Received: 17 September 2021 | Revised: 5 April 2022 | Accepted: 8 April 2022

DOI: 10.1002/nbm.4740

REVIEW ARTICLE

NMR IN BIOMEDICINE WILEY

Magnetic resonance spectroscopy ex vivo: A short historical review

John R. Griffiths

Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Cambridge, UK

Correspondence
John R. Griffiths, Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK.
Email: john.griffiths@cruk.cam.ac.uk

Over the last half century, there have been several periods during which magnetic resonance spectroscopy (MRS) has been used ex vivo, for a variety of reasons, on samples such as microorganisms, cells, animal or human tissue, tissue extracts or biological fluids. These studies began in the days before the acronym MRS had been invented, when all such methods were still called nuclear magnetic resonance (NMR), and have extended to the present day. I will describe the historical development of NMR methods used ex vivo, their influences on the development of MRS in vivo, and their longer-term uses. All the interpretations will be personal, based on what I saw, or discussed with colleagues at the time.

KEYWORDS
chemometrics, metabolomics, metabonomics, MRS ex vivo

1 | INTRODUCTION

Magnetic resonance spectroscopy (MRS) is the term that has come to be used for the nuclear magnetic resonance (NMR) spectroscopic studies of living organisms, in vivo, so why would there be any interest in using this method ex vivo on tissue samples, tissue extracts or biological fluids? Over the last half century there have been several periods during which MRS has been used ex vivo, for a variety of reasons, on samples such as microorganisms, cells, animal or human tissue, tissue extracts or biological fluids. These studies began in the days before the acronym MRS had been invented, when all such methods were still called NMR, and have extended to the present day.

I will describe the historical development of NMR methods used ex vivo, their influences on the development of MRS in vivo, and their longer-term uses. However, this will not be a formal review: all the interpretations will be personal, based on what I saw at the time or discussed with colleagues, initially when I was a graduate student in George Radda's laboratory in the early 1970s, and later as a young MRS researcher in London, working both ex vivo and in vivo. In the text, I shall mainly mention the names of researchers who were, or subsequently became, prominent in MRS or magnetic resonance imaging (MRI) research.

One further warning: 40 years ago, when I was starting out in scientific journal editing, as an Editorial Adviser, one of the subeditors admonished an author whose paper I was editing: 'You will recall, Professor, that the words "*ex vivo*" form a Latin adverbial phrase. They should therefore be used in that sense in English, following rather than preceding the words they modify.' That rigid piece of English grammar seems to have vanished, along with scientific subeditors trained in the classics, but just for old times' sake I shall revert to it in the following review.

Abbreviations used: HR-MAS, high-resolution magic angle spinning; MRS, magnetic resonance spectroscopy; Pi, inorganic phosphate.

2 | ^{13}C NMR STUDIES ON BIOLOGICAL SAMPLES

2.1 | Early ^{13}C NMR studies ex vivo

The first biological NMR spectra were obtained in the early 1970s. NMR instruments were already being used in chemistry departments to monitor the metabolic fate of ^{13}C tracers in extracts of biological tissues,[1] so using ^{13}C NMR spectroscopy to study the same molecules in living cells was an obvious next step. At that time, all chemistry-laboratory NMR experiments were routinely performed on tiny samples in rapidly spinning narrow-bore tubes, and it was generally assumed—at least by nonphysicists—that this configuration was essential to getting a spectrum. Consequently, the first studies on living biological specimens were performed ex vivo, on pellets of cells (see Figure 1, 1960s and 1970s).

Eakin et al.[2] reported metabolic NMR studies on anaerobic suspensions of *Candida utilis* yeast cells in 1972. They added ^{13}C labelled glucose and monitored its metabolism in real time by identifying ^{13}C labelled ethanol as a product. Adding D_2O induced osmotic shock and liberated metabolites that they speculated were amino acids. These experiments demonstrated the potential of ^{13}C NMR for studying living samples, but it was not until further advances in magnet technology took place a few years later that the method really began to deliver significant results.

2.2 | Metabolic ^{13}C tracer studies in real time

In the late 1970s, the magnet bore of some NMR instruments had increased to the point where the probe could accommodate an organ of a small animal. Robert Shulman's group, initially at Bell Labs and subsequently at Yale University, began an important series of studies using this technology to follow the metabolism of ^{13}C-labelled compounds in the perfused liver. Their results were of great interest at the time, because our understanding of mammalian metabolism had been transformed, over the previous decade, by the administration of compounds labelled with radioactive tracers, such as ^{14}C-labelled amino acids, to laboratory rodents in vivo.[3] These had been extremely laborious studies, in which the metabolic fates of the radioactive carbon atom in the labelled compound were followed by separating, and identifying each radioactive product, and then decomposing them so that the specific activity of each of their carbon atoms could be measured. That had been a formidable undertaking, requiring a dedicated laboratory with a large team of skilled technical staff, so there was great interest when, in 1979, Shulman and his collaborators reported experiments in which the metabolism of ^{13}C alanine was monitored in perfused rat livers, effectively in real time.[4] Shulman's group went on to perform several similar metabolic studies on liver and other tissues,[5,6] and they also collaborated with Joseph Katz's group, which had

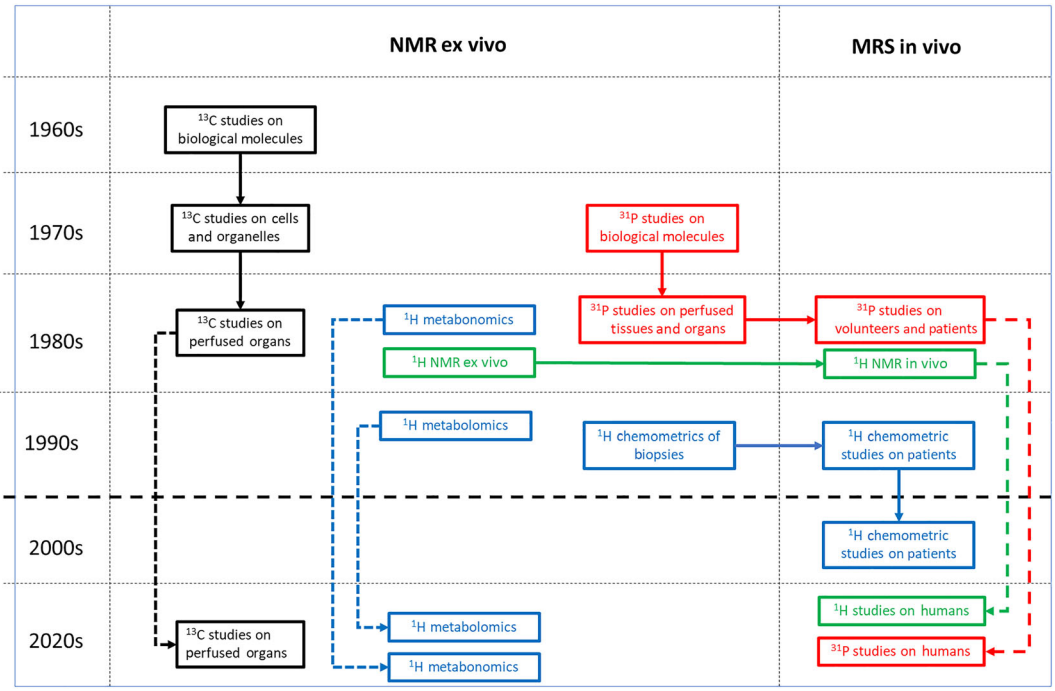

FIGURE 1 Simplified timeline of the development of ex vivo NMR methods. The dates indicate the approximate periods when the indicated methods were developed and extensively utilised ex vivo. Solid arrows indicate the utilisation of a method on progressively more 'biologically realistic' samples and eventually in vivo; dashed arrows indicate a method that has continued to be used ex vivo. MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance

performed much of the classic radiolabelled metabolite work, in a study demonstrating that the ^{13}C and ^{14}C tracer methods used by the two groups gave essentially identical results.[7]

Metabolic tracer studies using ^{13}C labels are still performed on biopsy samples ex vivo[8] (see Figure 1; broken lines indicate a technique that is still in use), but ^{13}C studies in vivo are now almost always performed using tracer compounds in which the ^{13}C signal has been enhanced by several orders of magnitude using hyperpolarisation techniques.[9]

3 | OTHER NUCLEI

3.1 | Choosing a nucleus to study

At the same time as these ^{13}C NMR methods were maturing, there were parallel developments on the study of biological samples with other nuclei. Most NMR instruments in chemistry departments were being used for ^{1}H work, so that initially seemed the most obvious nucleus to study. However, it was thought that ^{1}H NMR studies of living tissue, using the relatively inhomogeneous magnets available at that time, would be very challenging, and I was made fully aware of all these problems by several chemists whose instruments I requested to use in the 1970s. First, the signals from a large number of metabolites, each with several ^{1}H peaks, would have to be distinguished and assigned. Second, the ^{1}H chemical shift range is small, exacerbating the spectral overcrowding problem. To make matters even worse, the signals of the tissue metabolites, which are around 10^{-2} to 10^{-3} M in concentration, are superimposed on a $\sim$ 1 M rolling baseline signal from the protons in tissue proteins. And lastly, all of this jumble is superimposed on the ^{1}H signal from $\sim$80 M tissue water, creating a huge dynamic range problem. Probably for all of these reasons, ^{1}H NMR studies on living tissues were delayed until a new generation of more homogeneous magnets became available, and the main nucleus of interest in the early 1970s was ^{31}P.

3.2 | ^{31}P NMR of living cells ex vivo

As with the early ^{13}C studies, the initial ^{31}P work on living tissues was performed ex vivo (Figure 1). In 1973, Moon and Richards published a very influential paper,[10] in which they reported the use of ^{31}P MRS ex vivo to monitor phosphorus metabolites in living erythrocytes. The resulting spectra were good enough for them to identify the peaks of diphosphoglycerate, serum phospholipids, ATP and inorganic phosphate. They were also able to measure the intracellular pH of the erythrocytes from the chemical shift of the phosphate peak. The following year, Henderson and colleagues[11] showed that aged red blood cells could be induced to regenerate diphosphoglycerate when incubated with pyruvate and inosine.

These papers on erythrocyte NMR created much interest at the time, because they showed that ^{31}P NMR spectra of several metabolically important endogenous compounds could be detected within living cells, and also that their metabolism could be monitored in real time. The few peaks of high-concentration phosphate metabolites could be easily resolved, and several of them—the high-energy phosphate compounds ATP and phosphocreatine, as well as inorganic phosphate (Pi), their breakdown product—were important for energy metabolism. Simple metabolic perturbation studies could be performed by making the preparation hypoxic (initially, just by switching off the oxygen bubbling through the perfusion fluid) or inducing muscle contractions. The resulting changes in the peaks of nucleoside triphosphates and (in muscle) phosphocreatine, relative to that of inorganic phosphate, gave a simple readout of the state of cellular energy metabolism. Furthermore, as Moon and Richards[10] had shown, intracellular pH could be measured from the chemical shift of the intracellular Pi peak.

3.3 | Breaking new ground with ^{31}P NMR

^{31}P NMR also enabled studies on living tissues that were difficult, if not impossible, by other methods. In 1977, Truman Brown and Kamil Ugurbil, working in Shulman's laboratory, reported the use of saturation transfer methods to measure the unidirectional rate constant of phosphorus transfer between ATP and Pi in aerobic *Escherichia coli* cells.[12] A year later, Brown collaborated with several members of George Radda's group at Oxford University in another saturation transfer study that used ^{31}P NMR to analyse the kinetics of the creatine kinase reaction in perfused rat hearts.[13]

3.4 | ^{31}P NMR: from ex vivo towards in vivo

In 1974, a ^{31}P NMR study on a living tissue was performed in Oxford, in a collaboration between the research groups of George Radda and Rex Richards, using the latter group's newly completed, vertical-bore 7.5-T superconducting instrument. They obtained spectra ex vivo from excised

rat muscle; it was not perfused, so they were able to follow the falls in phosphocreatine and ATP, and the rise in Pi as it became hypoxic.[14] Two of the collaborators—David Hoult, who had built the 7.5-T instrument, and David Gadian—were to become well known in the field of biological MR. A comparative ^{31}P NMR study of frog, toad, abalone and human muscle was reported by Tyler Burt et al. in 1976.[15] Gadian subsequently collaborated with the physiologists Joan Dawson and Doug Wilkie in extensive ^{31}P NMR studies on perfused, excised muscles,[16,17] and similar work was performed in the same laboratory on perfused heart,[18] kidney[19] and liver.[20]

The use of NMR ex vivo on perfused cells, tissues and organs has a number of advantages, and this method is still in use (reviewed in[21]). However, the success of the early ^{31}P studies on tissues and organs immediately suggested the possibility of using that method to monitor the metabolism of whole animals, or even human subjects, once larger bore magnets became available. The direction of travel for ^{31}P studies, thereafter, was quite different from that of ^{13}C, because it soon led to studies on tissues and organs in living animals, and then on to studies on human volunteers and patients, the method that was to be called MRS.

4 | USE OF ^{1}H NMR, IN VIVO AND EX VIVO

It was not until the advent of more sophisticated instruments in the late 1970s that the many technical problems associated with obtaining ^{1}H NMR spectra of living tissues began to be addressed, and once again the initial work was performed on isolated cells (Figure 1). In 1977, Ian Campbell, Philip Kuchel and colleagues used ^{1}H spin echo NMR to study human erythrocyte metabolism.[22] They assigned resonances to purines, to oxidised and reduced glutathione (from which they followed changes in the redox status of the cells), and to lactate and pyruvate (which allowed assessment of glycolysis); they also measured intracellular pH from the chemical shift of the haemoglobin spectrum.

Another study, on membrane transport, was performed in Campbell's laboratory by Kevin Brindle and others.[23] They utilised extracellular Fe^{3+}, Mn^{2+} and Dy-DTPA to enhance the differential magnetic susceptibility between the intracellular and extracellular compartments. That work prophetically predated the use of paramagnetic agents in MRI for contrast enhancement, and it cites an even earlier paper[24] on water transport in erythrocytes that also utilised extracellular Mn^{2+}.

By the late 1980s, the more sophisticated research instruments that had become available also had large magnets that could accommodate whole animals or even humans. Consequently, most ^{1}H MRS research began to focus on methods that could be used in vivo, and although some early studies on solid tissues included data obtained both ex vivo and in vivo,[25] or were performed exclusively ex vivo,[26] there was no need for a prolonged preliminary period during which tiny tissue samples were studied.

5 | METABOLITE STUDIES EX VIVO

5.1 | Metabonomics

Beginning in the late 1980s, several long-lived methods that use ^{1}H NMR ex vivo were developed (Figure 1). The first was introduced by Jeremy Nicholson's group at Imperial College, London, which pioneered a method that they named 'metabonomics'. ^{1}H NMR was used ex vivo to monitor metabolic changes induced in biological systems by various perturbations, and mathematical methods were then applied to analyse the results.[27] The Nicholson group performed these studies in conventional, vertical-bore high-resolution NMR instruments, and in the early days they focused particularly on changes in body fluid metabolites and tissue extracts.[28] Their method was especially useful, therefore, for practical applications on topics such as toxicology and drug development,[29] where changes in the NMR spectrum of a body fluid can indicate the metabolic response of the animal or patient to a drug or toxin. Metabonomics has since been applied to numerous other systems, including microorganisms, with a particular focus on the intestinal microbiota.[30] In recent years, metabonomic studies of body fluids and tissue samples have also been used to improve the diagnosis of human diseases, such as liver.[31]

5.2 | Chemometrics

Some similar methods that used NMR ex vivo were developed, using different terminology (e.g., in the early 1990s, Sian Howells, who was then a student in my own laboratory, developed a method she called 'chemometrics',[32] a term that she had imported from chemistry). These approaches did not use the external perturbation that is fundamental to metabonomics. Instead, they focused on problems such as the diagnosis and prognosis of cancer, by developing methods for pattern recognition of the metabolite concentrations in tumour biopsies.[33] Another difference from metabonomics was that although they initially worked with biopsies, the long-term aim of these studies was their use with ^{1}H spectra of human cancers that were obtained in vivo.[34]

5.3 | Metabolomics

In the late 1990s, another alternative term—'metabolomics'—came into use.[35] This had a more general connotation than metabonomics: the metabolome is the totality of all the small-molecule metabolites in an organism, by analogy with its genome and proteome. Metabolomics, therefore, is the study of that system in totality, and once again NMR methods can have significant advantages. Because all the NMR-detectable metabolites can be quantified simultaneously in an extract of the original sample, with no further preparation or chemical derivatisation required, their relative concentrations can be measured with great precision. Furthermore, NMR is nondestructive, so the extracts can be retained and analysed by other methods. Compared with liquid chromatography–mass spectrometry, the other main technique in use for metabolomics, NMR is a simpler and more stable technique that yields peaks which are easily identified and quantified. However, NMR is much less sensitive, and detects relatively few metabolites. Nevertheless, the ^{1}H NMR method is still widely used ex vivo for studies that are nowadays generally termed 'metabolomics' (although some authors still use the term 'metabonomics'[31]).

5.4 | Medical uses of ^{1}H NMR ex vivo

In addition to metabonomics and metabolomics, the study of metabolites in body fluids for medical diagnosis is another strand that can be identified in the use of NMR—particularly ^{1}H NMR—ex vivo. There are important distinctions in the purpose of the studies between diagnostic NMR and metabolomics or metabonomics. Whereas the aim of metabolomics is essentially scientific, diagnostic studies on body fluids are aimed at medical utility. And although metabonomics can be used to study disease, it involves a deliberate perturbation of the system that is not usually necessary for diagnostic purposes.

Many body fluids are easy to monitor by ^{1}H NMR, as the metabolites are dissolved in a simple aqueous medium, giving a well-resolved spectrum with a flat baseline, and the myriad of metabolites that can be monitored makes subtle distinctions possible. Urine is particularly easy to study, and an early publication from Richard Iles and colleagues that focused on organic acidurias[36] led to many other studies on genetic disorders, both ex vivo and in vivo.[37] Recent NMR studies on urine have, for instance, identified biomarkers for the severity of Parkinson's disease,[38] early and late age-related macular degeneration,[39] and hepatocellular carcinoma.[40]

6 | HIGH-RESOLUTION MAGIC ANGLE SPINNING

All the applications discussed so far have used conventional solution-state NMR, but techniques for obtaining spectra from solid samples were also under development from the early days. In 1958, Raymond Andrew and colleagues first described 'magic angle' NMR for obtaining spectra from rapidly spinning crystals,[41] but it was not until the development of high-resolution magic angle spinning (HR-MAS) methods in the 1990s[42] that it became possible to obtain high-resolution spectra from biological samples. Compared with NMR spectroscopy of chemical extracts, HR-MAS has the advantage that the sample remains intact, so it can be used for further studies by other methods.

7 | THE PRESENT DAY

The use of NMR ex vivo continues to provide important results, as one can do things to an isolated sample that would be impractical or unethical with a patient, or even with an intact, living organism. Metabonomics and metabolomics continue to use NMR methods, and diagnostic ^{1}H NMR of body fluids is actively studied. This special issue discusses the state of the art in this important field.

ORCID

John R. Griffiths https://orcid.org/0000-0001-7369-6836

REFERENCES

1. Sequin U, Scott AI. Carbon-13 as a label in biosynthetic studies. *Science*. 1974;189:101-107. doi:10.1126/science.186.4159.101
2. Eakin RT, Morgan O, Gregg CT, Matwiyoff NA. Carbon-13 nuclear magnetic resonance spectroscopy of living cells and their metabolism of a specifically labeled ^{13}C substrate. *FEBS Lett*. 1972;28:259-264. doi:10.1016/0014-5793(72)80726-9
3. Katz J, Rongstad R. The labelling of pentose phosphate from glucose-^{14}C and estimation of the rates of transaldolase, transketolase, the contribution of the pentose cycle, and ribose phosphate synthesis. *Biochemistry*. 1967;6:2227-2247. doi:10.1021/bi00859a046
4. Cohen SM, Shulman RG, McLaughlin AC. Effects of ethanol on alanine metabolism in perfused mouse liver studied by ^{13}C NMR. *Proc Natl Acad Sci U S A*. 1979;76:4808-4812. doi:10.1073/pnas.76.10.4808

5. Cohen SM, Shulman RG. ^{13}C N.M.R. studies of gluconeogenesis in rat liver suspensions and perfused mouse livers. *Philos Trans R Soc Lond B Biol Sci*. 1980;289:407-411. doi:10.1098/rstb.1980.0057
6. Cohen SM, Glyn P, Shulman RG. ^{13}C NMR study of gluconeogenesis from labeled alanine in hepatocytes from euthyroid and hyperthyroid rats. *Proc Natl Acad Sci U S A*. 1981;78:60-64. doi:10.1073/pnas.78.1.60
7. Cohen SM, Rognstad R, Shulman RG, Katz J. A comparison of ^{13}C nuclear magnetic resonance and ^{14}C tracer studies of hepatic metabolism. *J Biol Chem*. 1981;256:3428-3432. doi:10.1016/S0021-9258(19)69626-2
8. Jalloh I, Carpenter KL, Helmy A, Carpenter TA, Menon DK, Hutchinson PJ. Glucose metabolism following human traumatic brain injury: methods of assessment and pathophysiological findings. *Metab Brain Dis*. 2015;30:615-632. doi:10.1007/s11011-014-9628-y
9. Cho A, Lau JYC, Geraghty BJ, Cunningham CH, Keshari KR. Noninvasive interrogation of cancer metabolism with hyperpolarized ^{13}C MRI. *J Nucl Med*. 2017;58:1201-1206. doi:10.2967/jnumed.116.182170
10. Moon RB, Richards JH. Determination of intracellular pH by ^{31}P magnetic resonance. *J Biol Chem*. 1973;248:7276-7278. doi:10.1016/S0021-9258(19)43389-9
11. Henderson TO, Costello AJR, Omachi A. Phosphate metabolism in intact human erythrocytes: determination by phosphorus-31 nuclear magnetic resonance spectroscopy. *Proc Natl Acad Sci U S A*. 1974;71:2487-2490. doi:10.1073/pnas.71.6.2487
12. Brown TR, Ugurbil K, Shulman RG. ^{31}P nuclear magnetic resonance measurements of ATPase kinetics in aerobic Escherichia coli cells. *Proc Natl Acad Sci U S A*. 1977;74:5551-5553. doi:10.1073/pnas.74.12.5551
13. Brown TR, Gadian DG, Garlick PB, Radda GK, Seeley PJ, Styles P. Creatine kinase activities in skeletal and cardiac muscle measured by saturation transfer NMR. In: Dutton L, Leigh J, Scarpa A, eds. *Frontiers of Biological Energetics: Electrons to Tissues*. Academic Press; 1978:1341-1351.
14. Hoult DI, Busby SJW, Gadian DG, Radda GK, Richards RE, Seeley PJ. Observation of tissue metabolites using ^{31}P nuclear magnetic resonance. *Nature*. 1974;252:285-287. doi:10.1038/252285a0
15. Burt CT, Glonek T, Barany M. Analysis of phosphate metabolites, the intracellular pH, and the state of adenosine triphosphate in intact muscle by phosphorus nuclear magnetic resonance. *J Biol Chem*. 1976;251:2584-2591. doi:10.1016/S0021-9258(17)33527-5
16. Dawson MJ, Gadian DG, Wilkie DR. Contraction and recovery of living muscles studies by ^{31}P nuclear magnetic resonance. *J Physiol*. 1977;267:703-735. doi:10.1113/jphysiol.1977.sp011835
17. Dawson MJ, Gadian DG, Wilkie DR. Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature*. 1978;274:861-866. doi:10.1038/274861a0
18. Garlick PB, Radda GK, Seeley PJ, Chance B. Phosphorus NMR studies on perfused heart. *Biochem Biophys Res Commun*. 1977;74:1256-1262. doi:10.1016/0006-291X(77)91653-9
19. Sehr PA, Radda GK, Bore PJ, Sells RA. A model kidney transplant studied by phosphorus nuclear magnetic resonance. *Biochem Biophys Res Commun*. 1977;77:195-202. doi:10.1016/S0006-291X(77)80182-4
20. Iles RA, Griffiths JR, Stevens AN, Gadian DG, Porteous R. Effects of fructose on the energy metabolism and acid-base status of the perfused starved rat liver. A ^{31}P NMR study. *Biochem J*. 1980;192:191-202. doi:10.1042/bj1920191
21. Milkevich M, Browning EA, James E. MRS of perfused cells, tissues and organs. In: Bottomley PA, Griffiths JR, eds. *Handbook of Magnetic Spectroscopy in Vivo: MRS Theory, Practice and Applications*. John Wiley & Sons; 2016:601-617.
22. Brown FR, Campbell ID, Kuchel PW, Rubenstein DC. Human erythrocyte metabolism studies by ^{1}H spin echo NMR. *FEBS Lett*. 1977;82:12-16. doi:10.1016/0014-5793(77)80875-2
23. Brindle KM, Brown FF, Campbell ID, Grathwohl C, Kuchel PW. Application of spin-echo nuclear magnetic resonance to whole-cell systems. Membrane transport. *Biochem J*. 1979;180:37-44.
24. Conlon T, Outhred R. Water diffusion permeability of erythrocytes using an NMR technique. *Biochim Biophys Acta*. 1972;288:354-361. doi:10.1016/0005-2736(72)90256-8
25. Petroff OAC, Spencer DD, Alger JR, Prichard JW. High-field proton magnetic resonance spectroscopy of human cerebrum obtained during surgery for epilepsy. *Neurology*. 1989;39(9):1197-1202. doi:10.1212/WNL.39.9.1197
26. Behar KL, Den Hollander JA, Stromski ME, et al. High-resolution ^{1}H nuclear magnetic resonance study of cerebral hypoxia in vivo. *Proc Natl Acad Sci U S A*. 1983;80:4945-4948. doi:10.1073/pnas.80.16.4945
27. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': Understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica*. 1999;29:1181-1189. doi:10.1080/004982599238047
28. Beckonert O, Keun HC, Ebbels TMD, et al. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat Protoc*. 2007;2:2692-2703. doi:10.1038/nprot.2007.376
29. Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. *Nat Rev Drug Discov*. 2002;1:153-161. doi:10.1038/nrd728
30. Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. *Science*. 2012;336:1262-1267. doi:10.1126/science.1223813
31. Yu M, Zhu Y, Cong Q, Wu C. Metabonomics research progress on liver diseases. *Can J Gastroenterol Hepatol*. 2017;2017:1-10. doi:10.1155/2017/8467192
32. Howells SL, Maxwell RJ, Peet AC, Griffiths JR. An investigation of tumour ^{1}H nuclear magnetic resonance spectra by the application of chemometric techniques. *Magn Reson Med*. 1992;28:214-236. doi:10.1002/mrm.1910280205
33. Griffiths JR. A multicentre trial on MR spectroscopy of human tissue biopsies. *Europsin Annual*. 1996;19:295-299.
34. Julià-Sapé M, Griffiths JR, Tate RA, et al. Classification of brain tumours from MR spectra: the INTERPRET collaboration and its outcomes. *NMR Biomed*. 2015;28:1772-1787. doi:10.1002/nbm.3439
35. Raamsdonk LM, Teusink B, Broadhurst D, et al. A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat Biotechnol*. 2001;19:45-50. doi:10.1038/83496
36. Iles RA, Hind AJ, Chalmers RA. Use of proton nuclear magnetic resonance spectroscopy in detection and study of organic acidurias. *Clin Chem*. 1985;31:1795-1801.
37. Iles RA. Nuclear magnetic resonance spectroscopy and genetic disorders. *Curr Med Chem*. 2008;15:15-36. doi:10.2174/092986708783330719
38. Kumari S, Kumaran SS, Goyal V, et al. Identification of potential urine biomarkers in idiopathic Parkinson's disease using NMR. *Clin Chim Acta*. 2020;510:442-449. doi:10.1016/j.cca.2020.08.005

39. Laíns I, Duarte D, Barros AS, et al. Urine nuclear magnetic resonance (NMR) metabolomics in age-related macular degeneration. *J Proteome Res*. 2019; 18(3):1278-1288. doi:10.1021/acs.jproteome.8b00877
40. Cartlidge CR, Alkhatib AM, Taylor-Robinson SD. The utility of biomarkers in hepatocellular carcinoma: review of urine-based (1)H-NMR studies - what the clinician needs to know. *Int J Gen Med*. 2017;10:431-442. doi:10.2147/IJGM.S150312
41. Andrew ER, Bradbury A, Eales RG. Nuclear magnetic resonance spectra from a crystal rotated at high speed. *Nature*. 1958;182:1659. doi:10.1038/1821659a0
42. DeFeo EM, Cheng LL. Characterizing human cancer metabolomics with ex vivo ^{1}H HRMAS MRS. *Technol Cancer Res Treat*. 2010;9(4):381-391. doi:10.1177/153303461000900407

How to cite this article: Griffiths JR. Magnetic resonance spectroscopy ex vivo: A short historical review. *NMR in Biomedicine*. 2022; e4740. doi:10.1002/nbm.4740