cm= 7.04 ± 0.05 to 6cm= 7.03 ± 0.03), Pi/ATP (2cm= 0.25 ± 0.12 to 6cm= 0.32 ± 0.09) and PME/ATP (2cm= 0.55 ± 0.1 to 6cm= 0.66 ± 0.18) was constant with depth. There was an increase in PDE/ATP with depth (2cm= 2.25 ± 0.43 to 6cm= 3.87 ± 0.81). The PCr/ATP did not vary (2cm= 1.03 ± 0.33 to 6cm= 1.16 ± 0.11). The proton line width (10Hz) was narrower in this region improving the signal with depth (6cm).

3.3.3 Frontal image

The frontal lobe image (n=4) was taken from lateral to medial, with the receiver coil positioned over a point 4cm above the lateral canthus of the eye. There are some anterior fibres of temporalis superficialis overlying skull and brain. This view was useful as a control for some of the frontal lobe convexity meningiomas studied, which in this small study were the most common meningiomas.

The results (Table 3.6) show no difference in the metabolite ratios between this image of the frontal lobe and images of the temporal and occipital regions. The metabolite ratios follow a similar trend (constant pHi, PME/ATP, PCr/ATP and

Pi/ATP; rising PDE/ATP). However the superficial (2cm) depth have lower PCr/ATP than seen in the other two images.

3.4.0 Absolute tissue concentrations

The temporoparietal image was used to obtain absolute tissue metabolite concentrations. 24 subjects were studied with a large age range (mean=48, 22-75yrs). All subjects were from either the MRS unit staff, or preoperative patients with no neurological history from a general surgical ward. The [ATP] (2cm=3.41mmol/L, 5cm=3.78mmol/L) showed no significant variation with depth. The [PCr] (2cm=5.21mmol/L, 5cm=4.85mmol/L) also showed no significant variation with depth into the brain, as with [PME], [Pi] or pHi. The observed [PDE] increased with depth (2cm=9.53mmol/L, 5cm=14.41mmol/L). The PDE peak measured using this technique only represents the narrow "tip of the iceberg" since much of the broad component has been removed by profiling (Gordon et al, 1982). Metabolite absolute tissue concentration coefficient of variation increased with depth (Table 3.4). No significant difference could be seen between left and right hemispheres.

Table 3.2 **VARIATION WITH DEPTH OF PHOSPHORUS METABOLITES**
IN THE TEMPOROPARIETAL LOBES *IN VIVO*.

Depth (cm)	pHi	PME/ATP	Pi/ATP	PDE/ATP	PCr/ATP
1 (muscle)	7.06 ±0.04	---	0.84 ±0.31	---	4.2 ±1.07
2 (Brain)	7.03 ±0.03	0.65 ±0.16	0.26 ±0.10	2.32 ±0.58	1.47 ±0.37
3	7.02 ±0.02	0.66 ±0.16	0.26 ±0.06	2.54 ±0.75	1.23 ±0.29
4	7.03 ±0.03	0.76 ±0.16	0.29 ±0.12	3.36 ±1.5	1.12 ±0.31
5	7.03 ±0.03	0.70 ±0.11	0.28 ±0.16	3.40 ±1.45	1.05 ±0.27
P+C	7.03 ±0.03	0.77 ±0.20	0.34 ±0.08	3.12 ±0.57	1.63 ±0.21

The temporoparietal lobes were studied in normal healthy subjects (n=10, mean age=34yrs, 22-55). Early normal studies were calibrated for depth only and metabolite concentrations are presented as ratios with respect to γ ATP peak area.

Table 3.3. **VARIATION WITH DEPTH INTO THE TEMPOROPARIETAL**
OF METABOLITE ABSOLUTE CONCENTRATION AND pHi *IN VIVO*

N=24, (Mean age = 48, range 22-75yrs)

Depth (cm)	pHi	PHOSPHORUS METABOLITES				
		PME	Pi	PDE	PCr	ATP
(Temporalis muscle, scalp and skull)						
1	7.05 ±0.04	n.d.	2.58 ±0.92	n.d.	10.19 ±3.9	2.86 ±0.8
(Brain)						
2	7.04 ±0.03	2.76 ±0.9	1.11 ±0.37	9.53 ±2.6	5.21 ±1.25	3.41 ±0.7
3	7.03 ±0.03	2.76 ±1.0	1.15 ±0.41	10.62 ±2.7	4.73 ±1.3	3.46 ±0.66
4	7.03 ±0.03	2.90 ±1.17	1.11 ±0.36	12.42 ±3.7	4.78 ±1.5	3.54 ±0.9
5	7.03 ±0.03	2.96 ±1.1	1.01 ±0.54	14.41 ±4.21	4.85 ±1.49	3.78 ±0.9

Table 3.4 **PHOSPHORUS METABOLITE COEFFICIENT OF VARIATION**

Depth (cm)	Phosphorus metabolite (%)				
	PME	Pi	PDE	PCr	ATP
Muscle					
1	0	35	0	38	31
Brain					
2	32	33	28	24	20
3	36	36	27	27	19
4	40	32	29	32	27
5	37	54	29	30	26

Later studies were performed with a calibrated receiver coil phantom for both depth and concentrations (see Table 3.1). The absolute tissue concentration (mmol/L) corresponds to the peak area under the metabolite peak (Table 3.3). The PME and PDE are composite peaks representing more than one compound. The range of variation is expressed as a standard deviation from the mean (±SD). The coefficient of variation is shown in Table 3.4.

Table 3.5 **VARIATION OF PHOSPHORUS METABOLITE RATIO IN OCCIPITOPARIETAL LOBE *IN VIVO*.**

Depth (cm)	pHi	PME/ATP	Pi/ATP	PDE/ATP	PCr/ATP
2 (Brain)	7.04 ±0.05	0.55 ±0.10	0.25 ±0.12	2.25 ±0.43	1.03 ±0.33
3	7.02 ±0.02	0.83 ±0.25	0.31 ±0.09	3.03 ±0.64	1.14 ±0.16
4	7.02 ±0.02	0.67 ±0.11	0.30 ±0.07	3.03 ±0.55	1.08 ±0.12
5	7.01 ±0.04	0.54 ±0.08	0.39 ±0.13	3.38 ±0.24	1.28 ±0.06
6	7.03 ±0.03	0.66 ±0.18	0.32 ±0.04	3.87 ±0.81	1.16 ±0.11

Table 3.6 **VARIATION OF PHOSPHORUS METABOLITE RATIOS IN THE FRONTAL LOBE *IN VIVO*.**

	pHi	PME/ATP	Pi/ATP	PDE/ATP	PCr/ATP
Depth (cm)					
2 (Brain)	7.01 ±0.03	0.83 ±0.14	0.29 ±0.11	2.49 ±0.70	1.08 ±0.26
3	7.02 ±0.03	0.83 ±0.30	0.37 ±0.19	2.71 ±0.71	1.14 ±0.26
4	7.04 ±0.03	0.77 ±0.30	0.35 ±0.19	3.34 ±0.25	1.27 ±0.13
5	7.02 ±0.02	1.04 ±0.14	0.28 ±0.15	3.90 ±1.16	1.25 ±0.22

The occipital (n=4)(Table 3.5) and frontal lobes (n=4)(Table 3.6) were also investigated, and the results are presented as ratios with respect to γ ATP.

3.5.0 Discussion of biochemical heterogeneity

The different regions of the cerebral hemispheres studied did not appear to differ in metabolite concentration with respect to ATP or pHi. Measurements of absolute tissue concentration did not differ significantly between left and right hemisphere. The coefficient of variation for tissue concentration in normal brain was 10-20% for ATP, and increased for other metabolites overlying the PDE peak.

Significant differences in the [PDE] concentration were found between superficial (mainly gray matter) and deep (mainly white matter) parts of the phosphorus image. The intensity of the PDE peak increased with depth (9.4-14.5mmol/L), reflecting a relative increase in line width and height. A small decrease was seen in [PCr] with depth, but no change in [ATP], [PME], inorganic phosphate concentration or pHi. An experiment using magnetization transfer was used to investigate the composition of this PDE *in vivo*. Magnetization transfer was also used to investigate the enzyme activity of creatine phosphokinase in the human brain *in vivo*.

3.6.0 Metabolic heterogeneity between gray and white matter

3.6.1 Imaging creatine kinase activity.

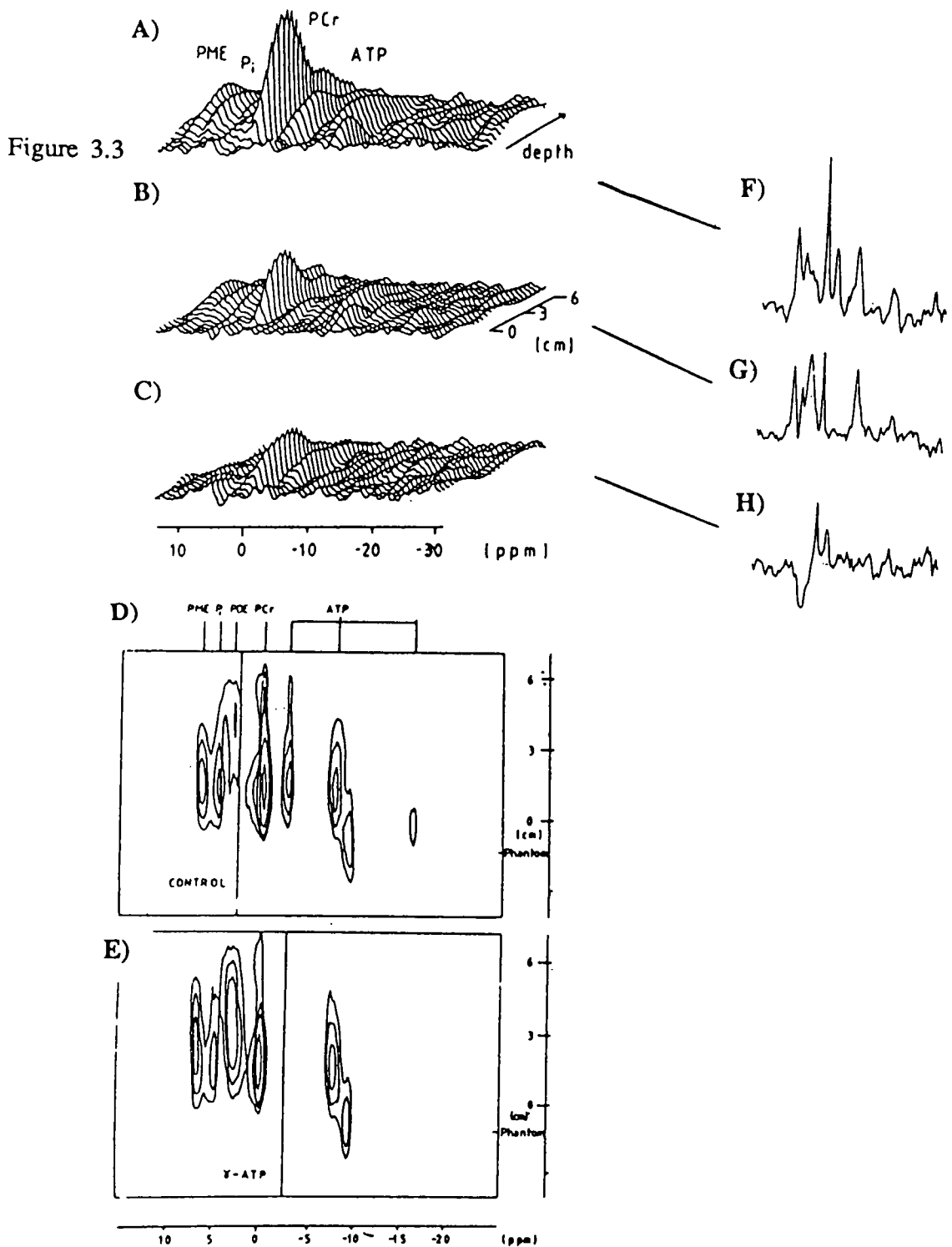
Six healthy subjects were studied (Figure 3.3) and the results of the magnetization transfer investigations of creatine phosphokinase activity show marked differences in forward flux rate of PCr between the superficial brain (2cm; mainly gray matter) and deep brain (4.5cm; mainly white matter) (Table 3.6). The γ ATP has been completely saturated with depth (Figure 3.3) using the "ear-phone" coil for the saturation pulse and the double surface coil for PMRFI. The γ phosphate of ATP

is transferred via the creatine kinase enzyme to phosphocreatine reducing the intensity of the PCr peak. Low rates of flux will produce a small reduction in the PCr peak area. Whilst rapid flux rates will produce a large reduction in PCr area with respect to the amount of ATP present.

The absolute tissue concentrations of ATP and PCr were not significantly different from those measured in the temporal image (Table 3.6). In the 3 subjects studied the saturated T_1 estimations showed a longer T_1 for PCr in white matter ($4.5\text{cm}=3.22\text{s}$), though the error in this measurement was large ($SD=\pm 0.81\text{s}$) (Table 3.7).

3.6.1 The phosphodiester peak

Three experiments were carried out investigating magnetization transfer within the large broad phosphodiester peak which overlies a very broad (3000Hz) signal from bone and a narrower (2000Hz) signal from phospholipids. The α ATP peak was saturated so that the efficiency of this off resonance saturation pulse with depth could be recorded in the phosphorus image (Figure 3.4). There is no biochemical reaction known which could transfer the α phosphate of ATP to the phosphodiester of phospholipid, but transfer can occur within the broad PDE peak. The off resonance saturation of the PDE peak resulted in a collapse of the sharp component of this peak (Figure 3.4). The sharp component of the PDE peak, as measured by the difference between the control and saturated images, was constant with depth into the brain (Table 3.8).



A radio frequency saturation pulse is set at the frequency of γ ATP and PMRFI is used to gain a one dimensional metabolite map shown as a stack plot (B) and contour plot (E). A control image is collected with radio frequency saturation set at a frequency equidistant from PCr shown as a stack plot (A) and contour plot (D). The difference between (A) and (B) is shown (C) as a stack plot. Selected spectra show the result of the γ ATP (G) and control (F) and difference (H).

Table 3.6 DEPTH VARIATION OF CPK ACTIVITY *IN VIVO*

	[PCr]* (mMol.l ⁻¹)		[ATP] (mMol.l ⁻¹)		[PCr]/[ATP]*		M _s /M _o ** (PCr)		KF** (s)		Flux** (mMol.l ⁻¹ .s ⁻¹)	
Depth	2.0	4.5	2.0	4.5	2.0	4.5	2.0	4.5	2.0	4.5	2.0	4.5
Subject												
1	5.5	4.9	3.2	2.5	1.7	1.9	0.3	0.5	0.3	0.1	1.6	0.7
2	4.6	4.2	3.4	3.3	1.3	1.3	0.4	0.5	0.2	0.2	1.1	0.7
3	5.1	4.6	3.2	3.6	1.6	1.3	0.4	0.6	0.2	0.1	1.3	0.6
4	5.3	5.0	3.6	3.4	1.5	1.4	0.3	0.5	0.3	0.2	1.7	0.8
5	4.8	4.3	2.8	3.0	1.7	1.4	0.2	0.5	0.3	0.14	1.6	0.6
6	5.8	4.7	3.6	3.7	1.6	1.3	0.2	0.5	0.3	0.2	2.0	0.8
Mean	5.2	4.6	3.3	3.3	1.6	1.4	0.3	0.5	0.3	0.2	1.6	0.7
±SD	0.4	0.3	0.2	0.4	0.1	0.2	0.1	0.05	0.04	0.02	0.3	0.12
* p<0.01												
** p<0.001												

Two depths have been selected from the 2DFT matrix of 6 subjects studied; superficial (mainly gray matter at 2cm) and deep brain (mainly white matter at 4.5cm). The absolute tissue concentrations for PCr and ATP, the PCr/ATP ratio, saturated PCr peak area (M_s)/control PCr peak area (M_o) ratio (M_s/M_o), forward flux rate constant (KF) and forward flux rate (Flux) have been measured at these two depths and compared. Significant differences were calculated using a Students t test for paired samples.

Table 3.7 **T_i RELAXATION TIME MEASURED IN THE PRESENCE OF
γATP SATURATION (T_{1APP}).**

(N=3)

	T_{1app} (s)	
Depth (cm)	2.0	4.5
Subject		
1	1.25	4.02
2	2.64	2.20
3	3.08	3.37

Mean	2.33	3.20
±S.D.	0.87	0.81

Figure 3.4

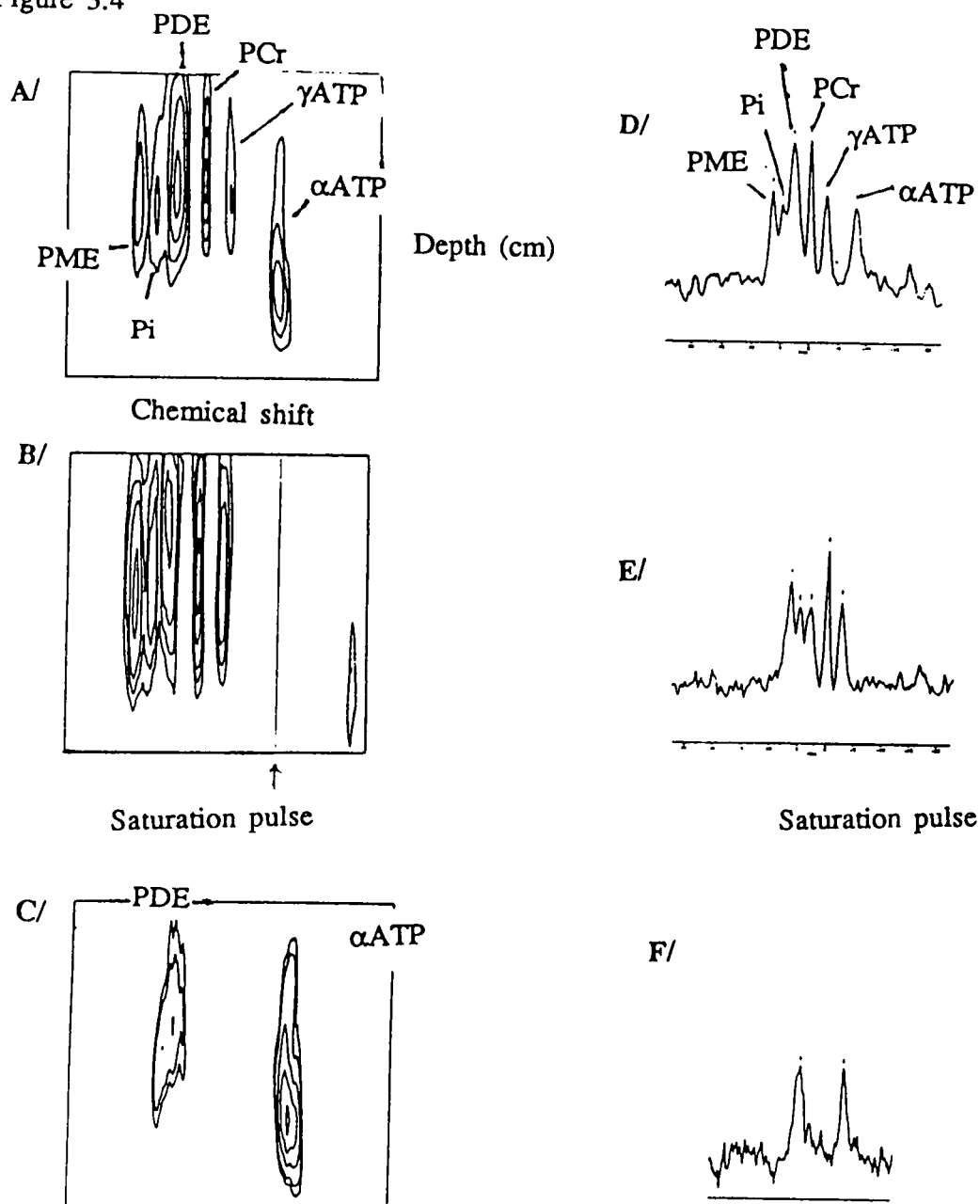


Figure 3.4 The saturation pulse is set on α ATP and a PMRFI experiment run. A contour plot (B) demonstrates the effect of off resonance saturation on the PDE peak. The difference (C) between the control matrix (A) and (B) demonstrates the off resonance effect with depth. Selected spectra at 3cm from the control (D), saturated (E) and difference (F) can be used to quantify the off resonance effect on the PDE peak.

Table 3.8 **OFF RESONANCE (at α ATP) SATURATION OF PDE WITH DEPTH *IN VIVO*.**

(N=2)		SUBJECT					
		A			B		
		[PDE']	[α ATP]	PDE//ATP	[PDE']	[α ATP]	PDE//ATP
		mmol/L			mmol/L		
Depth							
(cm)							
2		4.66	3.06	1.52	4.26	3.63	1.17
(Brain)							
3		5.35	3.86	1.39	4.96	3.64	1.36
4		4.87	3.33	1.46	4.34	3.66	1.18
5		4.19	2.42	1.73	4.80	4.05	1.29
Mean		4.68	3.46	1.39			
$\pm$ SD		0.4	0.52	0.18			
(all 8 data points)							

3.7.0 Summary

The PMRFI method was extended to measure absolute tissue concentrations of metabolites (ATP, Pi, PCr, and PME and PDE peaks) non-invasively *in vivo*. In ideal circumstances the coefficient of variation, as in studying well shimmed phantoms *in vitro*, is small *in vivo* (5-10%). The coefficient of variation for metabolite concentrations was small (10% for [ATP]) when studying a small group of subjects of similar age, with a narrow proton line width, as demonstrated in the CPK activity imaging studies. In the larger study, with a wider range of ages and proton line widths, the coefficient of variation increased (20% for [ATP]). No significant difference in absolute tissue concentration could be demonstrated between the left and right cerebral hemispheres. No regional difference in the concentration of metabolites with respect to ATP in the three images (frontal, temporoparietal or occipital) could be demonstrated. However a significant increase in the PDE peak was seen with depth into normal brain. Off-resonance saturation experiments of the PDE peak suggest that this rise in PDE is not due to an increase in mobile phospholipids such as found in endoplasmic reticulum but relatively immobile phospholipids probably found in myelin.

Images of CPK activity demonstrated greater forward flux rates in superficial tissues (mainly gray matter) than deep tissues (mainly white matter). Previous experimental studies of ATP utilization in muscle (Brindle et al, 1989) and CPK activity in the brain have been performed with magnetization transfer and MRS (Shourbridge et al, 1982; Balaban et al, 1983), showing similar flux rates for gray matter but were not able to map CPK activity through the brain or localise white

matter level. Immunohistochemistry has identified the CPK BB-isoenzyme in both neurones and glial cells (Yoshimine et al, 1983), and enzyme assays of CPK BB-isoenzyme have demonstrated more activity in cortical biopsies than corpus callosum (Miller and Wei, 1985). The function of PCr and CPK in the brain remains unknown. However some interesting theories have been proposed (Freidhoff and Lerner, 1977) based on the concept that PCr and CPK support subcellular [ATP] when local demand for ATP is greater than diffusion from mitochondria. This assumes that the ATP consumption is periodic and exceeds the diffusion rate of ATP from mitochondria as in synaptosomal discharge in the neurones. This hypothesis is supported by the greater CPK activity noted in the gray than white matter where neural cell bodies and synapses are concentrated.

Clinical investigations could be carried out imaging CPK flux rates for certain pathological processes involving increased synaptosomal activity such as epilepsy. Increased CPK flux rates may be associated with epilepsy and be used as a marker to localize the epilepsy epicentre in the future.

CHAPTER 4

BRAIN TUMOUR METABOLISM *IN VIVO*

4.1.0 Introduction

Many excellent reviews have discussed the biochemical and metabolic differences between normal and neoplastic tissues (Argiles and Azcon-Bieto, 1988). Differences in molecular biology have received particular attention with the demonstration of oncogenes (Freeman et al, 1989), which have been associated with specific biochemical changes in cytoplasmic metabolism (Nishizuha, 1984) such as expression of growth factor and oestrogen receptors in breast tumours. Primary brain tumours also express differences in cell surface receptors, with increased epidermal growth factor receptors on glial tumours (Reubi et al, 1989), and increased steroid receptors on meningiomas (Lesch and Gross, 1987).

The aim of this study was to investigate changes in intracellular biochemistry of primary brain tumours. Biochemical studies of biopsy specimens taken at the time of surgery have proved to be inconsistent (Lowry et al, 1977) leading to the concept of biochemical heterogeneity within a tumour group such as gliomas. This interpretation of tumour biochemistry could be misleading since the "ischaemia" time from removal to freezing must be variable due to the surgery. A solution to this problem was to study the tumours *in vivo* prior to surgery using a non-invasive technique.

Both positron emission tomography (Brooks et al, 1985) and magnetic resonance spectroscopy (Oberhaensli et al, 1986) studies have been performed on primary brain tumours *in vivo*, demonstrating differences in intracellular pH (pHi) between normal brain and tumour. Surprisingly the pHi of the gliomas and meningiomas was either alkaline or within the normal range for human brain; no

significantly acidic tumours were seen in these small studies. However the spatial localization techniques used in these studies were limited, allowing contamination from surrounding oedematous brain and skeletal muscle.

The PMRFI technique was used to investigate the cellular metabolism of primary brain tumours *in vivo* prior to surgical removal for histological diagnosis. In addition some patients with secondary tumours, breast carcinomas and melanomas were also studied for comparison.

A small group of patients with pituitary adenomas causing acromegaly were investigated to see if the large quantities of growth factors released by these tumours could induce a change in pH_i in nearby normal brain. Growth hormone breaks down to a series of growth factors which could modulate pH_i in normal cells via cytoplasmic membrane receptors increasing sodium/proton exchanger activity.

4.2.0 Glial tumours

4.2.1 Astrocytomas

The glial tumours in adults are derived from three types of glial cells; astrocytes, oligodendrocytes and ependymomas. Astrocytomas are the most common glial tumour and have been classified (Kernohan et al, 1949) into four grades, with de-differentiated tumours placed into a glioblastoma group. Seven patients with superficial (within 6cm of the skull surface by contrast enhancement on CT scan) astrocytomas (Grade II-IV) were studied prior to surgery. The results of the PMRFI data are presented (Figure 4.1, Table 4.1). Ratios with respect to ATP were used since the method for absolute tissue concentrations had not been developed at this stage.

Figure 4.1

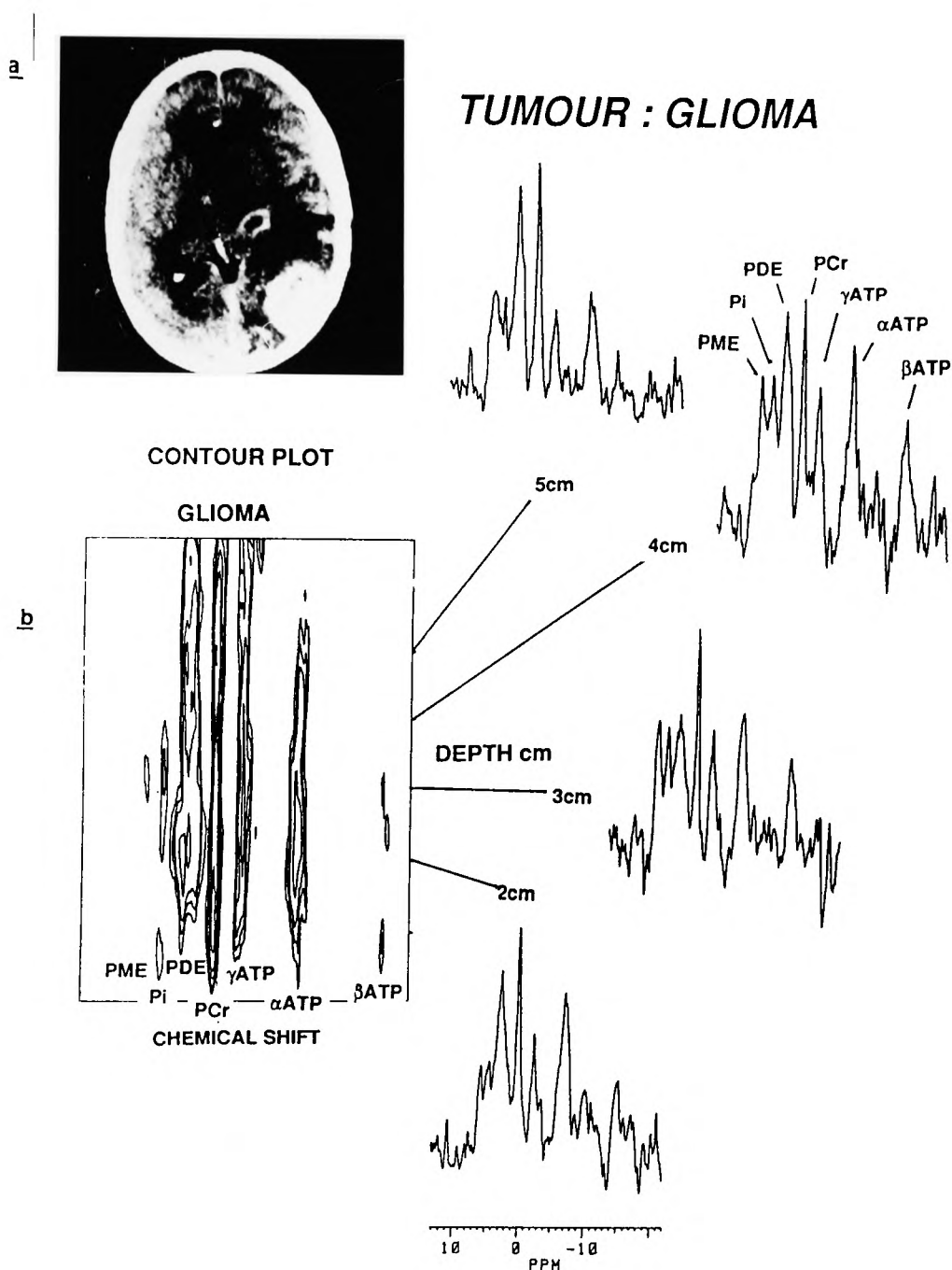


Figure 4.1 The PMRFI data set from an occipital glioma (Astrocytoma grade IV) was initially localized by Ct scan (A). The contour plot (B) and selected spectra show elevated PCr throughout the tumour, with raised PME and reduced PDE.

Figure 4.2

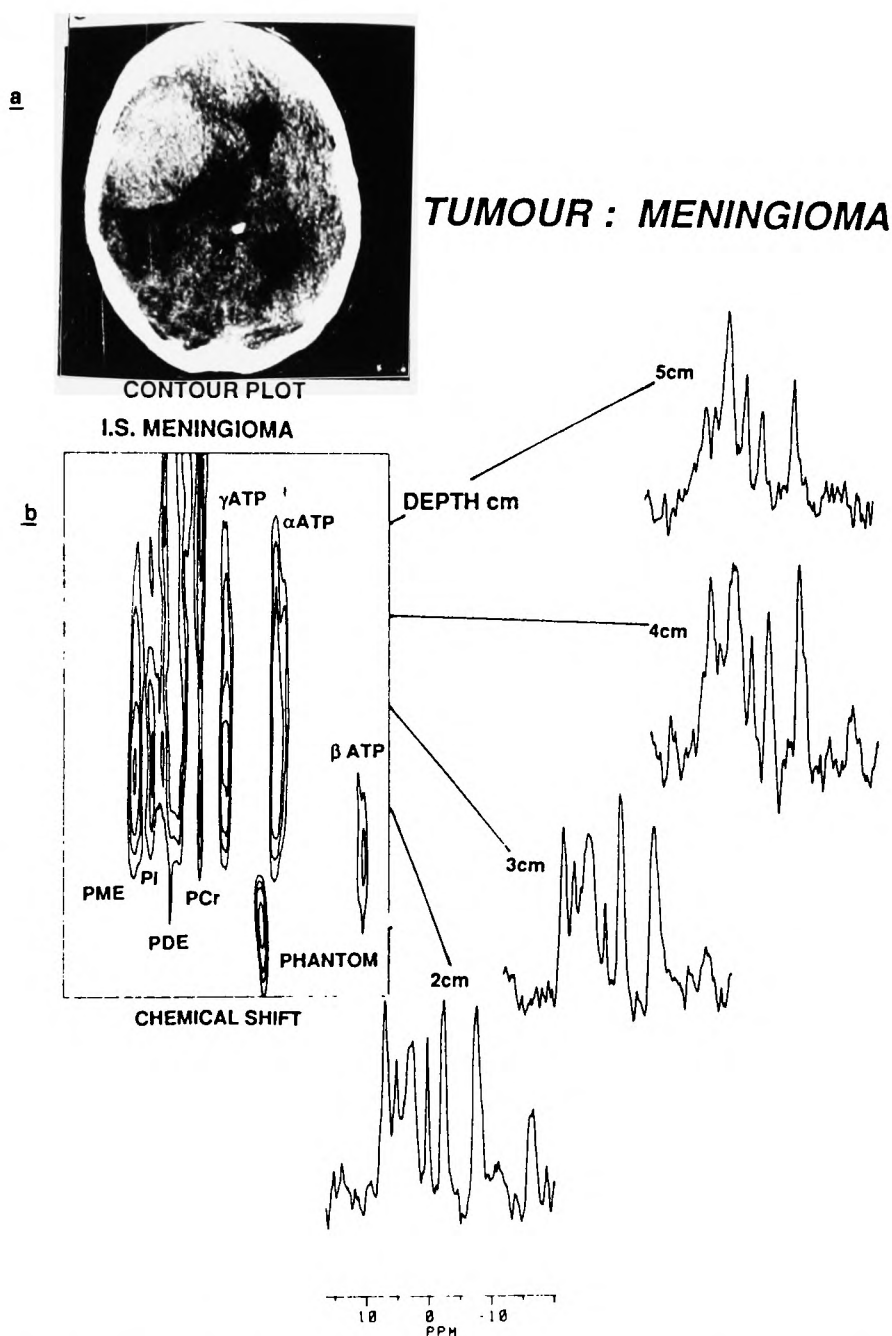


Figure 4.2 The PMRFI data (B) from a convexity meningioma, localized initially by Ct scan, shows a reduction in PCr at a depth corresponding to tumour centre. The PCr rises in displaced brain and the pHi is alkaline in the tumour.

Table 4.1 **PHOSPHORUS METABOLITE RATIOS AND pHi IN TUMOURS**

GLIOMAS (astrocytomas)

Name	Age	Dexam.	Grade	pHi	PME/ATP	PCr/ATP	PDE/ATP
1	50	+	IV	7.12	1.01	3.29	3.03
2	22	+	II	7.07	1.64	3.13	4.54
3	32	+	III	7.11	0.83	1.01	2.60
4	48	-	II	7.02	1.06	1.49	4.76
5	35	+	II	7.06	0.98	1.39	3.70
6	28	-	III	7.06	1.78	3.09	3.03
7	49	-	III	7.10	0.82	1.24	1.78
Mean	41			7.08	1.16	2.09	3.33
S.D.	5			±.03	±.4	±.5	±.4
P=				<.05	<.05	<.05	NS

MENINGIOMA

8	72	+	BGN	7.17	1.02	0.74	1.78
9	59	+	BGN	7.18	1.12	0.34	2.22
10	48	+	BGN	7.15	1.05	0.81	2.84
11	64	+	BGN	7.28	1.12	0.66	2.85
Mean	61			7.19	1.08	0.64	2.42
S.D.	10			±.02	±.05	±.1	±.5
P=				<.05	<.05	<.05	<0.1

Table 4.2 **ABSOLUTE TISSUE CONCENTRATION OF PHOSPHORUS METABOLITES WITH DEPTH AND pHi IN ASTROCYTOMAS AND MENINGIOMAS**

(A)	PRIMARY BRAIN TUMOURS					
	(N=8) (MEAN±SD)	Phosphorus metabolites				
	pHi	PME	Pi	PDE	PCr	ATP
Depth (cm)						
Muscle						
1	7.08 ±0.05	0	2.76 ±1.05	0	12.6 ±4.5	2.68 ±0.38
Brain						
2	7.07 ±0.05	3.31 ±0.94	1.11 ±0.4	9.26 ±3.2	6.46 ±2.8	3.37 ±1.06
3	7.095 ±0.05	3.57 ±0.84	1.15 ±0.46	9.58 ±3.56	4.84 ±1.52	3.14 ±1.15
4	7.11 ±0.07	3.16 ±1.16	1.26 ±0.56	10.53 ±2.5	4.78 ±1.3	3.26 ±0.98
5	7.17 ±0.15	3.73 ±1.47	1.3 ±0.8	12.6 ±4.74	4.46 ±1.41	3.14 ±1.09
(B)						
ABSOLUTE CONCENTRATION OF MENINGIOMA						
(N=2)						
	pHi	Phosphorus metabolite (mmol/L)				
		PME	Pi	PDE	PCr	ATP
Depth (cm)						
2 brain	24,6.96	2.21,4.53	1.26,1.21	7.16,11.5	2.56,4.79	1.42,3.37
3	7.24,7.08	2.61,4.66	1.85,1.18	6.76,9.36	2.26,2.93	2.46,4.04
4	7.33,7.12 '	4.15,3.94	0.77,1.72	7.49,12.0	1.68,2.15	2.61,4.88
5	-,7.13	3.37,5.59	-,1.92	5.69,12.39	2.61,3.64	2.71,4.52

The absolute tissue concentrations (Mean and SD) for astrocytomas (A) and meningiomas (B) are presented.

A further eight patients were studied prior to surgery, comprising a mixture of astrocytomas and glioblastomas, guided by MRI and absolute tissue concentrations were measured (Table 4.2). Significant differences between normal and pathologically affected brain were tested using the Wilcoxon test for non-paired non-parametric samples (SAS system).

The metabolite ratios with respect to ATP and absolute tissue concentrations showed a significant increase in the PME content (3.16-3.75mmo/L) in the tumours (Table 4.2). Three of the astrocytomas studied had increased PCr/ATP ratios (3.29, 3.13, 3.09), but the mean [PCr] was within the normal range (4.78 ± 0.78 mmol/L). The mean [ATP] concentration (3.26 ± 0.98 mmol/L) was similar to normal brain. There was a small insignificant reduction in PDE content in astrocytomas (10.53 ± 2.5 mmol/L). The astrocytomas were more alkaline (7.08 ± 0.03 , 7.11 ± 0.07) than normal brain, with pH_i increasing towards tumour centre.

Three cystic astrocytomas were also studied to investigate the cyst contents *in vivo*. The results are shown (Figure 4.3). Surprisingly the cysts contain a strong PDE signal, with reduced Pi and insignificant amounts of ATP, PCr or PME. Aspirates of the cyst fluid were placed in a small bore two tesla magnet to confirm these findings. The chemical composition of the cyst fluid aspirate is currently under investigation.

4.2.2 Oligodendrogliomas

The normal and tumour affected hemispheres of the patient with an oligodendroglioma were studied prior to surgery and the tumour showed similar biochemical changes to the astrocytomas (Figure 4.4). The tumour pH_i (7.08) was

Figure 4.3

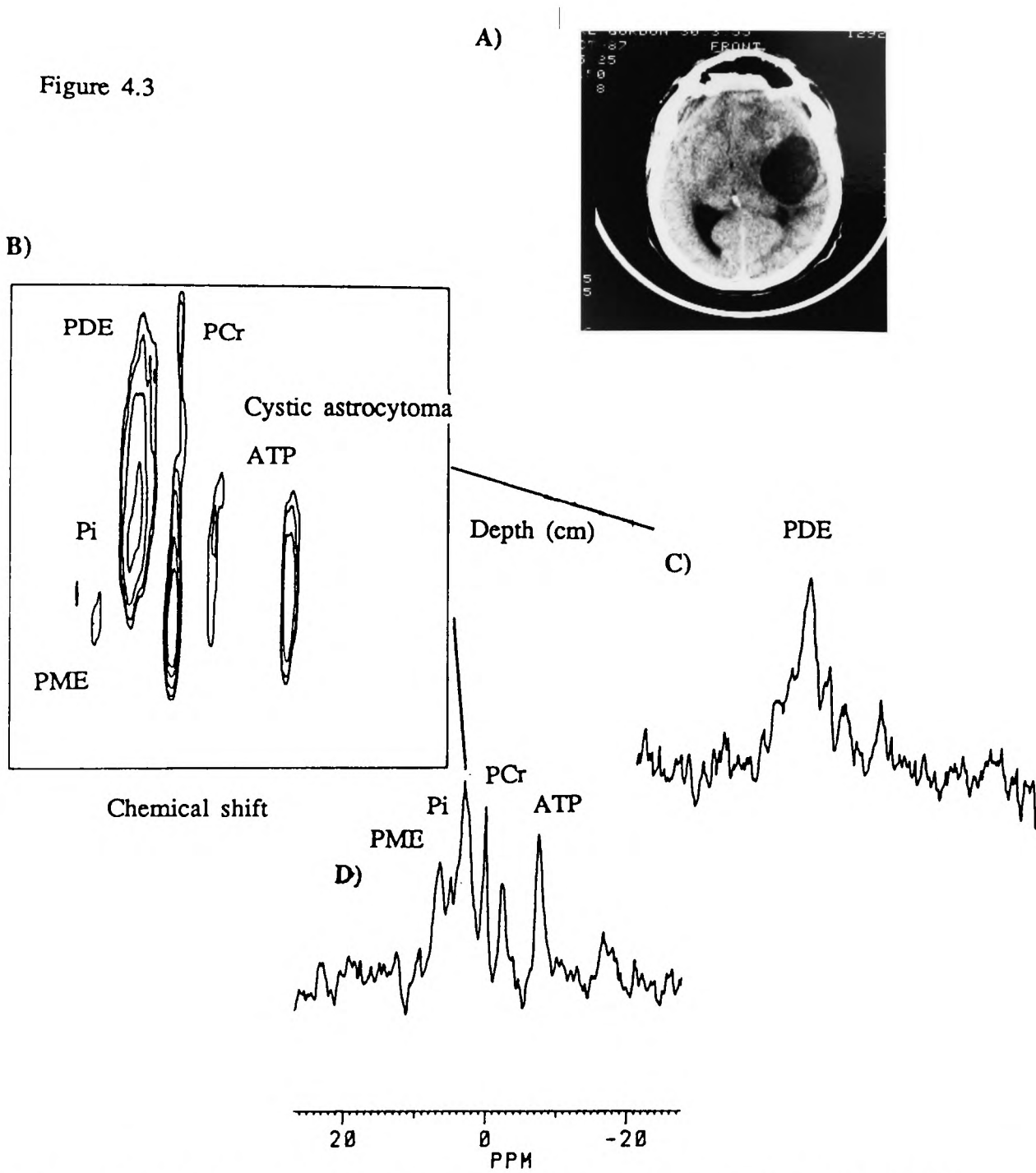


Figure 4.3 The Ct scan (A) demonstrates the large cystic astrocytoma. The PMRFI contour plot (B) shows a region at 4-5cm, corresponding to the depth of the cyst, with a large PDE peak and small PCr, ATP, and PME peaks, also shown in the spectra taken at 4cm (C). The superficial region (2cm) has a raised PME peak, typical of astrocytomas (D).

Figure 4.4

OLIGODENDROGLIOMA

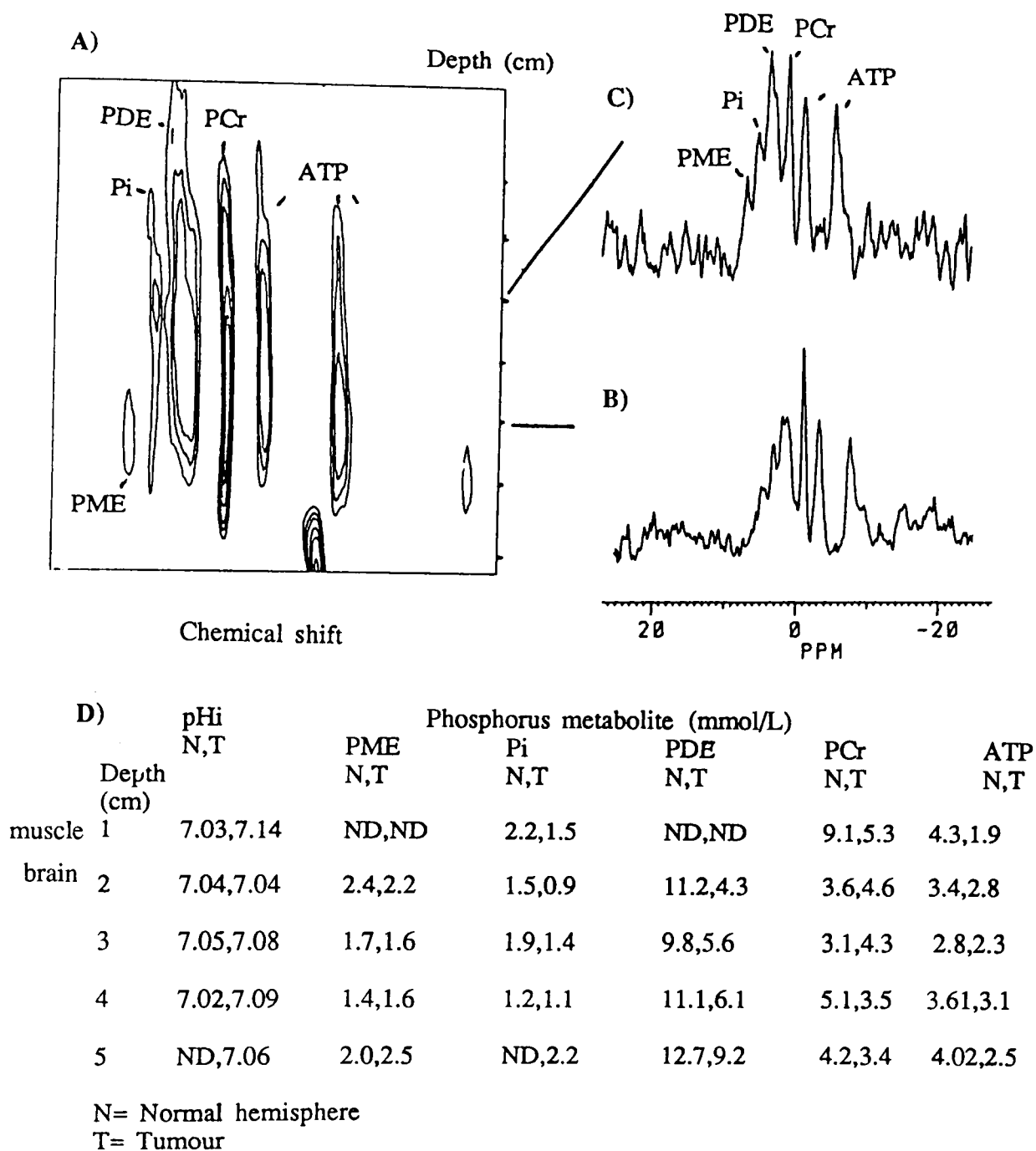


Figure 4.4 The contour plot (A) and spectra from 2cm (B) and 4cm (C) depths are shown from an oligodendroglioma. The absolute tissue concentrations from the tumour, and from the same position in the normal hemisphere are shown below (D).

Figure 4.5 EPENDYMOMA

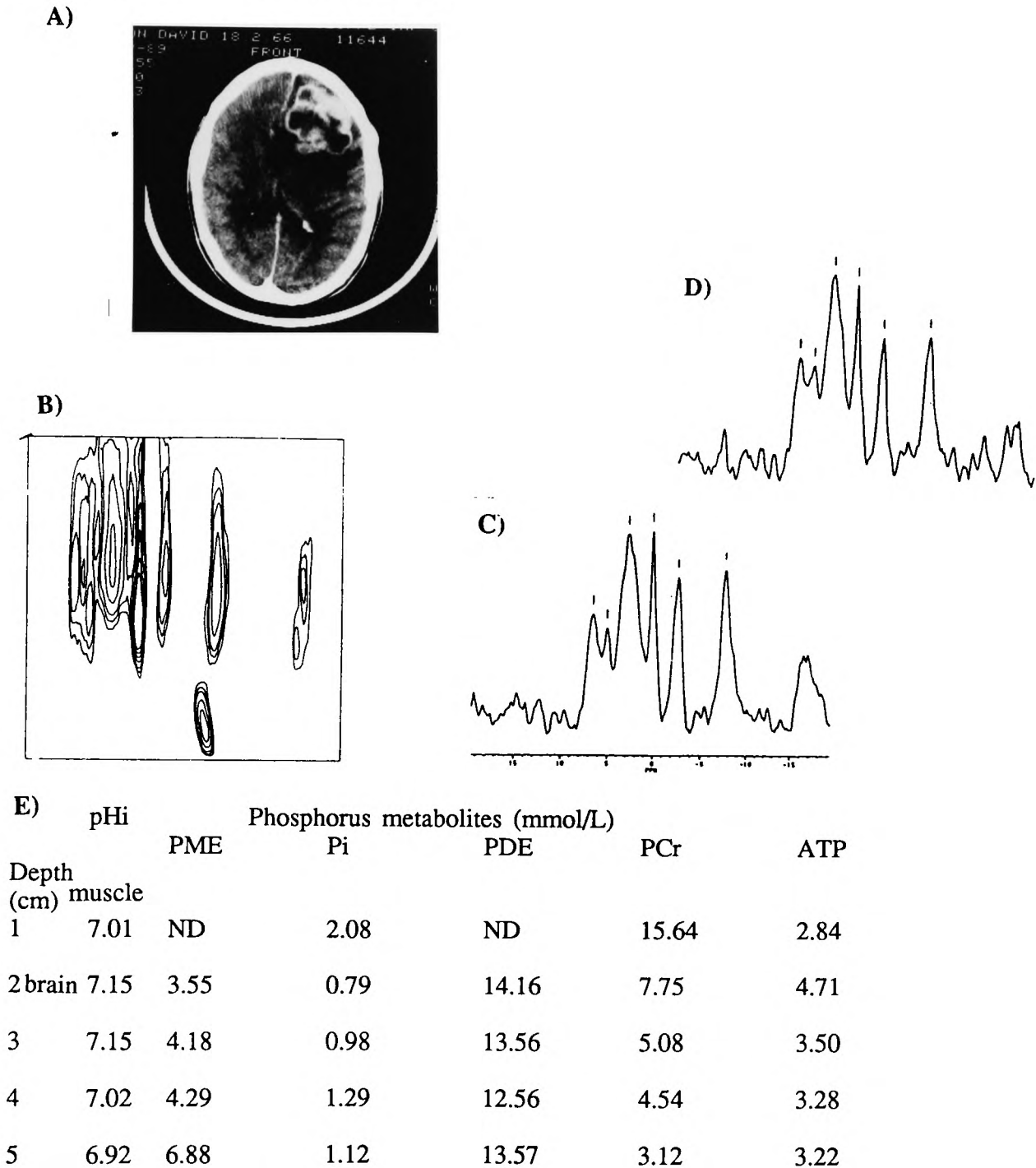


Figure 4.5 The Ct scan (A) shows a heterogenous contrast enhancement in tumour. The contour plot (B) demonstrates two distinct areas within the tumour, a near high pH (C) and distant low pH (D) regions. Spectra have been selected from these regions. The absolute concentrations are reported in the table below (E).

raised when compared to the normal hemisphere (7.02-7.05) in the same patient. The [PME] (1.6-2.5mmol/L) was only slightly elevated. The [PCr] (4.3-3.4mmol/L) was slightly reduced when compared to the normal hemisphere (3.6-5.3mmol/L). The [ATP] (2.8-2.5mmol/L) was also reduced, as was [PDE] (4.3-9.3mmol/L).

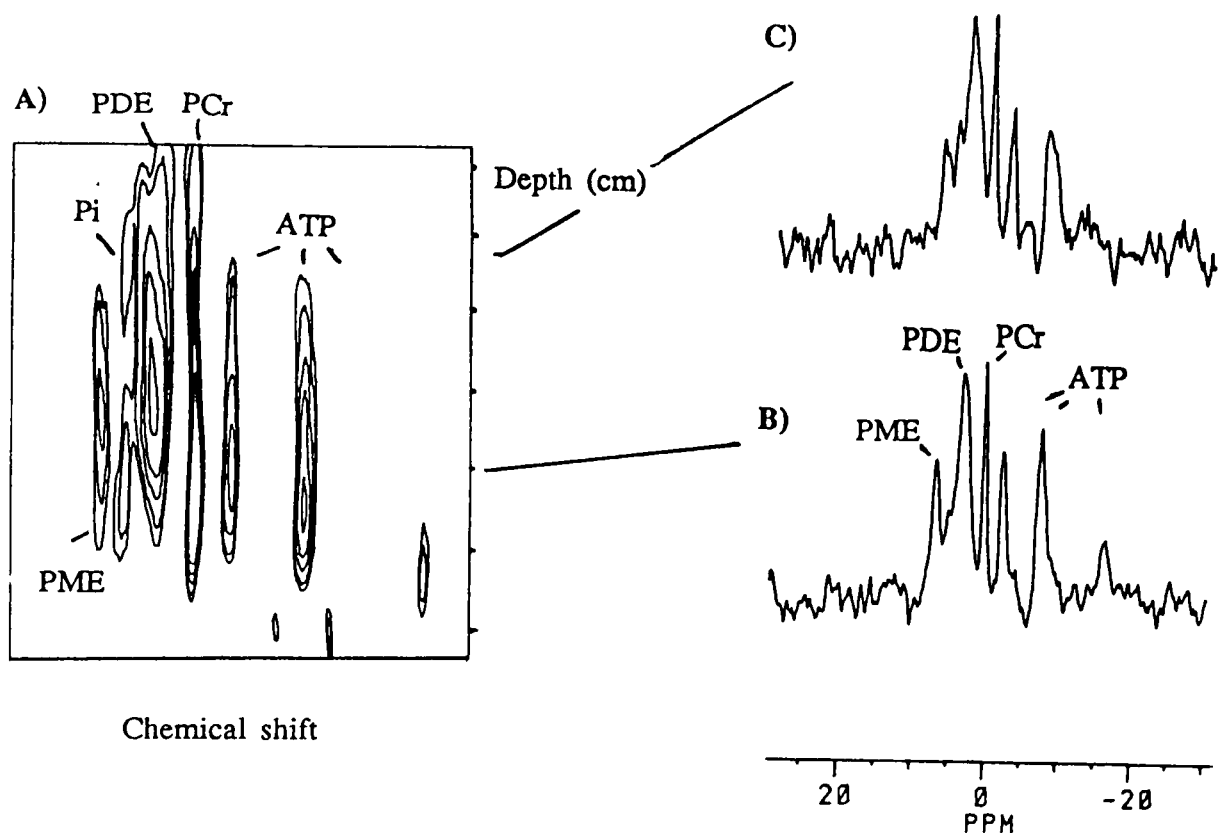
4.2.3 Ependymoma

One patient with an ependymoma was studied, reflecting the rarity of this tumour in the adult age group. The tumour had spread from the frontal pole of the lateral ventricle into the non-dominant frontal lobe. The CT scan with contrast enhancement showed a tumour with a well described superficial ring of radio density and a radiolucent central region. Considerable heterogeneity in the regional distribution of pHi within the tumour was demonstrated (Figure 4.5), correlating with the histology. The superficial growing edge of the tumour appeared alkaline (pHi 7.12) whilst the surviving cells in the necrotic centre were acidic (pHi 6.97), with a high [PME] (6.88mmol/L) but low [PCr] (3.12mmol/L) in this region.

4.3.0 Meningeal tumours

Six meningiomas were studied, all convexity tumours identified by CT scanning and studied 24hrs prior to surgery. The probe was placed directly over the tumours. The position was guided by previous CT scan topography or MRI using an inversion recovery sequence. A typical PMRFI data set is shown (Figure 4.2). The [PCr] (1.68, 2.15mmol/L) was greatly reduced in marked contrast to the glial tumours. Elevation of [PME] (4.15, 3.94mmol/L) and alkaline pHi (7.33, 7.12) was also seen (Tables 4.1 and 4.2).

Figure 4.6 MELANOMA; TEMPOROPARIETAL METASTASIS



D)	pHi	Phosphorus metabolites (mmol/L)				
Depth (cm)		PME	Pi	PDE	PCr	ATP
1 muscle	7.01	ND	1.58	ND	13.35	3.96
2 brain	7.10	2.54	0.98	8.13	6.36	3.70
3	7.08	2.92	1.02	8.20	3.01	2.33
4	7.11	3.59	1.1	8.28	2.29	2.28
5	7.04	2.62	1.93	8.76	3.85	2.26

ND = Not Detected

Figure 4.6 The PMRFI contour plot (A) and selected spectra at 2cm (B) and 4cm (C) show an alkaline pHi with the presence of PCr and PDE in this lesion. The absolute tissue concentrations are below normal brain levels.

4.4.0 Secondary tumours

A large (5cm diameter by Ct scan) melanoma secondary tumour was studied using PMRFI (Figure 4.6). The melanoma secondary also had a high pHi (7.08-7.11), a constant [PDE] (8.13-8.76mmol/L) with reduced [ATP] (3.70-2.26mmol/L) and [PCr] (6.36-2.29mmol/L). The [PME] (2.54-3.59mmol/L) was slightly elevated.

4.5.0 Acromegaly

Six patients with pituitary adenomas were studied before and three days after transphenoidal surgery (Figure 4.7). Three of the four patients had pituitary adenomas were secreting large quantities of growth hormone, as assessed by resting growth hormone levels and after suppression with an oral glucose load. One patient had a moderately raised growth hormone level, which was partially suppressed on oral glucose load.

The temporoparietal image using PMRFI was used to investigate the metabolism of cells near to the pituitary adenoma, but not in the tumour. Three of the four patients had elevated pHi in the deep white matter (5cm) as demonstrated by the chemical shift of Pi from PCr (Figure 4.7). The pHi was elevated in the deep white matter (5cm, 7.08, 7.08, 7.15) in 3 of the 4 patients prior to surgery, returning to normal levels after the tumours had been removed (7.04, 7.04). One patient (2) did not have a raised pHi and the growth hormone was moderately raised. The [PCr] was also raised both before (6.0, 6.7, 6.0, 6.4 mmol/L) and after (5.5, 7.1, 5.3 mmol/L) surgery.

Figure 4.7

ACROMEGALY BEFORE AND AFTER TRANSPHENOIDAL ADENECTOMY

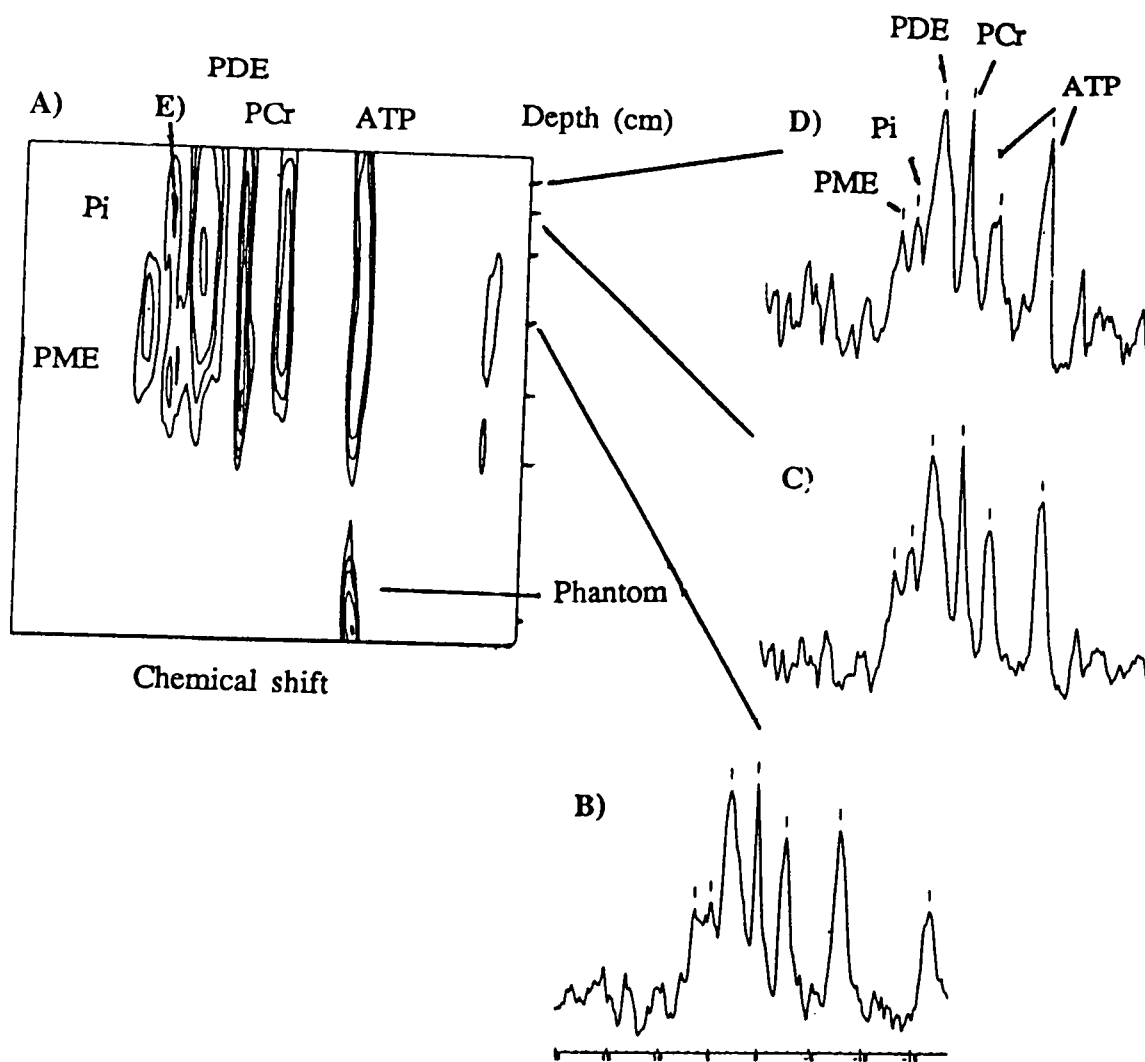


Figure 4.7 Four patients with acromegaly were studied with PMRFI before surgery and three of these patients were studied within 7 days following surgery. The PMRFI data is presented as a contour plot (A) and spectra are taken from 3cm (B) where the pHi was normal (7.04pH units) and 5cm (C) and 5.5cm (D) where the pHi was elevated at 7.15pH units. This shift in pHi can be seen on the contour plot as an island of Pi shift from PCr (E).

**Table 4.3 DEPTH VARIATION OF PHOSPHORUS METABOLITES AND
pHi IN ACROMEGALY BEFORE AND AFTER SURGERY**

Subject	GH mu/L R/S	pHi <u>B</u> A	Phosphorus metabolites (mmol/L)				
			PME	Pi	PDE	PCr	ATP
1	66/49	<u>7.08</u>	<u>3.1</u>	<u>0.8</u>	<u>11.9</u>	<u>6.0</u>	<u>3.9</u>
		7.04	2.4	0.7	10.5	5.5	3.7
2	21/10	<u>6.99</u>	<u>1.3</u>	<u>0.3</u>	<u>13.3</u>	<u>6.7</u>	<u>3.2</u>
		7.00	2.7	0.4	11.1	7.1	3.9
3	155/80	<u>7.08</u>	<u>2.3</u>	<u>1.4</u>	<u>13.1</u>	<u>6.0</u>	<u>3.2</u>
		7.05	3.2	1.3	12.9	5.3	3.5
4	95/45	<u>7.15</u>	<u>2.3</u>	<u>1.4</u>	<u>13.5</u>	<u>6.4</u>	<u>4.6</u>

R/S = Resting/ Suppressed Growth Hormone levels with an oral glucose load.
B = Before and
A After surgery metabolite levels

4.6.0 Summary

The metabolic changes observed in primary brain tumours using PMRFI MRS are similar to those observed by Oberhaensli (Oberhaensli et al, 1986). Increases were observed in the PME content and pHi. The glial and meningeal tumours differed from each other by the PCr content; glial tumours have normal or increased PCr, meningiomas have none or low levels of PCr. The increased coefficient of variation observed in the absolute concentration of ATP probably reflects the large range of cellular density seen in tumours. Some tumours have a high density of cells so that the absolute concentration of ATP will be high. Some tumours have a large degree of necrosis, reducing the [ATP].

Elevation in the PME content of the tumour cells has been observed in all types of MRS studies, from tissue culture models (Daly et al, 1987) to human studies (Maris et al, 1985). Extracts of these tissues (Pettegrew et al, 1986) have shown that the raised PME peak is due to elevation of phosphoethanolamine (PE), a precursor in the phospholipid pathway (Figure 4.8). An accumulation of this substrate prior to mitosis would allow for the rapid production of phospholipid. In addition PE may accumulate within the cell during break down of phospholipid. Further work is required to see if a raised PME peak is related to the rate of division of a tumour or merely a consequence of disordered regulation of phospholipid metabolism.

Some of the astrocytomas had an increase in the PCr signal, probably reflecting an increase in intracellular concentration of creatine. Creatine is pumped into the cell by the creatine/sodium exchanger (Loike et al, 1986). An increase in

Figure 4.8

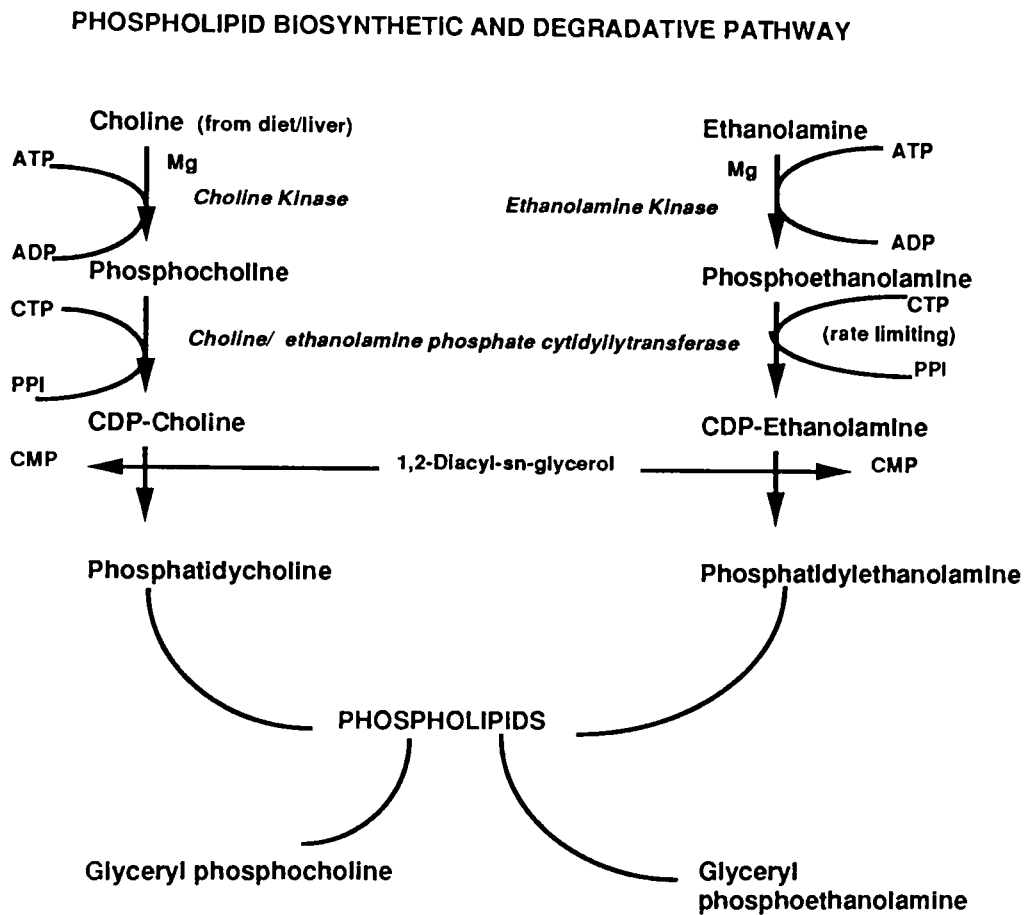


Figure 4.8 The phospholipid biosynthetic and degradative pathways are shown for phosphatidylcholine and phosphatidylethanolamine. The cytidylyltransferase step is thought to be rate limiting in the mammalian brain, allowing phosphoethanolamine to accumulate.

the activity of this pump may be present in these astrocytomas.

Elevation in the pHi of ^{most} tumours observed was the most consistent finding in this study. The majority of the acid load produced by the cell is removed rapidly as CO₂, assuming that the cell is aerobically respiring. A high pHi may be due to a switch of energy substrate or a change in pHi regulation by the Na⁺/H⁺ exchanger, or a combination of these factors. Mammalian cells in tissue culture require much greater quantities of glutamine in their growth medium than would normally be found *in vivo* (Reitzer et al, 1979). Deamination of glutamine by cytoplasmic glutaminase produces glutamate and ammonia. Glutamate can be converted to oxaloacetate which directly feeds into the Krebs cycle in the mitochondria. This pathway is a more direct and energetically simpler way of supplying substrates to the mitochondria, but does produce considerable quantities of ammonia. The majority of the ammonia will pass easily through the cytoplasmic membrane into the blood. Some ammonia will combine with water producing ammonium and hydroxyl ions, raising the cytoplasmic pH.

However the results from studying the white matter of acromegalic patients would suggest that other factors are also involved in alkalinization of tumours. Various growth factors have been shown to increase the activity of sodium/proton exchangers (L'Allemain et al, 1984; Moolenaar, 1986). Growth factors released directly into the white matter adjacent to the pituitary adenoma may be stimulating the sodium/proton exchanger, producing a more alkaline pHi as seen in the acromegalic patients. Primary brain tumours are known to have increase epidermal growth factor (EGF) receptors on the cell surface (Reubi et al, 1989). Variants of

transforming growth factor α (TGF α) have been shown to remain in the cytoplasmic membrane, continually stimulating EGF receptors (Bringman et al, 1987), via protein kinase C (Nishizuka, 1984). This cycle of events could leave the cell with a permanently raised pHi as observed in these investigations. Perona and Serrano (Perona and Serrano, 1988) have demonstrated that permanently raised pHi can cause a murine fibroblast cell line to divide more rapidly and loose contact inhibition, behaving like a tumour, suggesting that persistently elevated pHi is a direct cause of tumour development.

Significant metabolic differences were noted between normal brain and various tumours. These differences could be used to non-invasively assess rates of cell division via the PME peak and degree of alkalinization. The pHi difference between normal and neoplastic cells could be used therapeutically to kill tumour cells by activating a drug in an alkaline environment. A therapeutic agent which lowers pHi to normal levels may slow down the mitotic rate of the tumour and be therapeutically beneficial. Animal and human investigations could be developed to assess these possibilities.

CHAPTER 5

SOME NEURODEGENERATIVE CONDITIONS

5.1.0 Introduction

Destruction of the adult central nervous system can be caused by a variety of factors. Three CNS degenerative conditions were selected for MRS study namely; viral infections, multiple sclerosis and head injuries.

Viral infections may damage the nervous system directly by infection or indirectly by viral immune-complex syndromes. Herpes simplex virus (HSV), a DNA virus with two subtypes (HSV I + II)(Buchman et al, 1980), directly infects the nervous system (Corey and Spear, 1987), probably starting as a peripheral nervous system infection with intra-axonal spread to ganglia (Stevens and Cooke, 1971; Baringer and Swoveland, 1973). However HSV is the commonest cause of viral encephalitis, with HSV-1 accounting for 95% of cases (Whitely et al, 1977). The temporal lobe is a common site for HSV-1 infection in human brain, which has a close proximity but not directly connected to the trigeminal ganglion (Fraser et al, 1981) a possible site of entry. In contrast HSV-2 (Genital) is more commonly associated with meningitis (Olson et al, 1967). A patient with HSV infection of the right temporal lobe was studied using PMRFI during recovery to see if any biochemical changes are associated in this phase of recovery from viral infection.

Human immunodeficiency virus (HIV) infection result in immunosuppression and a progressive dementing disorder in patients with acquired immunodeficiency syndrome (AIDS) (Price et al, 1988), referred to as AIDS dementia complex (ADC). The ADC often occurs in association with opportune infections and neoplasms, but may also occur in the absence of significant immune dysfunction, suggesting that the HIV is the primary cause (Navia and Price, 1987), with 70% of ADC patients

showing neuropathological changes, including a "subacute encephalitis". Patients with HIV infection at various stages were investigated to see if intracellular biochemical changes were associated with this clinical condition.

The aetiology of multiple sclerosis remains unknown and many excellent reviews exist summarising this work (Matthews et al, 1984; McFarlin and McFarland, 1982). Three possible causes of multiple sclerosis have been widely investigated; lipid metabolism, autoimmune reaction, and infection. Characteristic changes associated with multiple sclerotic plaques have been noted by MRI (Young et al, 1981; Barnes et al, 1986), providing useful diagnostic investigation. The histopathology of MS plaques has been extensively reviewed (Prineas, 1975; Prineas and Connell, 1978), with perivenous demyelination containing macrophages, lymphocytes and plasma cells with lipid products found free or engulfed in macrophages. The majority of MS plaques are small (0.5-2cm diameter) and appear in the deep white matter. These lesions are too small and too deep to be studied by MRS *in vivo*. Large superficial plaques do occasionally occur involving the subcortical white matter and one such case was studied. The results are reported in this chapter. Further studies were performed on MS patients with normal appearing white matter in MRI studies as controls.

The intracellular biochemical changes associated with severe head injury have been studied in experimental models using MRS. In the acute phase of severe head injury lactate is produced, pH_i falls and PCr levels are reduced (Unterberg et al, 1988; Ishige et al, 1987; Inao et al, 1988). In human studies elevation in CSF lactate (King et al, 1974) with reduction in extracellular pH (DeSalles et al, 1987);

changes in cerebral blood flow (Obrist et al, 1979; Enevoldson and Taagehoj, 1977) and changes in glucose metabolism (Langfitt et al, 1986) have been noted in the acute stage. MRI studies have been used to follow up a series of patients after severe non-penetrating head injury (Wilson et al, 1988), and demonstrated persistent changes in white matter many months after injury, confirmed by histological changes (Strich, 1956; Adams et al, 1982; Castejon, 1985). A group of patients recovering from severe non penetrating head injury were selected to be studied by MRS to see if biochemical changes were associated with these MRI abnormalities.

5.2.0 Viral infections

5.2.1 Herpes simplex virus

A patient, with a reduced level of consciousness and increased irritability, was transferred to the Radcliffe Infirmary, Oxford. CT scan was performed showing translucency of the right temporal lobe. A working diagnosis of HSV infection of the right temporal lobe was made. The patient was started on Acyclovir and dexamethasone, but lapsed into coma for three days. Consciousness was regained by day five. The first MRS study was performed on day seven (Figure 5.5). The metabolite signal was reduced, with a raised pHi (7.13). The [PME] (3.14mmol/L) was elevated with a reduction in [PCr] (2.08mmol/L), [PDE] (6.54mmol/L) and [ATP] (2.21mmol/L). The second MRS study was performed two weeks later. The pHi (7.10) remained elevated and the [PME] (2.91mmol/L) had fallen slightly.

Figure 5.1 HERPES SIMPLEX ENCEPHALITIS OF THE RIGHT
TEMPORAL LOBE

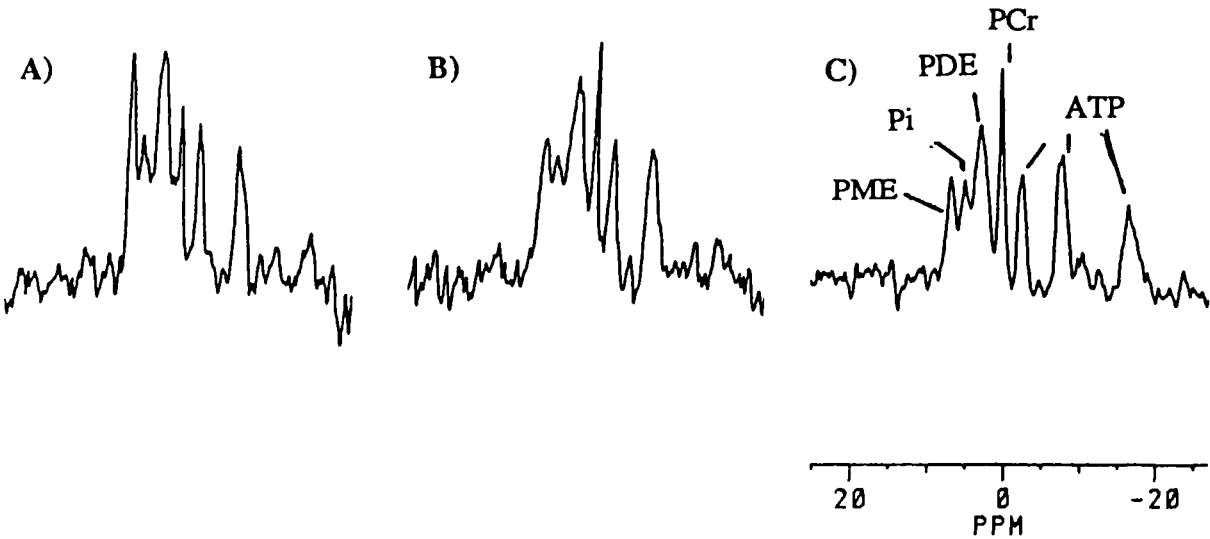


Figure 5.1 Three consecutive studies of the right temporal lobe HSV encephalitis were conducted at 1 week post coma (A), 2 weeks post coma (B) and 1 year post coma (C). Absolute concentration of metabolites at 3cm depth are reported (D).

The patient made a good recovery and left hospital to get married and return to normal work. Analysis of cerebrospinal fluid and blood samples showed an increase in herpes simplex viral (HSV) antibodies and a presumptive diagnosis of HSV encephalitis was made.

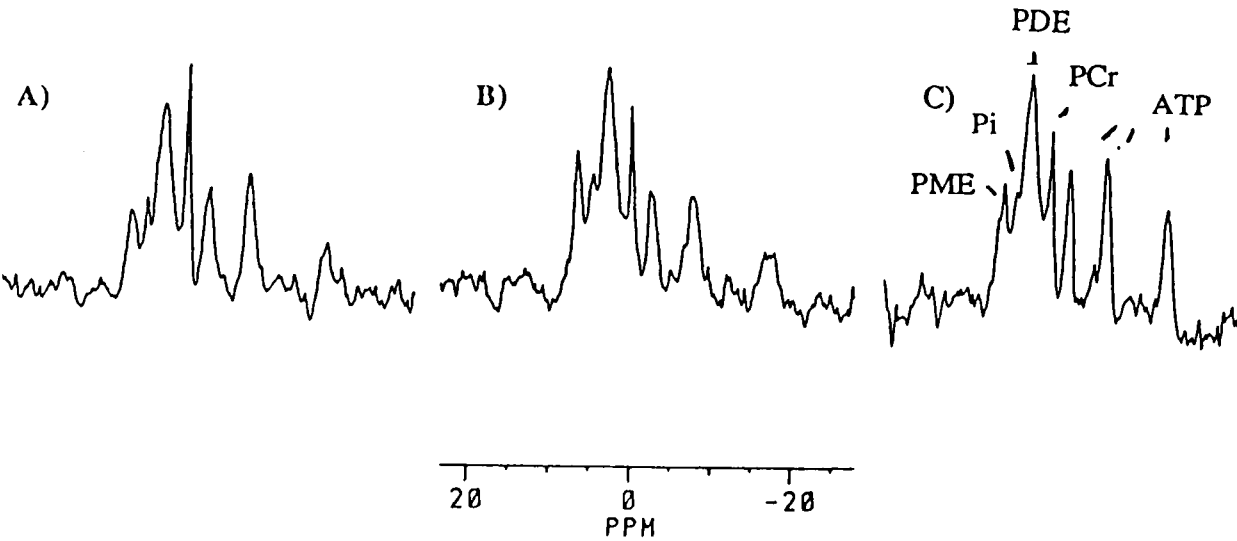
One year later a further combined MRS and MRI study was performed showing a right middle cranial fossa full of tissue. The pHi (7.01) was normal with a normal [PME] (1.92mmol/L) [Pi] (1.15mmol/L). The [PDE] (5.90mmol/L) [PCr] (3.60mmol/L) and [ATP] (2.50mmol/L) remained reduced.

5.2.2 Human immunodeficiency virus

Six patients with HIV positive serum were investigated with MRS. They represent a cross-section of the progression of this infection (Figure 5.2), from recent seroconversion to AIDS. The most severely affected patient clinically (5) had a calcified region in the left frontal lobe by CT scan. MRS studies of this region showed elevation of [PME] (3.9mmol/L) from 4-5cm depth, and a normal pHi (7.02). Subsequent post-mortem studies of this region show increased lymphocytic infiltration of necrotic white matter.

Patient (6), with insulin dependent diabetes, presented with uncontrollable hypertension and deteriorating renal function. Serology was HIV positive. Renal biopsy suggested glomerulonephritis consistent with diabetes. He developed early morning headaches and vomiting associated with failing vision and was transferred to the Churchill Hospital, Oxford. The CT scan was reported as normal. A combined MRS and MRI study was performed.

Figure 5.2 HUMAN IMMUNODEFICIENCY VIRUS



D)	Clinical				pHi	Phosphorus metabolites (mmol/L)				
	AZT	ARC	AIDS	CS		PME	Pi	PDE	PCr	ATP
Patient										
1(L)		+	-	-	7.03	2.5	0.6	9.7	4.0	2.4
(R)	-	+	-	-	7.00	2.0	1.9	10.8	5.1	3.4
2		+	-	-	6.99	1.7	0.6	10.2	4.1	2.6
3	+	-	+	+	7.01	2.1	0.9	11.3	5.3	3.8
4		-	-	-	6.99	2.3	1.0	9.6	3.3	2.3
5	+	-	+	+	7.02	3.9	1.1	9.3	3.6	2.5
6(R)		-	-	+	6.99	2.3	0.8	12.5	5.6	4.1
(L)	-	-	-	+	7.02	3.0	1.0	9.3	3.1	2.5
(L) _p	+	-	-	-	7.03	2.2	0.6	11.3	2.5	2.7

AZT = Zidovudine therapy, ARC = Aids related complex, AIDS = Acquired immunodeficiency syndrome, CS = Cerebral symptoms such as early morning headaches, vomiting and cognitive impairment. (R) or (L) = Right or left hemisphere studies, (L)_p = Post Zidovudine therapy of left hemisphere.

Figure 5.2 The elevated PME peak can be seen in the left (B) but not right (A) hemispheres at 3-4cm depth in the PMRFI study of patient 6 with cerebral symptoms prior to Zidovudine (AZT) therapy. The cerebral symptoms and PME peak (C) resolved with Zidovudine therapy. The clinical details, pHi and absolute concentrations are reported (D).

The MRI demonstrated a region of T_2 enhancement at 4cm depth in the left hemisphere. The right hemisphere appeared normal. MRS of the left hemisphere showed increased [PME] (3.0mmol/L) in the region at this depth (4cm), with normal pH_i (7.02). Metabolite concentrations in the left hemisphere were reduced compared to the right.

The patient was started on Zidovudine (AZT) and clinically improved with control of blood pressure and cessation of early morning headaches. The combined MRI and MRS studies were repeated. The [PME] (2.2mmol/L) was reduced, with a persistently low [PCr] (2.5mmol/L) and [ATP] (2.71mmol/L).

The other patients with HIV but minimal symptoms had metabolite concentrations in the normal range or just below, with normal pH_i.

5.3.0 Multiple sclerosis

A patient (22 yrs) with a recent history of weakness in her lower limbs was found to have a large superficial (5cm diameter) lesion in her left frontal region by MRI. Subsequent investigations at The National Hospital for Nervous Diseases, Queens Square, London, suggested multiple sclerosis as the cause for this lesion. MRS studies (Figure 5.3) of this region showed alteration in metabolite levels. The PCr/ATP ratio (0.56) was reduced with a small inorganic phosphate peak measuring an elevated pH_i. The PDE/ATP ratio (5.35) was greatly elevated.

The patient's symptoms resolved and she returned to work. A follow-up study one year later (Figure 5.3) showed a normal MRI and PMRFI phosphorus image, except for an elevated PDE/ATP ratio (5.29). Four further patients (M.S.) with multiple sclerosis and normal appearing white matter by MRI were studied (Table 5.1) and found to have normal phosphorus metabolite content.

Figure 5.3 MULTIPLE SCLEROSIS

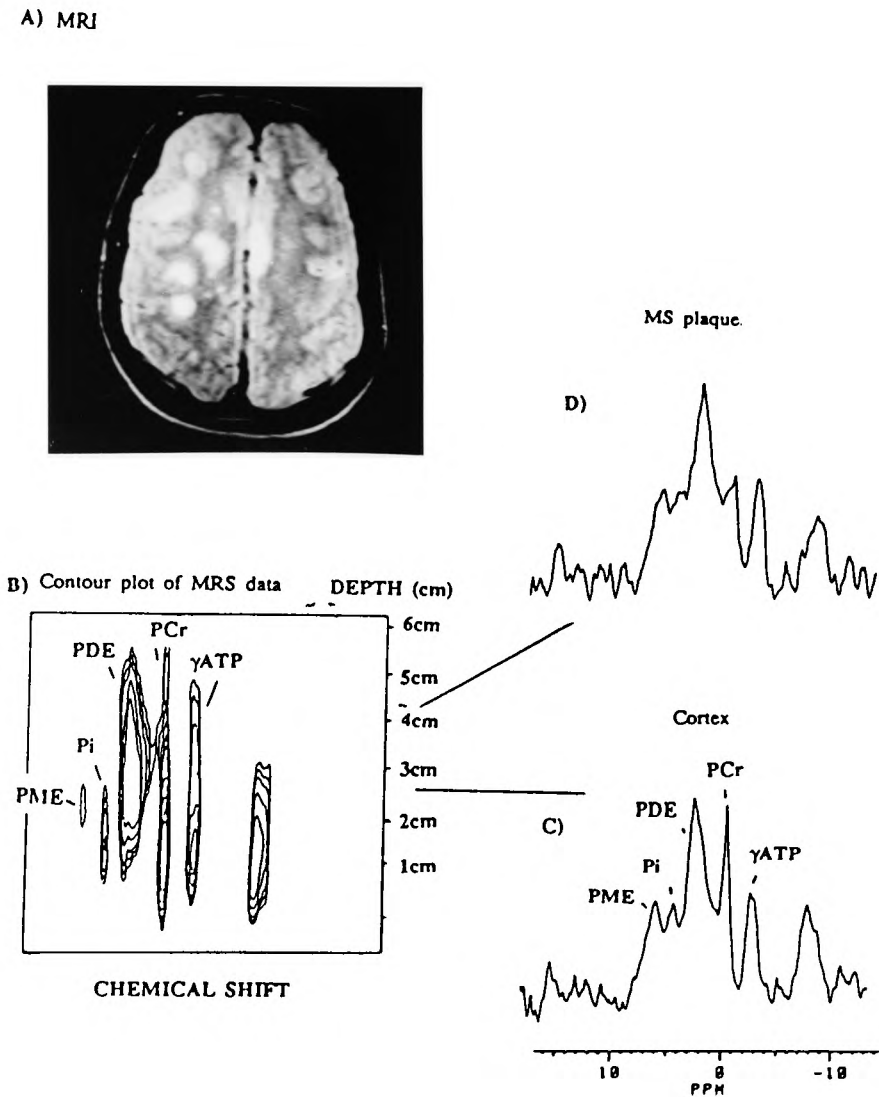


Figure 5.3 The MRI (A) (National Hospital, Queens Squ., London) demonstrated a large superficial lesion. The PMRFI study of this lesion shows a region at 4cm in the contour plot (B) with a reduced PCr content. Spectra taken from this region (D) show a low PCr/ATP ratio, but not in the most superficial layer of brain (C) which appears normal.

Table 5.1

PHOSPHORUS METABOLITE LEVELS IN NORMALS, CONTROLS AND MS BRAIN

Metabolite ratio	CONTROLS	M.S. Patient with plaque			
	Normal (n=7)	M.S. (n=4)	Acute lesion	Acute normal	Recovery lesion
PCr/ATP	1.40±0.2	1.50±0.2	0.56	0.99	1.62
Pi/ATP	0.33±0.2	0.37±0.1	0.24	NS	0.18
PDE/ATP	3.70±0.3	3.57±0.4	5.35	3.62	5.29
pHi	7.03±.02	7.03±.02	7.25	NS	7.02

5.4.0 Non-penetrating head-injury

Five patients recovering from non-penetrating head-injury (6-18 months) were studied. All patients had required ventilation after injury. No patients had suffered extra-dural haematomas. Four patients suffered from bilateral spasticity at the time of MRS study. Three patients were restricted to wheel chairs because of severe ataxia. Both hemispheres were studied by MRI (inversion recovery sequence) and MRS (PMRFI) in all patients and one hemisphere studied twice in one patient. The proton images showed considerable atrophy of the cerebral hemispheres in the temporoparietal region (Figure 5.4). The phosphorus metabolite results are presented with respect to the most spastic side of the body (Tables 5.3 and 5.4). The metabolite concentrations were reduced by 30% throughout the phosphorus image in both hemispheres (Figure 5.5), with a further reduction in PDE signal with depth (50% reduction, at 5cm), when compared to normal brain. The pHi was reduced (6.89 ± 0.06) in the most affected hemisphere (contra-lateral to worst spasticity), but normal in the least affected hemisphere (Figure 5.6).

5.5.0 Hypercapnia and normal brain pHi

Five normal healthy subjects volunteered to breath 5%CO₂/95%O₂ inspired gas through a circuit developed by Mr Gale in the Nuffield Department of Anaesthetic. This circuit allows the subject to breath air when no gas is delivered. When the inspired gas is delivered a valve shuts off the circuit from air. A capnometer (901-MK2, P.K. Morgan Ltd., Chatham, UK) was used to measure end-tidal CO₂. Temporoparietal MRS image were used to measure brain pHi on air and 5%CO₂/95%O₂ inspired gas.

Table 5.2

DEPTH VARIATION IN PHOSPHORUS METABOLITES AND pH_i
HEAD INJURY PATIENTS (contra-lateral hemisphere)

(N=7, mean age 24, 17-32yrs; 6-40 months post-injury)

Depth (cm)	pHi	Phosphorus metabolites (mmol/L, mean ±SD)				
	PME	Pi	PDE	PCr	ATP	
(Temporalis muscle, scalp and skull)						
1	7.05 ±0.05	n.d.	1.77 ±0.2	0	6.89 ±1.02	2.45 ±0.4
(Brain)						
2	7.05 ±0.02	1.98 ±0.6	0.94 ±0.4	6.16 ±1.9	3.57 ±1.2	2.45 ±1.03
3	7.02 ±0.02	2.21* ±0.5	0.75 ±0.3	7.40* ±2.5	3.44* ±0.8	2.53* ±1.01
4	6.98 ±0.04	2.38* ±0.8	0.91 ±0.3	8.12* ±2.6	3.98* ±0.8	2.75* ±0.76
5	6.89* ±0.06	1.95* ±0.5	0.8 ±0.3	8.40* ±3.4	4.19* ±0.8	2.76* ±0.5

*= P < 0.05, by the Wilcoxon test for non-parametric non-paired samples.

Table 5.3

<u>DEPTH VARIATION IN PHOSPHORUS METABOLITES AND pHi</u>						
<u>HEAD INJURY PATIENTS (Ipsi-lateral hemisphere)</u>						
(N=6)						
Depth	pHi	PME	Pi	PDE	PCr	ATP
(cm)						
(Temporalis muscle, scalp and skull)						
1	7.04 ±0.04	n.d.	1.37 ±0.55	n.d.	5.68 ±1.16	1.83 ±0.21
(Brain)						
2	7.08 ±0.05	2.06 ±0.9	0.94 ±0.5	8.27 ±4.5	3.62 ±1.76	2.93 ±1.37
3	7.06 ±0.06	2.18* ±0.62	1.03 ±0.44	8.21 ±3.5	3.62 ±1.29	2.43 ±0.9
4	7.07 ±0.06	2.13* ±0.37	1.18 ±0.4	9.08* ±2.5	3.38 ±1.3	2.72* ±1.2
5	7.08 ±0.07	2.25* ±0.48	1.06 ±0.5	9.61* ±2.84	4.01 ±0.9	3.06* ±1.1
*= P <0.05, by Wilcoxon test for non-parametric non-paired samples.						

Figure 5.4

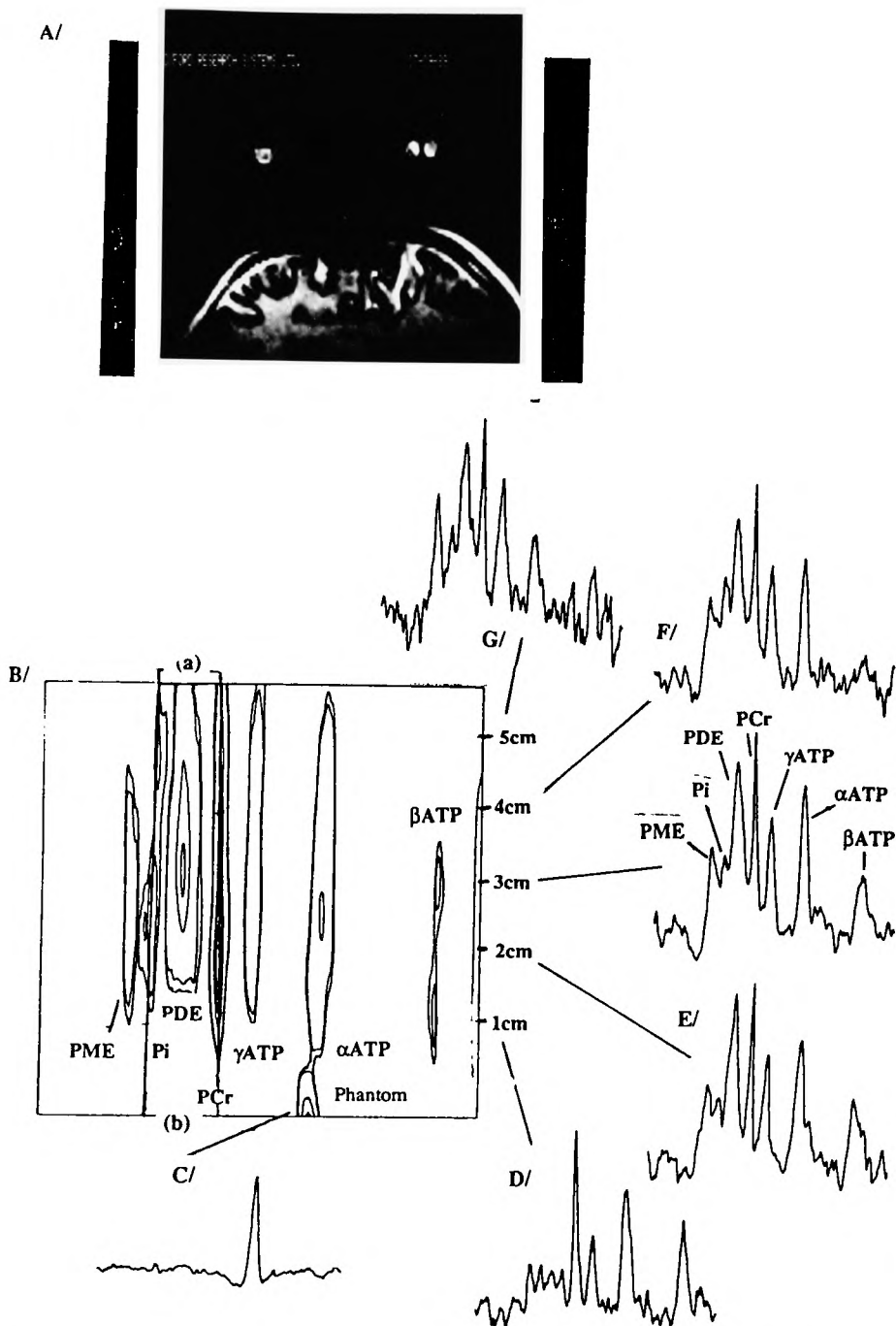


Figure 5.4 The proton imaging coil (Inversion recovery sequence) has been used to assess the structural changes in the parietal region of the brain (A). The phosphorus image (B) is shown as a contour plot, from which spectra have been selected at 1cm intervals (C-H).

Figure 5.5

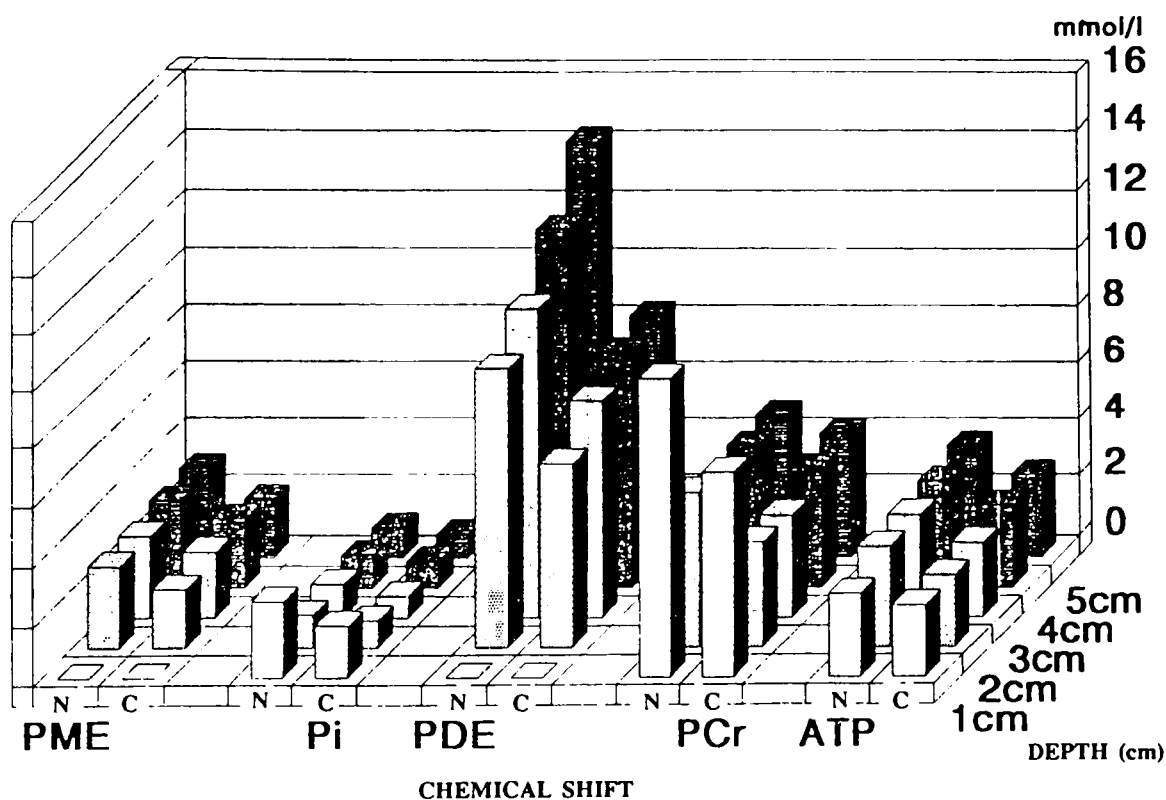


Figure 5.5 The bar chart compares the variation with depth of mean absolute tissue concentration between normal subjects (N) and the contra lateral hemisphere in the severely head injured patients (C) in the recovery phase. There is a greater loss of PDE with depth in the injured patients.

Figure 5.6

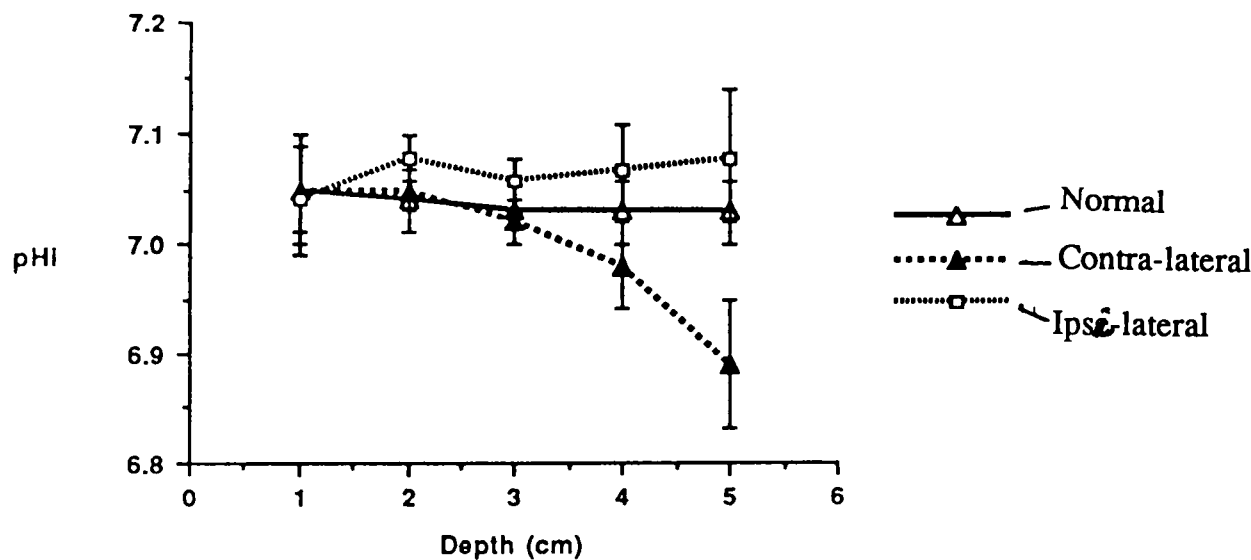


Figure 5.6 Variation of intracellular pH (pHi, mean $\pm$ SD) with depth (cm) into the brain is compared between normal subjects (**Normal**), and contra-lateral hemispheres to worst spasticity (**Contra-lateral**) and ipsilateral hemisphere (**Ipsilateral**).

Table 5.4 HYPERCAPNIA AND pHi OF THE CEREBRAL HEMISPHERES

Subject	End-tidal CO ₂ (%)			Depth (cm)	pHi			
	Air	5%CO ₂ Rise			Air	CO ₂	Fall	PostAir
1	4.75	5.80	1.05	2	7.02	6.99	0.03	ND
				5	7.03	6.92	0.11	ND
2	5.90	6.80	0.90	2	7.04	7.01	0.03	ND
				5	7.03	6.99	0.04	ND
3	5.40	6.45	1.05	2	7.03	6.99	0.04	ND
				5	7.01	6.95	0.06	ND
4	5.80	6.30	0.50	2	7.01	7.01	0	7.01
				5	7.02	6.99	0.03	7.03
5	6.00	6.70	0.70	2	7.02	6.99	0.03	ND
				5	7.02	6.97	0.05	ND
Mean ±SD		0.84 ±0.24		2	0.026±0.015			
				5	0.058±0.03			

ND = Not done

Figure 5.7

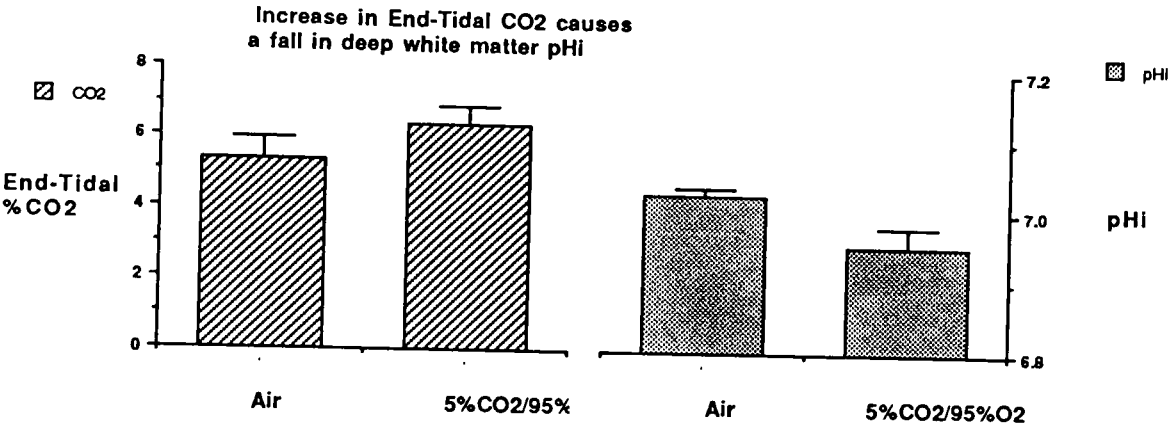


Figure 5.7 The bar chart shows the effect of raising end-tidal CO₂ on the white matter (5cm) pH_i.

The gas mixture 5%CO₂/95%O₂ increased end-tidal CO₂ by 0.84%±0.24. This rise in end-tidal CO₂ caused a fall in gray matter pHi (0.03±0.02) and white matter pHi (0.06±0.03) (Table 5.4 and Figure 5.7). A further PMRFI study on air, immediately after breathing 5%CO₂/95%O₂, showed a normal pHi (7.01) in gray and white matter despite a low end-tidal CO₂, due to hyperventilation which continued for 15 minutes.

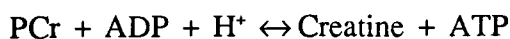
5.6.0 Summary

Herpes simplex infection of the right temporal lobe caused elevation in the [PME] and pHi with reduction in tissue concentrations, followed by clinical and biochemical recovery after one year, except for a low [PDE]. The HIV infected patients also showed reduced tissue metabolite concentrations. Two of these patients with cerebral pathology showed elevation of the [PME] but a normal pHi. MS patients with normal appearing white matter by MRI showed no difference from normal subjects. The one patient with a large superficial lesion showed reduced signal and a fall in the PCr/ATP at a corresponding depth. No elevation in the PME/ATP ratio was seen, but the pHi was elevated. The patients recovering from severe head-injury showed a significant reduction in pHi (6.89) in the deep white matter of the contra-lateral but not ipsi-lateral hemisphere to worst spasticity. The low metabolite levels probably reflect the cerebral atrophy seen in these patients.

The elevated [PME] shown in the patient with right temporal lobe HSV encephalitis may be due to rapid mitotic activity in gliosis immediately following the infection (MacCullam, 1967). The reduced tissue metabolite concentrations may reflect the extent of necrosis. Increases in EGF receptors have been noted on gliotic

cells (Nieto Sampedro et al, 1988), which may be associated with activation of the sodium/proton antiport exchanger causing an elevation in pHi. The pHi and [PME] returned to normal during recovery. The cerebral lesions associated with HIV showed an elevation in [PME], but normal pHi, with reduced tissue metabolite levels, possibly due to a combination of necrosis and gliosis. The [PME] returned to normal within two weeks in one patient who started AZT and showed signs of clinical improvement. The post-mortem study of one HIV infected patient with cerebral lesions by CT scan and MRS showed infiltration of altered lymphocytes and areas of necrosis in the white matter. Post mortem studies of the gray matter appeared normal, confirming the findings of the MRS phosphorus images.

The large superficial lesion associated with multiple sclerosis had an elevated pHi, reduced PCr/ATP and normal PME/ATP ratio. The elevated pHi may be a alteration in pH regulation of the cell, or reflect the increase in extracellular space seen in acute MS lesions histologically (Prineas and Connell, 1978) with changes on MRI (Bydder et al, 1982). An increase in a more alkaline extracellular space would increase the contribution of extracellular Pi to the observed signal, making the observed inorganic phosphate peak more alkaline. The reduced PCr/ATP may reflect reduced mitochondrial activity in the cells, with a an elevated ADP level causing a reduction in PCr;



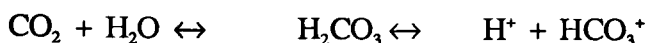
The reduction in mitochondrial activity may be due to a failure of oxygen or energy substrate supply or a primary mitochondrial failure. Alternatively a reduction in the cell creatine concentration could also reduce PCr. Creatine is pumped into the cell

by the creatine/sodium exchanger (Loike et al, 1986). Reduced activity of this pump would deplete cellular creatine and PCr levels. The normal PME/ATP ratio suggests that MS lesions differs biochemically from viral encephalitis, casting doubt on a viral aetiology.

The patients recovering from severe head-injuries were all studied some time after injury. In these "steady-state" conditions the pH_i of the deep white matter in the worst affected hemisphere was significantly reduced. This reduction in pH_i may be due to alteration in blood flow or tissue structure. Structural changes have been predicted to occur in the white matter (Holbourn, 1948) and shown histologically (Strich, 1956; Adams et al, 1982). However no studies exist on the cerebral blood flow so long after injury and acute studies of cerebral blood flow are inconsistent. Reduced levels of blood flow have been seen in regions most affected by severe injury (Obrist et al, 1979). This reduction in flow may persist long after the acute phase, causing a reduction in pH_i. However the PCr/ATP ratio was not reduced as might be expected in conditions of ischaemia. The hypercapnia studies showed that white matter pH_i can be reduced when blood and therefore tissue CO₂ rises. A similar situation may arise in the head injuries patients, where a rise in tissue CO₂ content is caused by a reduced tissue clearance due to a low blood flow. The reduction in blood flow is probably most severe in regions of maximal damage such as deep white matter as previously proposed (Holbourn, 1948; Strich, 1956; Adams et al, 1982).

The hypercapnia study demonstrated a difference in pH_i response to an acid load between gray and white matter, as seen in experimental models (Petroff et al,

1987; Cady et al, 1986). CO₂ rapidly dissolves and passes into the blood (Effros et al, 1978) and in to CSF (Pappenheimer et al, 1965), presumably also enters cells of the cerebral hemispheres. Carbonic anhydrase is found in both astrocytes and oligodendrocytes (Ghandour et al, 1980), catalysing the following reaction;



The rise in end-tidal CO₂ of 1% should cause a rise in PaCO₂ of approximately 8mmHg. A similar rise in PCO₂ in cell, unbuffered, would cause a fall in pH of 0.08. Assuming an equal and rapid influx of CO₂ in to the gray and white matter, the gray matter appears to experience a smaller fall in pHi than cells in the white matter. This differential response to CO₂ may be due to either differences in blood flow to the two regions or different pHi regulation or buffering. The gray matter receives four times the blood flow to a 5%CO₂ load than white matter (James et al, 1967) in experimental models clearing the build up of CO₂ in gray matter faster than white matter.

The pHi of cells may be buffered by passive or active systems. There are a variety of active ion transport channels in the cell membrane which could provide an active buffering mechanism to changes in cell pHi. The gray matter appears to be more effective at buffering against changes in pHi than white matter, by utilising either mechanism. Further work is required to investigate which buffering system predominates.

CHAPTER 6

DISCUSSION

Technical advances in this thesis have seen the PMRFI technique extended to include measurement of absolute tissue concentrations, imaging of creatine phosphokinase flux rates and addition of a proton imaging coil for identifying lesions within the brain. These technical advances were then applied to the study of intracellular metabolism of normal and pathologically affected tissue *in vivo*. In normal cerebral hemisphere significant biochemical and metabolic differences were demonstrated between gray and white matter, but not between different regions of the cerebral hemispheres. Certain pathological conditions were shown to be associated with significant biochemical differences from normal tissues.

The advantages and disadvantages of PMRFI as a technique for localized spectroscopy have been discussed. The significant advantages of PMRFI which have permitted the measurement of absolute metabolite tissue concentrations and measure enzyme flux rates *in vivo* are as follows:

a) The pulse sequence and data collection are relatively short so the intensity of the signal is collected is not significantly affected by the short T_2 relaxation time of phosphorus, increasing the accuracy of measuring absolute tissue metabolite concentrations.

b) The PMRFI technique does not require fast switching gradients to localize signal, which can cause serious artifacts at high magnet field (>2 tesla). At present artifacts induced by fast switching gradients make accurate estimation of metabolite

concentrations difficult.

c) Metabolite imaging methods for MRS record signal from multiple points. Progression of biochemical alterations can be followed from normal into pathological tissue. Multiple point analysis of a region of tissue allows direct comparison of one point with another in the same subject.

d) The simultaneous collection of signal from an external phantom and a localized point in a sample considerably reduces variables and increases the accuracy of measuring tissue metabolite concentrations.

The measurement of absolute tissue metabolite concentrations in the cerebral hemispheres *in vivo* has been performed. The [ATP] (3.4mmol/L), similar to wet biochemical analysis of freeze-blown brain (2.45 μ moles/g wet wt)(Veech et al, 1973) was found to be the same in both superficial and deep brain, reflecting similar total water content between these two layers of the brain (Gore, 1982). At present there is no magnetic resonance technique for measuring regional differences in cytoplasmic volume. The similarity between gray and white matter [ATP] makes the comparison of metabolite peak areas with ATP, metabolite/ATP ratios, a useful evaluation. The [PCr] (5.1mmol/L), [PME] (2.5mmol/L), inorganic phosphate concentration (0.9mmol/L) and pHi (7.03 ± 0.03) were found to be similar between superficial and deep regions of the cerebral hemispheres. The [PDE] was found to rise significantly from superficial (9.41mmol/L) to deep brain (14.12mmol/L). The data processing used in these studies involves removing the broad components (>200Hz line width) associated with the PDE peak. This leaves only the narrow component (<50Hz line width). The majority of this narrow PDE component is

formed by phospholipid bilayers (Murphy et al, 1989) and some signal from glyceryl phosphocholine (GPC) and (GPE) (Radda et al, 1989). The magnetization transfer experiment investigating the content of mobile phospholipids showed similar amounts of transfer in superficial and deep brain, suggesting that the mobile phospholipids found in cytoplasmic organelles such as endoplasmic reticulum (Murphy et al, 1989) does not change with depth into the cerebral hemispheres. The increase in the observed PDE signal is probably due to phospholipids in myelin. Further experiments could be performed on tissue extracts of mammalian brain to confirm or refute this suggestion.

Phosphodiester magnetization transfer could be developed to demonstrate demyelination in clinical conditions as in multiple sclerosis, contributing to this difficult diagnosis. Myelin fragments found in multiple sclerosis lesions may have increased magnetization transfer within the PDE peak than normal brain. A project using MRI and gadolinium-DTPA to establish early demyelination (Kermode et al, 1990;in press) and ^{31}P MRS to demonstrate increased phospholipid mobility could be established along side routine clinical practice.

The PMRFI technique was successfully applied to the investigation of certain pathological lesions found in the human cerebral hemispheres *in vivo*. Previous MRS work suggested difference in cellular biochemistry between normal brain and primary brain tumours (Oberhaensli et al, 1986), supporting previous PET studies (Brooks et al, 1985). The elevation of pHi (7.07-7.17pH units) and [PME] was found in gliomas and meningiomas studied with PMRFI. The mechanism causing the elevation of the pHi in these tumours remains unknown. Disruption of the blood

brain barrier in primary brain tumours could be a cause. However positron emission tomography studies (Brooks et al, 1985) have shown alkaline pHi in primary brain tumours with intact blood brain barriers as assessed by contrast enhancement, and normal or low pHi in tumours ^{with} damaged blood brain barriers.

These tumours may have switched energy substrate supply from glucose to glutamine. Mammalian cells in tissue culture require large quantities of glutamine (20mmol/L) to grow (Reitzer et al, 1979) with similar findings *in vivo* (Sauer et al, 1984). The utilisation of glutamine by tumours *in vivo* will produce large quantities of ammonia, of which the majority will leave the cell directly, but some will form ammonium and hydroxyl ions, contributing to an alkaline pHi. Proton spectroscopy could be used to measure tumour glutamine and ammonium/ammonia levels in the region of these tumours *in vivo*, and relate these levels to the alkaline pHi.

The sodium/proton (Na^+/H^+) antiport exchanger has been identified on glial cells (Kimelberg et al, 1979). Due to the negative charge found within cells positively charged ions will tend to be attracted into the cell, including sodium and hydrogen ions, giving normal cells a possible pHi of 6.2pH units. The Na^+/H^+ antiport exchanger is probably continually active in maintaining a normal pHi (7.03 ± 0.03). Phosphorylation of the Na^+/H^+ antiporter by protein kinase C and certain growth factors have been shown to alter Na^+/H^+ antiport activity and increase pHi (L'Allemain et al, 1984). Glial tumours have increased epidermal growth factor receptors (Reubi et al, 1989) which may be capable of increasing the efflux of hydrogen ions from the tumour cell via the Na^+/H^+ antiporter. Increased V_{MAX} and/or alteration in the K_m of the Na^+/H^+ antiport exchanger by various growth factors may

be a further cause of the alkaline pHi of brain tumours.

The study of deep white matter in patients with acromegaly suggests that normal cells, presumably oligodendrocytes, respond to a factor released from the pituitary adenoma by a rise in pHi. A growth factor produced from the breakdown of growth hormone may be responsible for this phenomenon. This hypothesis could be tested *in vitro* by modulating growth factor activity in human tumour slices or tissue culture models with simultaneous pHi measurement. A direct relationship between growth factor level and pHi would be supportive of the role of Na^+/H^+ antiporter activity in regulating pHi in tumour cells.

Perano and Serrano (Perano and Serrano, 1988) have suggested a causal relationship between elevated pHi and tumour development. Implantation of DNA coding for a Na^+/H^+ ATPase into murine fibroblasts was associated with increased pHi and mitotic activity and loss of contact inhibition. These cells implanted into nude mice resulted in rapid spread of the tumours. Implantation of the same DNA but without the genetic material for the Na^+/H^+ ATPase resulted in normal pHi and no change in mitotic activity. These experiments suggest that elevation of pHi is causally related to mitotic activity, implying that reduction of pHi to normal would result in normal rates of mitosis. This hypothesis could also be tested by reducing Na^+/H^+ antiporter activity and measuring the S phase fraction of the tumour by BUDr (Nishizaki et al, 1988) *in vivo* or *in vitro*. If there is a causal relationship between pHi and mitotic activity new chemotherapeutic agents such as antibodies to Na^+/H^+ antiport exchangers or growth factor receptors, could be used to manipulate tumour growth.

Elevation of tumour PME levels have been noted in a variety of tumour models from cells in tissue culture (Daly et al, 1987), human tumours (Oberhaesli et al, 1986, Maris et al, 1985) and rapidly dividing normal cells (Brenton et al, 1985). Tissue extracts have suggested that phosphoethanolamine (PE) levels are elevated more than phosphocholine (PC) (Murphy et al, 1989b). Both the gliomas and meningiomas had elevation in PME content. However there was no correlation between histological grading of the glioma and PME content. The elevation in PME content may be a result of abnormal regulation of phospholipid synthesis during mitosis, whether in normal or abnormal cells (Radda et al, 1989). Further work could be carried out on other tumour type *in vivo*, such as breast cancer, to see if histological grading was related to PME content or other clinical indices of growth.

Elevation of the PME content was also seen in response to herpes simplex encephalitis one week after the start of infection. This elevation in PME and pHi may be related to the rapid mitotic rate seen with gliosis which follows such viral infections (Olson et al, 1967). The follow up study one year later showed that the pHi and PME content had fallen to normal levels. In the HIV patients with cerebral symptoms and signs the PME level, with respect to ATP concentration, was also elevated. Post mortem study of one of these patients showed widespread white matter necrosis and abnormal lymphocyte infiltration with gliosis. Elevation of the PME content with viral related lesions in the brain is probably due to the gliotic response rather than a specific marker of infection, but could be useful in clinical practice.

Patients recovering from severe head injuries showed reduction in metabolite concentrations, presumably due to the cerebral atrophy which commonly ensues (Levin et al, 1981). The PDE peak showed a greater reduction in deep brain (50%) than superficial (30%), suggesting loss of myelin phospholipid, greater in the contralateral hemisphere to worst spasticity. This region of the brain also had significantly reduced pHi (6.82pH units), an unexpected and surprising observation, since this study was carried as a control group for acutely injured patients. Two possible causes could explain this steady-state alteration in pHi.

Studies of regional cerebral blood flow in acutely head injured have been inconsistent in the acute phase with both increased and decreased blood flow reported (Messeter et al, 1986; Enevoldson et al, 1977; Obrist et al, 1979; Langfitt et al, 1986), and with no long term follow up studies. However the steady-state reduction in pHi may be due to a chronic inadequate blood flow. Reduced blood flow may either reduce oxygen and energy substrate supply or reduce the removal of waste products such as CO₂. The human brain has an excessive blood supply with respect to oxygen and energy substrate supply. A reduction in blood flow (to 40%) may significantly raise venous CO₂ without affecting the oxygen and glucose supply. The elevation of venous CO₂ would be expected to increase cellular CO₂ content, causing a fall in pHi.

This hypothesis was tested in normal subjects by increasing inspired CO₂ (5%CO₂/95%O₂). This gas mixture caused end-tidal CO₂ to increase by 0.84% (± 0.24 , from 5 to 6%), producing an 8 mmHg rise in PaCO₂. The arteriovenous difference of CO₂ between carotid artery and jugular venous bulb has been measured

at 7mmHg (Severinghaus and Lassen, 1967). The rise in end-tidal CO_2 obtained (8mmHg) could not be compensated for by simply increasing blood flow alone exposing the brain to an ~~rapid~~ increased level of CO_2 with rapid passage through the blood brain (Pappenheimer et al, 1965). The CO_2 will be rapidly converted to hydrogen and bicarbonate ions by carbonic anhydrase found in glial cells (Ghandour et al ,1980). The bicarbonate ions could leave the cell via the chloride/bicarbonate exchanger, leaving behind the hydrogen ion which would lower pHi. The expected fall in pHi would be 0.08 units (Log 6%/5% end-tidal CO_2). The white matter pHi was observed to fall by 0.06pH units, whilst the gray matter pHi fell by only 0.03pH units. This differential fall in pHi could be explained by differences in the rise in venous and therefore cellular CO_2 , due to difference in blood flow response between gray and white matter (James et al, 1969). The gray matter, in experimental models, has been shown to receiver a greater rise (1.46ml/100g/min/%rise CO_2) in flow than white matter (0.46ml/100g/min/% CO_2) in response to 5% CO_2 . This differential increase in flow may result in a smaller rise in venous CO_2 in gray than white matter, explaining the difference in pHi fall in these two regions of the brain, assuming minimal cellular pHi buffering.

If the cells are experiencing the same rise in CO_2 then the ability of these to regions to regulate changes in pHi must be different. The gray matter may be more effective at buffering pH changes than white matter either by passive or active mechanisms (Roos and Boron, 1981). Passive mechanisms include proteins with in the cell. Active buffering mechanisms involve the cell membrane ion exchangers such as the Na^+/H^+ antiporter and $\text{Cl}^-/\text{CO}_3^-$ exchanger. These active systems may be

able to respond to small changes in pHi. Experimental work, measuring changes in pHi and hydrogen ion rate efflux in human lymphocytes (Ng and Dudley, 1988) has shown that changes in pHi of less than 0.1pH units produce/ a small increase in hydrogen efflux. With these small changes in pHi the Na^+/H^+ antiporter may not be sensitive enough to return pHi to normal with respect to the influx of CO_2 and the experimental time (18 minutes per phosphorus image).

The hypercapnia study suggests that human brain pHi is sensitive to changes in blood CO_2 . This model, of rising venous CO_2 causing reduction in pHi, may explain the low pHi observed in the white matter of patients recovering from head injury. Low blood flow, causing low pHi, may persist in regions of maximal damage, reflected by loss of PDE, such as deep white matter in global head injuries (Holbourn, 1943). This hypothesis could be tested in these patients by measuring cerebral blood flow by PET. Certain drugs such as the calcium antagonist Nimodopine may be able to improve the blood flow in the deep white matter of these patients.

Studies of the acute phase of head injury require the combination of ventilation and MRS. A ventilator and suitable circuit were constructed and tested in the magnetic field (by Dr. L. Loh, Dr. C. Garrod, Dr. D. Young and Mr L. Gale, Nuffield department of anaesthetics, Oxford) and a patient with status epilepticus of unknown cause was studied twice. The first study after one week of thiopentone anaesthesia was normal (Figure 6.1). However a further study at three weeks was abnormal with loss of superficial metabolites, raised PME and reduced pHi at depth. The selective loss of superficial metabolites may be due to gray matter

Figure 6.1

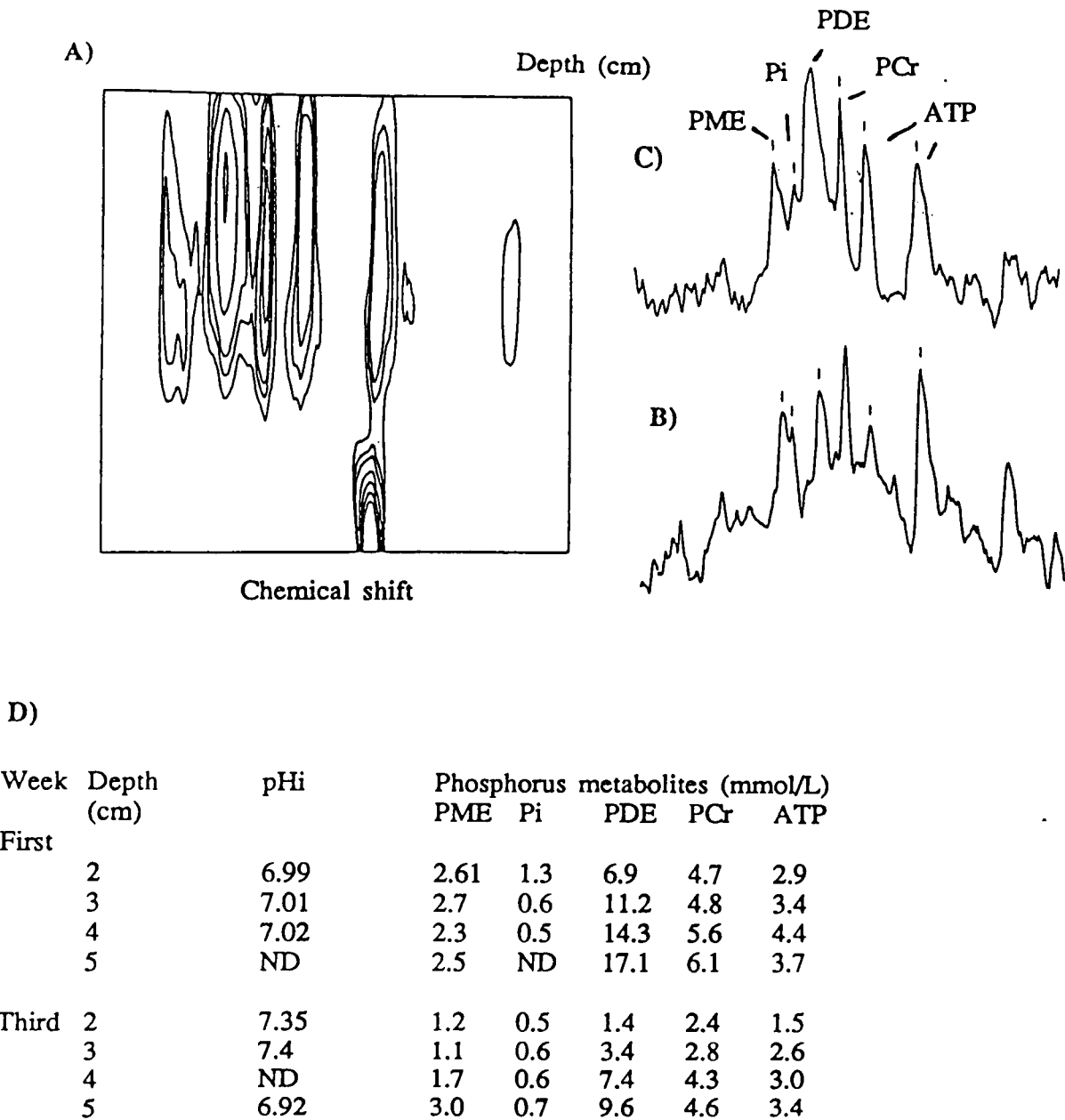


Figure 6.1 The left parietal lobe was studied in this 35 year old taxi driver who was found in status epilepticus of unknown cause. The contour plot (A) shows loss of phosphorus metabolites in the first few centimetres of brain (mainly gray matter). The selected spectra at 2cm (B) shows loss of PDE. The spectra at 4cm (C) appeared normal. The absolute tissue concentrations confirm these observation (D).

necrosis in the presence of epilepsy. The Ct scan appeared normal at three weeks. A similar ^{31}P MRS study of acute cerebral pathology *in vivo* could prove clinically beneficial. Specifically a study of acute severe head injuries may provide useful information on changes in intracellular metabolism with fluctuations in intracranial pressure and various therapeutic measures used to control pressure waves, such as hyperventilation and diuresis.

The metabolic events following sub-arachnoid haemorrhages could also be followed by ^{31}P MRS and help in the timing of surgery, which remains controversial. A pilot study with daily MRS studies of patients with sub-arachnoid haemorrhage could be carried out along side routine clinical practice.

Developments in the PMRFI experiment have been applied to the study of human brain metabolism *in vivo*. A method was developed to measure absolute tissue concentrations, imaging enzyme rate fluxes, and examination of certain characteristics of phospholipid structure. Biochemical and metabolic differences have been demonstrated between gray and white matter in normal cerebral hemispheres and pathological lesions. The possible mechanisms behind these alterations have been discussed and some further experiments described at both the human, animal and tissue culture level. The most consistent finding and observation of therapeutic relevance was the alteration in pHi shown in primary brain tumours. The lowered pHi observed in patients recovering from severe head injuries may be related to the low blood flow in deep white matter.

Magnetic resonance is a unique technique for the study of human cellular metabolism, simultaneously providing structural, biochemical and metabolic

information. Relevant biochemical and metabolic abnormalities can be demonstrated non-invasively, with the possibility of wide clinical applications. The same experiment can be used at all levels of clinical science from human to animal and tissue culture models, unifying these different levels of medical research.

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Publications : The papers and abstracts which have arisen out of this work are listed below.

Papers

Cadoux-Hudson, TAD, Blackledge, MJ, Rajagopalan, B, Taylor, DJ, Radda, GK (1989) Human primary brain metabolism *in vivo*: a phosphorus magnetic resonance spectroscopy study. *Br J Cancer* **60**:430-436.

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Abstracts

- Cadoux-Hudson, TAD, Blackledge, MJ, Rajagopalan, B, Taylor, DJ, Radda, GK (1988) Measurement of phosphorus metabolites in patients with intracranial tumours. *Proc Sac Magi Reson Med (San Francisco, USA)* 1:614.
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