Phys. Med. Biol. **51** (2006) R61–R82 doi:10.1088/0031-9155/51/13/R05

REVIEW

Magnetic resonance spectroscopy (MRS) in the investigation of cancer at The Royal Marsden Hospital and The Institute of Cancer Research

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Received 16 March 2006, in final form 23 May 2006
Published 20 June 2006
Online at stacks.iop.org/PMB/51/R61

Abstract
Developments in magnetic resonance spectroscopy (MRS) at The Royal Marsden Hospital and The Institute of Cancer Research are reviewed in the context of preceding developments in nuclear magnetic resonance (NMR) and MRS, and some of the early developments in this field, particularly those leading to human measurements. The early development of technology, and associated techniques for human measurement and assessment will be discussed, with particular reference to experience at out institutions. Applications using particular nuclei will then be described and related to other experimental work where appropriate. Contributions to the development of MRS that have been published in *Physics in Medicine and Biology* will be discussed.

Introduction

This account of magnetic resonance spectroscopy focuses on developments and investigations at The Institute of Cancer Research and The Royal Marsden Hospital, in the context of the development of the field and progress elsewhere, over the last 20 years. As the assessment and treatment of cancer are our major interests, the review concentrates on this area. Given that we are celebrating the contribution *Physics in Medicine and Biology* has made to biological understanding, medical science and practice over the past 50 years, the emphasis will be on physics and technological development, and the applications this has led to. However, naturally the applications and the underlying science straddle a much wider range of topics, and inevitably this account will stray into these related areas.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy describes the use of nuclear magnetic resonance (NMR) techniques to investigate the metabolism of intrinsic or exogenous chemicals in the living

body. Different chemicals containing the same nucleus exhibit characteristic chemical shifts in resonance frequency, allowing the chemical form of the element to be identified and so providing a non-invasive window on metabolism within the body. The bulk of research involves investigations of molecules containing ^{1}H or ^{31}P, both 100% naturally abundant, but with ^{31}P having a lower gyromagnetic ratio and lower sensitivity. A number of important metabolites contain either ^{1}H or ^{31}P, and these can be observed in tissue spectra. ^{19}F has similar sensitivity to ^{1}H but is observed in exogenous agents such as drugs, ^{13}C has low isotopic abundance as well as a low gyromagnetic ratio, and is usually observed as an extrinsic label, although some compounds can be detected at naturally occurring levels. Several other nuclei have also been used *in vivo*. *In vivo* applications using MRS may be accompanied by laboratory NMR or other analytical measurements to better understand processes and results, and some examples will be provided below.

MRS at The Institute of Cancer Research and The Royal Marsden Hospital

As an institution, our involvement in MRS started in 1983, when we participated in the first measurement of a human tumour (Griffiths *et al* 1983). At about this time, at the suggestion of Professor Ged Adams, I and several colleagues visited Oxford Instruments to see the new MRS system they had developed for *in vivo* investigations, and were impressed with its potential. However in our setting our major interest was in clinical investigations, and we were aware that MRI would also be required. My practical involvement started in about 1984 when together with colleagues, particularly Professor Janet Husband, I developed proposals for a high field (at that time) 1.5 T magnetic resonance scanner together with related research proposals which were submitted to the then Cancer Research Campaign. This provided the opportunity to purchase a device capable of both imaging and spectroscopy, at that time a new concept, as the techniques of magnetic resonance imaging (MRI) and MRS had followed separate developmental paths (see below). We therefore, supported by an appeal run by the Royal Marsden Hospital, raised the funding for a leading edge scanner and a building to house it, making this new technology and its use available to the NHS for a considerable period at no additional cost. In 1986 we installed the first high field clinical scanner capable of MRI and MRS within a hospital in the UK. The ability to combine imaging techniques with spectroscopy was of great value in applying this new technique to cancer.

Developing equipment, cancer diagnosis and treatment

By the 1980s the sophistication and complexity of many medical imaging devices had meant that the most appropriate way to explore the clinical application of new equipment was to base such work on a manufacturer's development programme. There remains a case for laboratory instrumental development, but time scales, resources and teams required, together with complexity, have to be balanced against the speed of commercial development. This is particularly the case when it is necessary to have on the same platform the wide range of current MR sequences. Research including collaboration with manufacturers, to develop and exploit a basic tool, extending its functionality, is a very important way of advancing medical science. The objectives of this are to attack new medical problems not provided for in the equipment and to thereby develop tools that could be included in such equipment in the future. With MRS, the early equipment had very limited facilities as well as various limitations and was consequently not well suited to complex examinations. As understanding has grown, and applications have been demonstrated, more sophisticated equipment and facilities have become available, providing what is now an increasingly valuable investigational tool.

Since we started research in this area, equipment has advanced from providing no spatial localization other than that offered by coils, to three-dimensional (3D) spectroscopic imaging supported by a wide range of automation. There have been considerable advances in cancer evaluation over the same period. Initially, disease management decisions were based on volume and extent of disease based on relatively low resolution and contrast images, mostly derived from x-rays and radioisotopes, together with conventional histology and some histological markers of cellular function. Conventional therapies included radiotherapy based on the above, with limited shaping to target structures; and cytotoxic agents with limited discrimination between normal and abnormal cells. Now much higher resolution images are available, aided by contrast agents that are increasingly specific, and complemented by functional information gained from imaging and spectroscopy. Characterization of disease is increasingly based on identifying specific genetic and expression abnormalities, and developing therapeutics to target these specific changes, moving from class treatments to individualized therapy. MRI and MRS play an important role in trials of these new agents, as well as supporting more accurate planning of radiotherapy treatments.

Outline

This paper will firstly briefly review the background of nuclear magnetic resonance (NMR) developments leading to the development of MRS, and some of the early developments in this field, particularly those leading to human measurements. The early development of technology, and associated techniques for measurement and assessment will be discussed, with particular reference to our own experience. Applications using particular nuclei will then be described, related to other experimental work where appropriate. This will relate to the progressive development of technologies. Contributions to the development of MRS in *Physics in Medicine and Biology* will be discussed.

Historical background

Development of NMR

Nuclear magnetism was first detected in solid hydrogen by Lasarew and Schubnikow (1937) with the independent observations of nuclear magnetic resonance (NMR) in bulk material being reported by Bloch *et al* (1946) in water and Purcell *et al* (1946) in paraffin wax. This discovery resulted in the joint award of the Nobel Prize for Physics in 1952 to Felix Bloch and Edward Purcell. The measurement by Bloch of a strong proton signal from a finger was the first *in vivo* observation of nuclear magnetic resonance. Following on from these early developments, NMR developed firstly as a tool for understanding the properties of the nucleus, and then, with the identification of a characteristic chemical shift for different molecular configurations containing an NMR observable nucleus, as a powerful analysis method for chemical samples, providing information on both molecular composition and on configuration.

Any isotope with an odd number of protons and/or neutrons will have a nuclear magnetic moment, and when exposed to a magnetic field will consequently display a characteristic resonant absorption of electromagnetic energy at its Larmor precession frequency. Different isotopes can have widely different Larmor precession frequencies, but these are generally in the radiofrequency range (1–300 MHz) for commonly used magnetic fields. Once these isotopes are bound in molecules, the external magnetic field causes a characteristic interaction with the molecular electron cloud, which causes a proportional change in the magnetic field

experienced by the nucleus. This results in a small frequency shift in the Larmor precession frequency, generally measured in parts per million, that can be detected. Clearly, to identify these small frequency shifts requires that the magnetic field across the sample is extremely homogeneous. By expressing the chemical shift in parts per million (ppm) this is then independent of magnetic field, which is helpful in comparing results between magnets of differing field strength.

Initially NMR analytical methods used a continuous wave approach, where the magnetic field's amplitude, or the radio frequency, was swept, with the radio frequency, or the magnetic field respectively, kept constant. This approach has now largely been superseded by pulsed Fourier transform NMR, where the sample is maintained in a uniform fixed magnetic field, and the frequencies detected following a brief radiofrequency pulse are analysed from the time signal using a Fourier transform. This is a highly efficient approach, allowing simultaneous identification of many resonances. Solution state NMR spectrometers are commonplace, and NMR is also widely used for understanding the configuration of complex proteins, and properties of solid mixtures. Much insight into biochemical processes can be gained by extracting tissue samples, and by investigating biofluids. Intact tissues can be examined using the technique of magic angle spinning.

Measurements in living tissues

Following on from the unpublished observation of signal from a finger by Bloch and colleagues, there have been a number of references to measurements in intact cells and tissues (Bratton *et al* 1965, Jackson and Langham 1968, Odeblad *et al* 1956). However, the research that most closely presaged the development of clinical MRS was that of Richards, Radda and colleagues showing that metabolism and pH could be measured in intact rat tissue (Hoult *et al* 1974). While early experiments made use of standard vertical bore NMR systems and perfused organs (Gadian *et al* 1976, McLaughlin *et al* 1979), the realization that it was possible to perform *in vivo* measurements on live animals led to the development of horizontal superconducting magnets with a wider bore for *in vivo* spectroscopy (Alger *et al* 1981, Balaban *et al* 1981, Gordon *et al* 1980, Grove *et al* 1980). Compared with developments in MRI, where a lower field, often with resistive magnet coils, was used, this development of relatively large bore higher field magnets was a major innovation.

Performing measurements in an intact animal led to new problems compared with experiments in conventional magnets—how to localize signal to an area of interest. Two developments in 1980 addressed these problems. One was the use of special field profiling coils to destroy magnetic field homogeneity other than in an area of interest—topical magnetic resonance (Gordon *et al* 1980, Gordon 1982). The other was the introduction of the surface coil, to localize signal to the immediate vicinity of a small RF coil, at the same time reducing RF power and considerably improving signal to noise compared with a volume coil (Ackerman *et al* 1980). The potential of these new MR systems to perform human measurements was quickly recognized, leading to systems that could accommodate a human limb for muscle investigations (Chance *et al* 1981, Radda *et al* 1982), and larger systems capable of measuring neonates (Cady *et al* 1983, Delpy *et al* 1982), and adult humans (Blackledge *et al* 1987, Hanstock *et al* 1988, Jue *et al* 1988, Lawson *et al* 1987, Oberhaensli *et al* 1986a, 1986b), rapidly became available. The first whole body high field magnets for MRS (at 1.9 T) presaged the development of the 1.5 T magnets soon to be offered by major medical manufacturers. Due to the interest in muscle energetics, most of these early measurements were performed on ^{31}P, but the potential to observe other nuclei, such as ^{1}H and ^{13}C, with the potential to use ^{13}C labelled compounds, was quickly recognized.

Technological developments for MRS

Selecting a system and designing a building

In 1984 we were able to commence discussions with manufacturers to select a system. This involved viewing the systems, which at that time were not completed and were in part only concepts. It was evident to us that we required a system capable of both imaging and spectroscopy, and this dictated that it should operate at 1.5 T. This was contrary to the advice given to us by some manufacturers, who did not have such a product. A decision was made to purchase a Siemens GBS1 Magnetom system. This resulted in the Hospital receiving a letter of displeasure from the Prime Minister, Mrs Thatcher, as we had not bought from a British company. However, as the system was not bought from public funds, and as we were confident in the rationale for the choice, we reiterated our reasons.

At that time, the manufacturers also had very limited experience of spectroscopy, and their requirements regarding nearby steel probably incorporated a significant safety margin, but required some unusual features in our building, including a laminated wood support for the roof. The building incorporated the usual safety features, in terms of restricted entry, and the area nearest the magnet was steel free. As the magnet was unshielded, it had a substantial footprint requiring a fence to enclose the 0.5 mT field contour. Unusually, we chose a stainless steel RF shield, which is still providing good service. Our architects had previously designed sound studios for the BBC, and they were most concerned about the gradient noise, and included significant structural sound damping. Whilst this may have been overkill at 6 mT m^{-1}, it certainly has more value now. While we had taken good care to avoid any nearby sensitive imaging and therapy equipment, it came as a surprise to find that there was an electron microscope in the adjacent Institute Haddow Laboratories. We calculated that the field would be a little over the Earth's field at this point, and considered the possibility of providing a compensating field. However on ramping the magnet, there was only a small shift in the focal spot position, which was easily adjustable. We also monitored the radiotherapy treatment linacs carefully, as there was considerable structural steel in the vicinity. Again we expected a field of the order of the Earth's field, and this produced no problems.

Provision for spectroscopy

The MR system was equipped with a number of facilities to support spectroscopy. This included a broadband transmit system, and a phosphorus pre-amplifier, and appropriate in-line filters on the receive channel, with switchable options for a number of other pre-amplifiers to be supplied. The system had high order, user adjustable shims. These were adjustable by potentiometers in the (then) very large equipment room, requiring a remote oscilloscope (somewhat affected by the field) to observe the free induction decay. This was somewhat less ergonomic than some dedicated experimental systems. Two phosphorus coils were provided, both in large plastic enclosures, with 1 m long tuning rods so that they could be tuned and matched when the patient was in position. The coils quickly became known as the shoeboxes. Simple pulse and acquire sequences were provided. In initial studies it became clear that we needed to include imaging in the protocol, to identify whether the coil was appropriately positioned. In time we incorporated markers for position read-out, and calibration samples to aid quantification, in the coils. However the basic coils did function well, although they were not optimized and were hard to locate. The magnet demonstrated good homogeneity, and it was possible to shim off centre. Although spectra could be phased and plotted, no software to quantify the spectra was provided, which was not unusual, even for purpose made spectrometers, at that time. However in order to progress, a number of improvements

were required, including coils, filters, further channels, measurement sequences providing localization, analysis software and eventually the hardware to support ^{1}H decoupling.

RF coils

Early in the programme of work in collaboration with Siemens we installed a ^{19}F channel, together with a further similar surface coil. Siemens also brought in a much more flexible switched ^{31}P/^{1}H transmission line resonator coil, which became a main stay for our research. A prototype dual frequency coil that allowed simultaneous ^{1}H and ^{31}P measurements was also developed in collaboration with the coil laboratory at Siemens (Leach *et al* 1986). Several simple coils were also developed in house, but for much of our research work particularly on breast tumours, we used the transmission line resonator, together with a marker ring and a calibration sample, using a home designed mounting that provided much greater flexibility than the hoop system provided to us. Later as our requirements became more demanding, as part of a collaborative project with J Murphy-Boesch and T Brown, a set of ^{31}P loop coils with ^{1}H flexible butterfly elements were built. D Collins developed our in-house coils and as part of our collaboration built a set of similar smaller coils for neck nodes (Klomp *et al* 2001), which had a fixed ^{1}H element. These coils are still in use. To ensure (particularly for decoupling) that the power deposition for our sequences would be within IEC standards, we performed extensive RF field simulations, confirmed by temperature measurements (Prock *et al* 2001, Prock *et al* 2002, Schwarz *et al* 2000). This was necessary as the manufacturer could not provide such support for user built coils. In addition, a number of further coils including ^{19}F designs, decoupled ^{19}F coils, ^{129}Xe coils, ^{129}Xe/^{1}H coils, ^{31}P/^{1}H birdcage coils have been constructed to support a range of projects.

Localization

Initially no localization software was provided, and it was necessary to rely on surface coils. A number of surface coil based localization schemes were implemented and investigated. We rapidly came to the conclusion that these were not sufficient for cancer measurements. In time a prototype implementation of ISIS, recently developed by R Ordidge (Ordidge *et al* 1986), was provided. This was not particularly appropriate to the geometry of many of our tumours, and did not have a very sophisticated graphical interface for set-up. We therefore embarked upon developing and implementing an improved version of ISIS, Conformal ISIS (Sharp and Leach 1992), which addressed the ability to select angulated regions and more complex volumes, with aspects having application to a range of localization methods. In principle, the method could produce shapes approximating to ovoid or spherical. This also incorporated hyperbolic secant pulses for accurate inversion, and a half-hyperbolic secant adiabatic read-out pulse, to reduce sensitivity to the surface coil B1 profile, providing a robust tool for clinical examinations. In time we also received a version of the recently implemented STEAM (Frahm *et al* 1987) and PRESS (Bottomley 1987, Ordidge *et al* 1985) sequences, for ^{1}H spectroscopy. From T Brown, we received an implementation of 3D spectroscopic imaging (SI) (Brown *et al* 1982, Maudsley *et al* 1983), together with a prototype analysis package, which enabled us to investigate its application. None of these techniques were optimized for our system, and they required considerable input to effectively implement them.

Shimming

As described above, the shimming provided was manual, and initially only localized by the receive sensitivity of the coil, irradiation being provided by the ^{1}H body coil (or head coil for brain measurements). We felt that this would not provide the quality of result required in

the localized region, so localized shimming was developed (Sharp *et al* 1992), based on noise pulses (Connelly *et al* 1988, Ordidge 1987) using identical gradient patterns to those employed for ISIS (to ensure equivalent eddy current effects from our unshielded gradients). This approach enabled a good localized shim over the region of interest. A further scheme allowed an identical shimming approach to be used for both STEAM and ISIS, whilst maintaining eddy current correction (Yongbi *et al* 1994). Shimming remained a fairly time-consuming part of the measurement, but at a later stage in our work we were able to make use of software developed by T Brown and colleagues at the Fox Chase Cancer Center. This used imaging software to measure image phase shifts, and based on these provided a first-order shim adjustment that could be manually loaded into the imaging gradient offsets, providing a more rapid and robust manual shimming solution. With proton spectroscopy, the localization sequence itself was effectively used for shimming, without water suppression. Our more recent systems have been provided with effective localized automated shimming programs.

Water suppression

A prerequisite for analysis of metabolites by ^{1}H MRS was suppression of the large water peak. The most commonly used approach to this, using chemical shift selective (CHESS) pulses to excite the water signal, followed by dephasing, was published by Haase and colleagues in *Physics in Medicine and Biology* (Haase *et al* 1985). While a number of other approaches have been used, and the technique has been optimized, this approach remains a main stay of ^{1}H MRS.

Analysis

For analysis we initially performed phasing with the manufacturer's software, but rapidly implemented a calculated first-order phase correction, based on the parameters of the measurement sequence and the frequency. A range of commercial software provided further solutions to analysis, together with some in-house developments, including a fitting method based on a superposition of spectral elements (Webb *et al* 1992). A pH calibration curve was measured (Madden *et al* 1989). With 3D CSI, we implemented software provided by T Brown, and also developed in-house software. For proton measurements we explored several software approaches, including HSDV (De Beer *et al* 1992), MRUI (Naressi *et al* 2001) and LCmodel (Provencher 1993).

Decoupling

Based on our interest in ^{31}P measurements, and experiments on tumour extracts from some of the tumours we measured *in vivo*, it was clear that a number of lines of interest to us were overlapping multiplets which, if decoupled, might be separable *in vivo*. Our MR system was not equipped to perform decoupling, or NOE enhancement, which offered the potential for some additional signal. We therefore decided to add a further broadband channel to our system, providing flexibility for future applications. This was based on a Surrey Medical Imaging Systems (SMIS) spectrometer and broadband amplifier, allowing independent transmit and receive (van Sluis *et al* 1995). The independent channel needed to be synchronized with our Siemens system, which was achieved by isolating a reliable programmable signal line in the Siemens, that could be accessed via the pulse programming language. The SMIS system could be set to wait for this pulse, and would then run a specified pulse sequence in synchrony with the RF pulses and gradient pulses provided by the Siemens system. As the system was to be used for human investigations, it was also necessary to monitor the RF output, to ensure

it conformed to predetermined limits dependent on the coil and sequence used. This was achieved using an RF power monitoring unit provided by SMIS.

With this system we initially implemented a range of decoupling approaches and irradiation to provide for NOE enhancement, led by G Payne. This included the bi-level decoupling scheme required for a collaborative study of ^{31}P in cancer, supported by the NCI (Arias-Mendoza *et al* 2004). Ensuring that we did not get additional RF noise in the receive channels of either system required effective filters, which we were able to source commercially. We were able to demonstrate simultaneous measurement of ^{1}H and ^{31}P (van Sluis *et al* 1996). The potential to decouple fluorine signals was demonstrated (Li *et al* 2000), a further example where some of the metabolites are line broadened. More recently, having transferred the SMIS channel to a Siemens Vision system, we have demonstrated the use of ^{1}H decoupling of ^{31}P in clinical studies of ifosfamide (Payne *et al* 2000, Schwarz *et al* 2000), studying a range of tumours, particularly non-Hodgkin's lymphoma, and in clinical trials evaluating a heat shock protein 90 inhibitor, 17AAG. The success of these basic heteronuclear experiments suggested that some advantages might be gained by transferring polarization from ^{1}H to ^{31}P, and a number of approaches to this were investigated, showing that some sensitivity could be gained (Mancini *et al* 2003b, Mancini *et al* 2005).

Eddy currents

Our original GBS1 Siemens Magnetom was designed before the clinical advent of fast imaging sequences, and hence the need for very small eddy currents, was recognized. For ^{31}P and ^{19}F localized spectroscopy, the gradient performance was adequate, and the pre-emphasis circuits provided sufficient compensation. In evaluating our conformal ISIS software on a later model, very long gradient eddy currents were apparent, resulting in the need for additional compensation (or gradient balancing) in the sequences. However, with the advent of ^{1}H localized spectroscopy for intercranial studies, together with the relatively short echo time measurements that were of interest, the problems of non-shielded gradients were clearly apparent. In order to implement these new techniques on our scanner, we had to put considerable effort into optimizing our eddy current correction, by manually optimizing each pre-emphasis circuit in turn. This did achieve a considerable improvement in quality, to the extent that we could implement and use ^{1}H localized spectroscopy, performing several clinical studies. a replacement scanner with shielded gradients markedly improved the performance of ^{1}H spectroscopy and allowed short echo times to be used.

Quality assurance and phantoms

Our interest in spectroscopy of cancer naturally led to a need for quantification. Conventionally, most ^{31}P spectroscopy in animal models was performed by comparing ratios of metabolites, obviating the need for quantification. Generally, such measurements were only (at that time) localized by means of a surface coil. While this approach might suffice for muscle energetic measurements, it was a different situation for tumours. Tumours were often sited adjacent to other tissues that also contained metabolites, thus contamination from adjacent tissues was an issue and we needed to know how spatially selective and how efficient the localization was (Leach 1992). While the behaviour of energetic metabolites was well known, particularly in muscle, in tumour there were further metabolites, and the way in which these, and energetic metabolites, were likely to behave was less well established. Therefore two metabolites of interest might change in the same direction, resulting in no change on a metabolite ratio, despite an important change in absolute levels occurring. There was therefore a need for a method of quantification not dependent on measuring ratios of metabolites. Spectra needed to be

quantified, although at that time the standard method was 'cut and weigh', this being a particular problem where a number of broad peaks overlapped. Collaborating groups tackled a number of these problems in a very effective series of European concerted actions, led by F Podo from Rome (Podo *et al* 1998). This established a number of spectroscopy test objects (Bovee *et al* 1998, Howe *et al* 1995, Leach *et al* 1995), evaluated a range of processing algorithms (De Beer *et al* 1995), and tested spectral quantification methods (Keevil *et al* 1995, 1998) developing quality assurance protocols (Bovee *et al* 1995), laying the foundation for many of the methods now in use. The INTERPRET project (Julia-Sape *et al* 2006, Tate *et al* 2003) also grew out of this collaboration. The test objects facilitated evaluation of localization sequences, and enabled evaluation of spectroscopy across manufacturers' platforms, sometimes revealing unexpected behaviour of the measurement sequences. The requirement for spectroscopic standardization was considered (Leach *et al* 1994a, 1994b). Quality assurance measurements were developed for collaborative clinical studies, to ensure that different centres were performing multi-centre studies equivalently, and a report of the consistency obtained has been published (Arias-Mendoza *et al* 2004).

^{31}P spectroscopy

^{31}P spectroscopy provides information on metabolites important in providing energy for cellular processes (Nucleotide tri- and di-phosphates (NTPs, NDPs), phosphocreatine (PCr), and the result of breakdown of phosphocreatine, inorganic phosphate (Pi)), and has been extensively used to investigate these processes, particularly in muscle. In other tissues further metabolites may be present, including sugar phosphates in the liver, 2,3-diphosphoglycerate in the heart. In the brain, lines relating to phospholipid metabolites, phosphomonoesters (PMEs) and phosphodiesters (PDEs) could also be observed. In tumour studies at the time we started measurements, assessment of tumour models had concentrated on the metabolites concerned with energy metabolism. The first published *in vivo* human spectrum of a tumour was performed by colleagues at the Royal Marsden Hospital with J Griffiths 1983, showing a spectrum from a rhabdomyosarcoma in the hand (Griffiths *et al* 1983). Shortly afterwards a paper from J Maris, B Chance and colleagues from Philadelphia (Maris *et al* 1985) reported changes in other metabolites, the phosphomonoesters, in a child with neuroblastoma, in response to treatment.

In the early stage of research, we evaluated a range of tumours to establish their MR characteristics, and how these changed with therapy. While we had expected to see consistent energetic changes, these in fact were not well defined. Our first observation was that the PME signal appeared to be reduced with chemotherapy in breast cancer (Glaholm *et al* 1989). While we saw such changes in a number of other tumour types, breast cancer was a major focus of our work. We noted that many tumours did not show PCr in the spectra, and reported a method of referencing pH measurements to the water resonance frequency (Madden *et al* 1991) that was subsequently used for much of our research. In a group of breast cancer patients, we were able to measure spectra *in vivo* prior to resection, and then evaluate samples from the same tumours following extraction, using high-resolution NMR (Smith *et al* 1991b), confirming that the major metabolites in the PME peak were phosphoethanolamine (PE) and phosphocholine (PC), with the PDE peak containing glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC). These are precursors and degradation products of the major membrane phospholipids phosphatidylethanolamine and phosphatidylcholine. We extended this work to investigate the heterogeneity of these metabolites in human breast cancer (Smith *et al* 1991c), comparing with normal breast tissue and inflammatory breast disease, showing that some tumours demonstrated considerable heterogeneity, that normal breast tissue exhibited

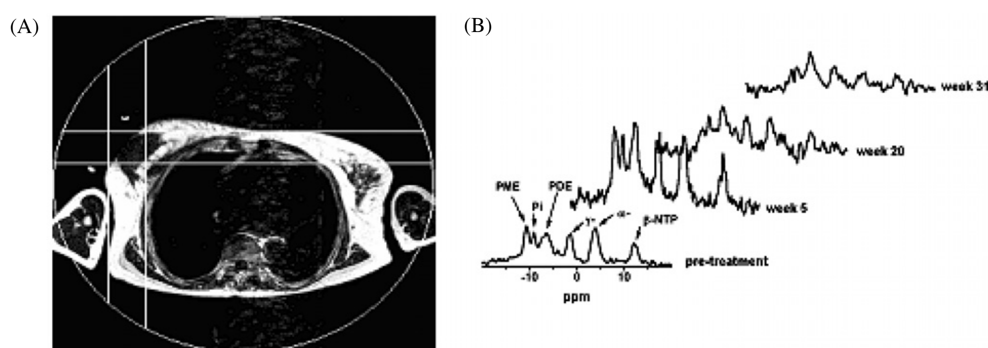

Figure 1. (A) Localization ^{1}H image for ^{31}P MRS showing a single voxel, positioned using in-house software, in a breast tumour. (B) ^{31}P magnetic resonance spectra obtained from the region defined in A before (pre-treatment), during (week 5 and week 20), and after (week 31) a course of chemotherapy, showing an initial increase in all metabolites (week 3) followed by a marked reduction in metabolite signals with treatment (weeks 20, 31). (Reproduced with permission from Leach *et al* (1998), copyright Wiley.)

low levels of these metabolites, and that inflammatory breast disease, with high levels of macrophages, demonstrated high levels of phosphorus containing metabolites.

In a hormone-dependent experimental tumour model we were able to show that PC were directly, and GPC levels inversely, correlated with S-phase fraction and bromodeoxyuridine (BrdU) labelling, both being measures of proliferation (Smith *et al* 1991a). In further work we showed that in this model oestrogen ablation reduced levels of PC, and increased GPC, whereas in an *N*-methyl *N*-nitrosourea (NMU) induced tumour there was no correlation between phospholipid metabolites and S-phase fraction or oestrogen ablation (Smith *et al* 1993a). In rat sarcoma cells we were also able to demonstrate that PC was elevated in faster growing cell populations (Smith *et al* 1996).

In vitro NMR assessment was performed on a large number of human breast tumours. In these, phosphorus metabolites did not correlate with indices of proliferation, reflecting the considerable heterogeneity in human tumours (Smith *et al* 1993b). However, PC levels were higher in high-grade tumours compared with low-grade tumours. In a large study of patients monitored before and during chemotherapy, we showed that levels of ^{31}P containing metabolites correlated with early measures of response, with PME showing the most significant changes (Leach *et al* 1998). Figure 1 shows an example of a localization image of a breast tumour, and sequential spectra obtained from the tumour before and after therapy. Interestingly PCr was generally absent from these tumour spectra, requiring a different approach to calculating the pH-dependent shift of the Pi peak (Madden *et al* 1991).

The reproducibility of localized ^{31}P MRS spectroscopy was also assessed. By studying metabolite levels in the breast during the menstrual cycle, we were able to define the levels that could be seen in normal tissues, and the hormone-dependent changes in these during the menstrual cycle (Payne *et al* 1994). Interestingly, detectable levels of PME were seen in normal breast, and the levels changed with the menstrual cycle. The use of MRS in breast cancer has been reviewed (Ronen and Leach 2001). We also investigated whether the use of an anti-cancer drug, Lonidamine, in patients with breast cancer resulted in a change in ^{31}P metabolism in muscle that was consistent with an increase in myalgia (Mansi *et al* 1991), believed to result from inhibition of lactate export and resultant intracellular acidification (Ben Horin *et al* 1995). Although some response was seen in this phase II trial, no association of metabolic change with MR spectra was seen.

We have been partners in an international trial of proton decoupled ^{31}P spectroscopy using 3D spectroscopic imaging, assessing its contribution to predicting and assessing response to treatment in a number of human tumours. Results to date from this trial have shown that pre-treatment levels of phosphocholine predict response of non-Hodgkin's lymphoma patients to chemotherapy. When these metabolic data are added to the International Prognostic Index for lymphoma, it is possible to segregate good responders from those with a poor prognosis, with increased power. Results from an associated quality assurance programme have been published, demonstrating the reproducibility possible between centres on a range of equipment (Arias-Mendoza *et al* 2004).

Cellular processes

Apoptosis. Cancer diagnosis and treatment is increasingly utilizing our growing understanding of the cellular mechanisms controlling processes such as cell growth and death, and the increasing information about the cancer genome. One important process is apoptosis or programmed cell death, an inherent mechanism that allows aberrant cells to be killed and disposed of in an orderly way. This process can be actuated by internal processes such as detection of DNA damage within the cell, or by external signals. Examples of its use include many changes, such as the removal of amphibious features that occur in the developing embryo and the removal of potential cancer cells. Many forms of cancer are characterized by mutations of the genes that produce proteins responsible for detecting cellular damage (so-called tumour suppressor genes), and correcting for this loss of function and finding means of initiating apoptosis in cancer are objectives of current therapeutics. We investigated whether there were MR visible changes in metabolites when apoptosis was induced in leukaemia and colon cancer cell lines, showing that a build up of a glycolytic intermediate, fructose 1,6-bisphosphate, was an early event (seen at 3 h) in the generation of the apoptotic signalling process (Ronen *et al* 1999). A further effect observed in apoptosis by MRS is the presence of lipid signals. By observing Jurkat T-cells, following induction of apoptosis, a build up of methylene was seen, with an increase in triacylglycerols and a concomitant increase in lipid droplets (Al Saffar *et al* 2002). A fall in cellular phosphatidylcholine suggested that the lipid droplets resulted from breakdown of cellular membranes. These observations help to explain MR effects seen in model systems, and may be applicable to the *in vivo* evaluation of apoptosis.

Pathways and inhibition. In a range of studies in cells we investigated the effects of oncogene expression, investigating cell lines over expressing mutations in ras, a gene expressing a protein important in cellular signalling which when mutated leads to increased activity in pathways controlling proliferation. To investigate whether over expression of ras affects the MR signal, we investigated two fibroblast cell lines, one of which had been transfected with mutant ras, the second being wild type with normal ras (Ronen *et al* 2001). Comparison of the spectra showed a fourfold increase in PC in the mutant line, of considerable interest as elevated PC (or PME) is commonly seen in human cancers. Treatment of the cells with three inhibitors of the ras pathway reduced PC in the transfected line, but not in the wild type cells, showing that MRS could detect effects when inhibiting this pathway, potentially providing a means of non-invasively monitoring new treatments designed to target this important pathway. The molecular chaperone, heat shock protein 90 (HSP90), is responsible for ensuring correct folding of a range of proteins on several important pathways in tumour cells. We evaluated the effects of inhibiting HSP90 with a new agent, 17AAG, in three cell colon cancer cell lines, and in a xenograft model in collaboration with Y-L Chung and J Griffiths (Chung *et al* 2003). This showed that PC increased following treatment, compared with control and a similar effect could be seen *in vivo*, where the PME/PDE ratio increased. We have performed

a pilot evaluation of this approach using ^{1}H decoupled ^{31}P spectroscopic imaging in patients in a phase I trial of 17AAG, showing detectable changes in one patient (Beloueche-Babari *et al* 2003). This technique is now being applied to further clinical trials of HSP90 inhibitors.

We have recently investigated a number of further inhibitors using these techniques. Mitogen activated protein kinases (MAPK) are proteins several steps below ras in the important ras/raf/MAPK signalling cascade that provide an important target for new anti-cancer therapeutics. Inhibition of MAPK resulted in a significant drop in PC levels that was associated with inhibition of ERK1/2 phosphorylation, a measure of successful MAPK inhibition, with PC changes following a similar time course to inhibition of ERK1/2 phosphorylation (Beloueche-Babari *et al* 2005). In the related PI3K pathway, which is also a target for new therapeutics, we showed that PC was reduced in breast cancer cell lines following inhibition of this pathway with two different inhibitors (Beloueche-Babari *et al* 2006). These results demonstrate that PC may provide a potential biomarker for investigating new inhibitors of this pathway in clinical studies, where a major objective with these new agents is to demonstrate that they are working by the intended mechanism *in vivo*, and we plan to incorporate these methods into forthcoming early stage clinical trials. We have used similar techniques to investigate a new anti-cancer therapeutic approach which targets the phosphorylation of choline by choline kinase (Al Saffar *et al* 2006). Our investigation showed that PC and choline levels fell when choline kinase was inhibited, with PC levels correlating with choline kinase activity, and that these changes were reflected by a fall in phosphocholine and choline in xenograft models (in collaboration with Y-L Chung and J Griffiths), in this case providing a direct report on the mechanism of action of the drug.

Drug pharmacokinetics

As well as reporting on the effects of drugs on tumour metabolism, in some cases ^{31}P MRS can be used to directly measure the drug and its behaviour. Two widely used anti-cancer drugs, ifosfamide and cyclophosphamide, contain phosphorus, and are given at sufficiently high doses to be measured *in vivo*. They are used in the treatment of sarcoma. We have shown that these compounds can be measured in patients *in vivo*, and that sensitivity can be improved by decoupling and polarization transfer (Payne *et al* 2000). Measurements in a model system showed that there was biliary uptake, not expected on the basis of the current literature, and that several metabolites could be observed in bile (Mancini *et al* 2003a). We have identified these (Payne *et al* 2005b) providing information that may contribute to our understanding of the toxicity of this drug.

^{19}F spectroscopy

Clinical investigations of the anti-cancer drug 5-fluorouracil metabolism

While naturally occurring fluorine is not normally visible in *in vivo* MR spectra, MRS has a high sensitivity for this element. For chemical reasons, it is often a component of anti-cancer drugs. This was the case for the first rationally designed anti-cancer drug, 5-fluorouracil (5FU), an anti-metabolite which mimics the behaviour of the nucleotide uracil, but which fails to be correctly incorporated into DNA. Stevens *et al* (1984) showed in a pre-clinical study that MRS could be used to observe the metabolism of 5FU. Shortly afterwards Wolf *et al* (1987) reported the first *in vivo* observation of 5FU in patients. The metabolism of 5FU is quite complex, and MRS allows the time course of the pharmacokinetics to be followed *in vivo*, as well as providing signals from many of the metabolites. As cells are continuously metabolizing 5FU, with misincorporation detected at mitosis, the time course of 5FU availability is an important

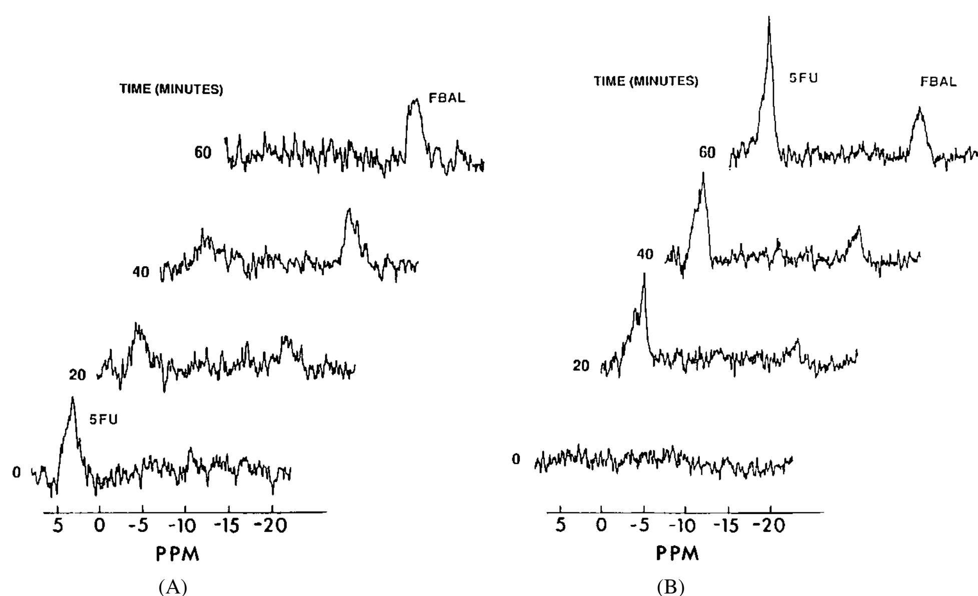

Figure 2. The pharmacokinetics and metabolism of the drug 5-fluorouracil (5FU) in the liver of a patient with colorectal cancer as a function of time after (A) intravenous and (B) intraperitoneal bolus administration. Measurements were obtained using a surface coil in separate treatments of the same patient. The development of the principal catabolite of 5FU, fluoro-beta-alanine (FBAL) can be seen, with different time courses of 5FU appearance and conversion depending on administration route. (Reproduced with permission from Glaholm *et al* (1990).)

issue. The route of administration can play an important role in maintaining access, and we demonstrated that MRS in patients receiving 5FU can be used to monitor the pharmacokinetics and metabolism of the drug (figure 2) (Glaholm *et al* 1990).

To maximize the temporal exposure of cells, 5FU therapy switched to continuous infusion via a portable pump. We demonstrated that although the dose rate was low, we could monitor uptake in tumour and liver by MRS, and that patients who demonstrated 5FU in the spectra were likely to respond to the treatment (Findlay *et al* 1993). The study design included a second phase of treatment in those patients whose disease became refractory to 5FU alone, whereby α-interferon was added to modulate the behaviour of 5FU. A number of these patients receiving the combined treatment showed active metabolites of 5FU in the spectra, the first time these had been observed in human studies, confirming that the α-interferon was changing the metabolism of the 5FU. Patients showing a new or increased 5FU signal were likely to show a response to the combined treatment.

By examining plasma levels of 5FU by HPLC we were able to correlate liver concentrations of metabolites with plasma measurements (Findlay *et al* 1996). These showed that plasma levels of 5FU increased when α-interferon was administered, that plasma levels of 5FU correlated with normal liver catabolite concentrations, and log plasma 5FU concentrations correlated inversely with normal liver 5FU concentrations. Plasma 5FU correlated with toxicity but not with response. These results were surprising in that pre-clinical studies had shown that exposure levels of the drug correlated with effectiveness. However, in these clinical studies we were observing drug following continuous infusion, and those patients with high levels of 5FU in the plasma might have impaired liver catabolism, and tumour may not be anabolizing

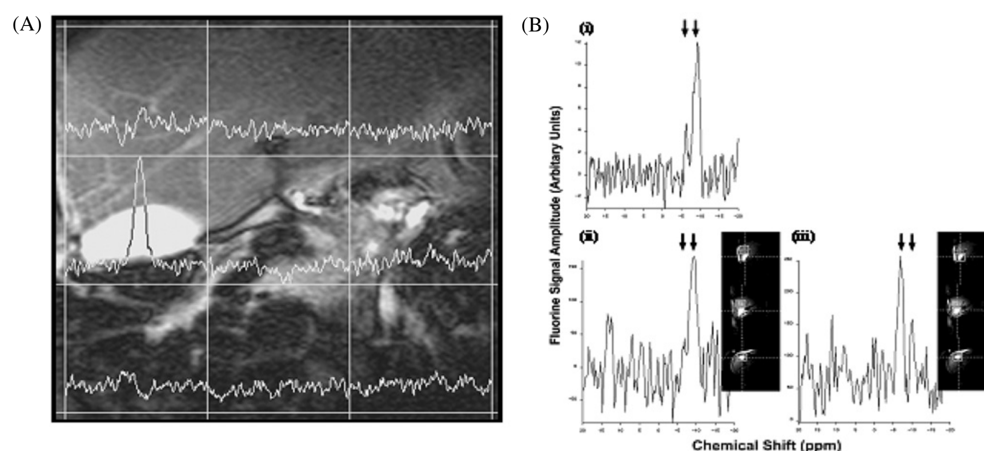

Figure 3. (A) Two-dimensional magnetic resonance spectroscopic map of the liver superimposed on a proton image showing drug metabolite signal localized to the gall bladder. (B) Chemical specificity of the localized metabolite signals from (A): (i) unlocalized signal from whole liver, (ii) spectrum from a voxel positioned in liver, (iii) spectrum from a voxel localized in the gall bladder showing a shift in the resonant frequency of the FBAL in the gall bladder (left-hand arrow) due to conjugation with bile acid compared with unconjugated FBAL in the liver (right-hand arrow). (Reproduced with permission from Dzik-Jurasz *et al* (2000).)

the drug to active fluoronucleotides. It was possible to also infer that patients with toxicity were not anabolizing drug but tended to have high catabolite signals, although these catabolite signals could vary considerably. A review of MRS of 5FU was also published (Findlay and Leach 1994). While some of these results were unexpected, later research, where we employed 3D localized ^{19}F spectroscopic imaging in patients receiving bolus and continuous infusion 5FU, identified a likely explanation. This showed that the major catabolite of 5FU, fluoro-beta-alanine (FBAL), was sequestered by the gall bladder, where it was conjugated to bile acids, producing a characteristic chemical shift (Dzik-Jurasz *et al* 2000), figure 3. This indicated that the catabolites seen in our earlier continuous infusion measurements were likely largely to originate in the gall bladder, and that toxicity might be related to high levels of hepatobiliary recirculation.

Pre-clinical investigation of 5-fluorouracil behaviour

In parallel pre-clinical experiments in collaboration with J Griffiths, we have shown that in HT29 cells, thymidine and interferon both increase the intratumoural T1/2 of 5FU, with an increase in the intracellular pH with interferon, increasing the negative pH gradient across the cell membrane (McSheehy *et al* 1997). The combination of interferon with 5FU provided an effective treatment, whereas each alone did not produce a response. This suggestion that drug residence time was affected by cellular pH gradient might explain clinical MRS findings that tumour T1/2 predicts response. Further experiments in cells confirmed the effect of the pH gradient on 5FU uptake (Ojugo *et al* 1998). Tumour uptake can be modulated by carbogen (McSheehy *et al* 2005) and bafilomycin (McSheehy *et al* 2003), which both affect the intra-, extra-cellular pH gradient. 19F MRS can also be used to assess the metabolism of pro-drugs of 5FU, such as capcitebine, showing that conversion depends on the level of thymidine phosphorylase in the tumour (Chung *et al* 2004).

Investigation of other fluorinated compounds

^{19}F MRS has also been used to study a range of other compounds. One example is the hepatic uptake of an anti-microbial sitafloxacin, where there were regulatory concerns about liver uptake and changes in function. In a clinical investigational study we were able to demonstrate that there was no evidence of liver uptake (Payne *et al* 2005a). An interesting application has been in evaluating a fluorinated hypoxic marker, SR4554, which is reduced and binds in hypoxic areas. Pre-clinical investigations have demonstrated the retention of the agent in hypoxic areas as planned (Aboagye *et al* 1996, 1997, Seddon *et al* 2002), and based on this, clinical ^{19}F MRS has been used to investigate retention in a clinical trial of the agent, demonstrating that it was retained in most tumours compared with plasma kinetics (Seddon *et al* 2003).

^{1}H spectroscopy

Investigations in the brain

In 1987 Frahm *et al* published their paper describing STEAM (Frahm *et al* 1987), and work describing the PRESS method of ^{1}H localized spectroscopy was reported (Bottomley 1987, Ordidge *et al* 1985). Together with water suppression (Haase *et al* 1985), these provided methods of measuring ^{1}H spectra in the body. These techniques were first applied in the brain, where the technical challenges were most tractable. The small chemical shift range of ^{1}H places high requirements on homogeneity and the method of localization and quality of water suppression can be adversely affected by gradient eddy currents. ^{31}P studies of tumour had been rare in the brain, as the sensitivity of the technique has been limited. ^{1}H spectroscopy provided a sensitive method, allowing smaller voxels, and providing a technique that has been very widely used. Our initial interest was in the assessment of treatment toxicity on paediatric brain (Davidson *et al* 2000a, 2000b). Patients treated with methatrexate showed low total choline (Cho) to water ratios. In patients treated with radiation, it was not possible to demonstrate a radiation-related metabolite change, although there was an association between low IQ and reduced Cho to water ratio. We have also assessed response to therapy in patients with low-grade glioma, showing that a reduction in Cho provided a metabolic marker of treatment response (Murphy *et al* 2004) As low-grade glioma generally shows a small response to therapy, the ability to be able to combine volume change with an independent metabolic change provided increased confidence in assessing response (figure 4). The presence of a methylene signal in brain tumour aided detection of transformation from low- to high-grade glioma (Murphy *et al* 2003). The potential to use contrast agents as a probe for compartmentalization of Cho was investigated, demonstrating that Gd-DTPA did not cause enhancement of metabolite signals, suggesting that the choline was intracellular, but that some broadening of peaks was evident, as a result of susceptibility effects (Murphy *et al* 2002).

^{1}H spectroscopy in the body

We have also investigated the use of ^{1}H MRS to investigate extra-cranial tumours. In lymphoma and germ cell tumours we have demonstrated that Cho is visible in spectra, and that changes in the Cho:water ratio predicted response to chemotherapy (Schwarz *et al* 2002). We also demonstrated Cho signal from human rectal carcinoma *in vivo*, documenting some of the practical issues involved in such measurements (Dzik-Jurasz *et al* 2002). Following on from our assessment of drug metabolites in the human gall bladder, we developed an

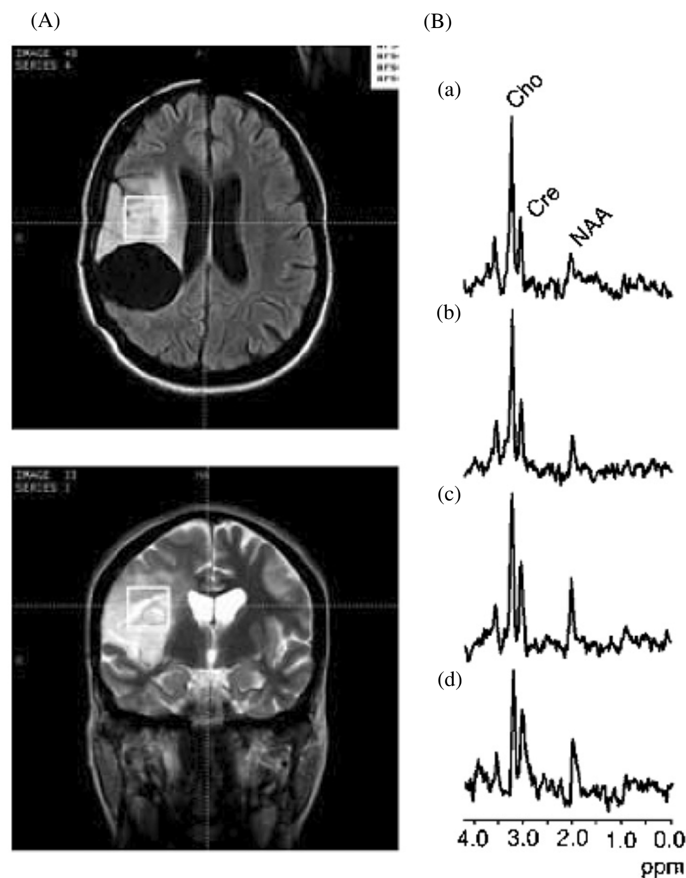

Figure 4. (A) Pre-treatment FLAIR (fluid-attenuated inversion recovery) (top) and T2W-FSE (T2 weighted fast spin–echo) (bottom) pre-treatment images from a patient with low-grade glioma receiving treatment for recurrent disease, showing the position of voxel selected for spectroscopy and (B) serial ^{1}H spectroscopy measurements from the same patient. Panel showing long echo time (TE = 135 ms) STEAM (stimulated-echo acquisition mode) spectra obtained before (a) and at (b) 3 months, (c) 6 months, and (d) 9 months after initiation of temozolomide treatment. Within both series, a progressive decrease in the choline/creatine (Cho/Cre) ratio was observed, suggesting reduced membrane metabolism and diminishing cellular density. Also note the increasing conspicuity of the N-acetyl aspartate (NAA) peak, a specific neuronal marker whose level may reflect the regression of tumoural tissue and repopulation of normal brain matter. (Reproduced with permission from Murphy *et al* (2004).)

MRS approach for investigating the gall bladder, using PRESS localization, and sequential spectral acquisition, showing that biliary metabolites could be identified (Prescot *et al* 2003). We have also implemented two-dimensional spectroscopic techniques (Localized COSY and DQF-COSY) and applied these to the evaluation of bone marrow in patients with leukaemia, comparing these with normal controls. This and other examples illustrate how far *in vivo* spectroscopy has progressed compared with the initial use of simple surface coil spectroscopy. The study assessed the degree to which the triacylglyceride (TAG) acyl chains were unsaturated, finding no difference between controls and patients with leukaemia (Prescot *et al* 2005).

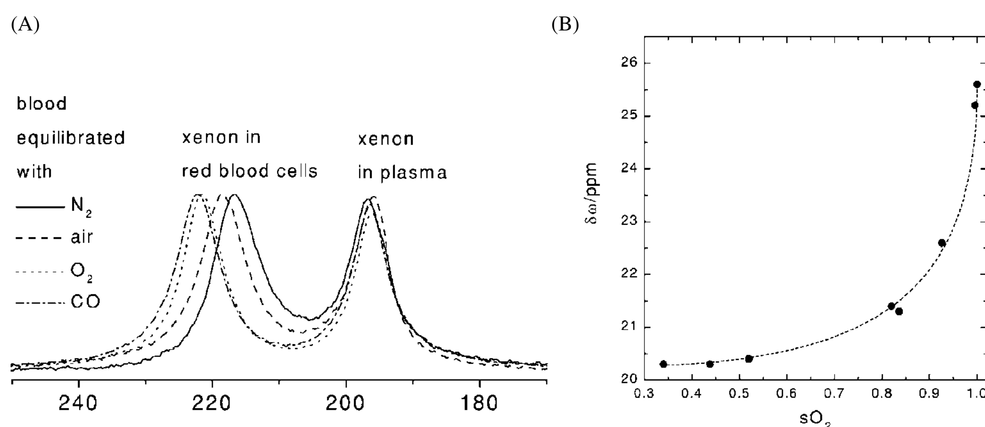

Figure 5. (A) ^{129}Xe spectra from whole blood equilibrated with different gases, showing that the chemical shift of ^{129}Xe in blood (in ppm) depends on the oxygen content. (B) A graph of chemical shift separation of the red blood cell signal and the plasma signal as a function of the blood oxygenation level sO_2. (Published with permission from Wolber *et al* (2000a).)

Hyperpolarized ^{129}Xe and ^{13}C

MRS is often limited by the low sensitivity of NMR, particularly where nuclei with low isotopic and/or natural abundance are considered. One approach to address this shortcoming is by hyperpolarizing the spin population, an approach that has been applied most commonly to two isotopes of nobel gases, ^{3}He and ^{129}Xe, as well as more recently to ^{13}C. We were able to establish the first hyperpolarization facility at a biomedical laboratory and hospital in the UK, measuring the diffusion of hyperpolarized ^{129}Xe in a range of solutions (Wolber *et al* 1998a), reviewing and investigating the properties of hyperpolarized ^{129}Xe in potential vascular delivery media (Wolber *et al* 1998b, 1999b), where the dependence of the ^{129}Xe signal on emulsion droplet size is shown. We were able to show that in blood, the T1 relaxation time of xenon is sensitive to the conformation of the haemoglobin molecule, detecting changes that result from oxygen binding (Wolber *et al* 1999a, 2000b). The chemical shift of ^{129}Xe is also sensitive to blood oxygenation levels, and to the binding of other molecules to haemoglobin (figure 5) (Wolber *et al* 2000a), with potential value in a range of applications. ^{129}Xe was investigated for the first time in two tumour models *in vivo*, showing characteristic differences between the two tumour types (Wolber *et al* 2001). While xenon is very sensitive to its environment, as these studies have shown, it does not form part of biologically important molecules. We have investigated the potential to transfer ^{129}Xe polarization to ^{13}C, showing, via thermal mixing, that the ^{13}C signal can be enhanced by a factor of 390 (Cherubini *et al* 2003). Much interest is now focussed on the use of dynamic nuclear polarization to polarize ^{13}C (Golman *et al* 2003).

MRS in *Physics in Medicine and Biology*

While *Physics in Medicine and Biology* published a number of early papers in the development of magnetic resonance imaging, its contribution to the development of MRS has been less marked, as much work was published in biochemical or general journals, and specialist journals already existed for NMR, and developed rapidly to support this new field of biomedical applications. Pioneers of the techniques of MRI published a number of early papers, and some

of these techniques also later supported aspects of spectroscopic measurements (Andrew *et al* 1977, Bottomley *et al* 1978, Edelstein *et al* 1980, Grannell and Mansfield 1975, Haase *et al* 1985, Lai and Lauterbur 1981, Mansfield and Maudsley 1976, Pykett and Mansfield 1978), including spin–warp imaging (Edelstein *et al* 1980) and CHESS chemical shift selective imaging (Haase *et al* 1985). One study examined the RF magnetic field distribution and power deposition with frequency, raising concerns regarding field amplitude and phase above 30 MHz (Bottomley and Andrew 1978). A number of investigators measured water relaxation times of biological materials (Bakker and Vriend 1984, Koivula *et al* 1982, Ling *et al* 1980, Ling and Foster 1982, McLachlan 1980), including reporting reduced relaxation times in the plasma of cancer patients (McLachlan 1980). Several papers examined aspects of high-resolution NMR spectroscopy (Kimmich *et al* 1984, 1987). A review by Gordon in 1985 provided an introduction to the growing field of MRS, providing a valuable reference for those in this developing field (Gordon 1985). Papers reporting applications of MRS to biological tissues (Grunder *et al* 1989, Klammler and Kimmich 1990), or methodological aspects of measurements, including spectroscopic quantification (Harpen *et al* 1987), the properties of RF pulses (Van Cauteren *et al* 1992) and localized shimming (Sharp *et al* 1992) were published in the 1980s and early 1990s. *Physics in Medicine and Biology* continues to contribute to the growth of this important field.

Conclusions

While this review has concentrated on applications of MRS in cancer at The Institute of Cancer Research and The Royal Marsden Hospital, it has provided many examples of the growing range of applications of this technique, which now plays a role in the investigation and study of many diseases, particularly in the brain in neurological and psychiatric diseases and the study of brain function, but with growing roles outside the brain in the assessment of, for example, diseases of cardiac tissues, muscle and liver. With the provision of stable and automated MR systems, which make spectroscopy reproducible and easy to include in a standard examination, together with the availability of higher fields, we can expect to see its use continue to expand.

Acknowledgments

The research described in this review has been performed by many of the staff and students who have been members of the MR Unit of The Institute of Cancer Research and The Royal Marsden Hospital. In particular David Collins and Geoffrey Payne developed many of the techniques described and used, and performed many of the developmental and clinical studies. Angelo Bifone developed research on hyperpolarized gases, Sabrina Ronen led many of the pre-clinical investigations. Many clinical fellows, post-doctoral staff and research students have contributed to this research. Our radiography staff provided essential support in designing and performing the clinical studies. Ralph McCready provided encouragement for the development of MRS. Janet Husband played a key role in developing and jointly directing the MR Unit and its programme of research. Our industrial partners provided access to key technologies. Cancer Research Campaign and its successor, Cancer Research UK have provided support and encouragement for our programme of research, together with the US National Cancer Institute and the International Association for Cancer Research.

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Biography

Martin Leach joined the Institute of Cancer Research and Royal Marsden Hospital in 1978 after completing Physics degrees at the Universities of Surrey and Birmingham. Initially he worked on *in vivo* activation analysis, the development of CT devices for radiotherapy planning, and on applications of isotope imaging in cancer diagnosis. He has developed the programme of research into applications of MR in cancer together with provision of an MR Service to the Royal Marsden since the installation of the first clinical imaging and spectroscopy system in 1986. He previously was Honorary Editor of *Physics in Medicine and Biology* from 1996–1999. He was appointed Professor of Physics as Applied to Medicine in 1996 and was elected to the Academy of Medical Sciences in 2002.