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THE DEVELOPMENT OF MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY

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A lifetime with magnetic resonance

Part 1. Exciting times – the dawn of tissue MRS

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Introduction

I am writing this memoir almost 50 years after the publication in *Nature* of our discovery that we could use ^{31}P NMR spectroscopy* to detect metabolites in intact muscle (1). I was in the final year of my DPhil when we carried out this work. It was an exciting time for us, and I'd like to tell the story, from my perspective, of how this all came about.

I have referred back wherever possible to near-contemporary material, including the paper itself (1), as well as my DPhil Thesis (2), written a few months after the experiments were carried out, and Steve Busby's DPhil thesis (3), which was written in the summer of 1975. In addition, I mention reminiscences published in the 1990s (4). I have also had invaluable input from three of the other authors of our *Nature* paper – Steve Busby, John Seeley, and David Hoult – as well as another member of the team, John Griffiths. And I refer to another paper from my time as a DPhil student (5), a paper that faded rather rapidly into obscurity but that does have a tale to tell.

*****For studies of tissue metabolism, the acronym MRS (magnetic resonance spectroscopy) now tends to be used rather than NMR (nuclear magnetic resonance) spectroscopy; here I use the contemporaneous terminology.

Background - Rex Richards and George Radda

The experiments that were published in the *Nature* paper involved close collaboration between two research groups, one led by Rex Richards and the other by George Radda. I think it would be helpful to begin by describing how this collaboration came about. As with many aspects of the story, there was a considerable element of serendipity. Rex was a physical chemist who

had a stellar rise through Oxford academia (6). In the late 1940s, not long after the pioneering NMR experiments of the Bloch and Purcell groups, Rex had foreseen that NMR might play an important role in chemistry – at the time the technique was firmly in the grip of physicists. So he embarked on a career that involved the design and building of NMR spectrometers as well as their use in solving chemical problems. His 1951 paper on acid hydrates with his student John Smith was arguably the first in which a chemical problem was solved by NMR (7). Rex rapidly rose up the ranks and, in 1964, while still in his early 40s, he was appointed Dr Lee's Professor of Chemistry, a post that was associated with a departmental role as head of the Physical Chemistry Laboratory in Oxford.

Alongside his scientific achievements, Rex's administrative skills became widely recognised, and in 1968 he was invited to be a candidate for the wardenship of Merton College, Oxford. He realised that, if appointed, he would have less time for research and would have to stand down from his professorship in physical chemistry. But he recognised that it might provide an excellent opportunity for him to move into a research area that he had been considering for some time, namely biological applications of NMR. So he applied for the wardenship, was duly appointed, and by 1970 had relocated his lab to the Department of Biochemistry, courtesy of Rodney Porter, who was the Head of Department at the time. In his memoirs (8), Rex spoke highly of Porter's generosity and vision in facilitating this move.

One of the young lecturers in Rodney Porter's department was George Radda. George had escaped from Hungary in 1956, and on arriving in the UK had been awarded a place as a chemistry student at Merton College. Within ten years, while still in his 20s, he was appointed tutor and fellow at Merton and lecturer in the Department of Biochemistry. By 1970, when Rex relocated to the Biochemistry Department, George had built up a large research group in the department, with a focus on bioenergetics and the structure and function of glycolytic enzymes. This was one component of a much larger interdisciplinary research programme within Oxford investigating glycolytic enzymes, a programme that led to the establishment of the Oxford Enzyme Group. George was a prominent member of this group and Rex was its chairman. Rex's links with George, via the Biochemistry Department, Merton College and the Oxford Enzyme Group, led to their two teams working together on NMR approaches to the investigation of glycolytic enzymes. They also established a productive collaboration studying membrane phospholipids, though this does not form part of the present story.

The NMR spectrometers

Rex's group moved to the Biochemistry Department with two NMR spectrometers, somewhat unimaginatively known as *Mark 1* and *Mark 2*. To give some background, in 1959 Martin Wood of the Clarendon Laboratory in Oxford had established a spin-off company, Oxford Instruments Ltd, to design and manufacture magnets for academia. When superconducting technology became available, Martin immediately saw its potential. He and Rex set up close links, and Rex obtained funding from the Science Research Council for two high-homogeneity superconducting magnets that Oxford Instruments would build The first, operating at 5T and built with niobium/zirconium wire, was delivered to Rex's laboratory in Physical Chemistry in 1966, while the second was delivered a couple of years later. It was a larger system, operating at 7.5T, and was built with niobium/titanium wire, which is much more stable than niobium/zirconium and works at higher fields. Rex's team built the two spectrometers incorporating these magnets. *Mark 1*, the 5T system, was more-or-less fully operational by the time I joined the group, though it was somewhat limited in capability because of the relatively modest sample size and field homogeneity. *Mark 2*, although it was superior in principle, seemed to have perennial problems with field homogeneity, more of which later!

How I joined Rex's lab

I entered Merton as a physics undergraduate in 1968. It was a wonderful place to be a student – academically very strong, with excellent physics tutors, socially very easy-going, and lots of sporting activities. My own enthusiasm was for soccer, and perhaps my main college achievement was that, under my captaincy, we won the inter-college league championship (Fig. 1).

Figure 1. Merton College AFC 1970-71

Time seemed to pass by very quickly, and around half-way through my final (third) year, I realised that I needed to consider what to do next. The easy option was to stay on for DPhil research in some aspect of physics, but none of the options that I looked at was particularly appealing to me. I wondered whether I might be able to use my physics instead in some area of biology or medicine, and I approached Rex, my head of college, for advice. Rex suggested that I might consider joining his group in the Department of Biochemistry, or that alternatively I might be interested in X-ray crystallography of proteins, a field of research in which Oxford excelled. So I went to see Professor Sir David Phillips, the head of the crystallography team, who was also very welcoming. Without knowing very much at all about either NMR or X-ray crystallography, I felt that NMR was more appealing, if only because it seemed to involve a more flexible approach to research. So in October 1971 I joined Rex's lab as a DPhil student.

The topics suggested for my DPhil research

Although Rex was formally my supervisor, he was very busy with other matters too, including his wardenship of Merton, and on a more day-to-day basis the physics components of my doctoral work were overseen by David Hoult. David had joined Rex's group as a DPhil student just 3 years earlier, but he was rapidly gaining a reputation as an exceptionally bright innovator in NMR physics and technology. Many of the readers of this article will be aware of the seminal papers he published in the 1970s, and I was extremely fortunate to be able to work with and learn from him, even if at times it was somewhat dispiriting for me to struggle with concepts that seemed to come so easily to him. I was set to work on two main topics that occupied much of David's stream of thought, namely the design of radiofrequency coils suitable for use with superconducting magnets, as well as analyses of a new system of field-correcting shim coils that David had designed.

There was also a biochemical component to my research, the subject matter of which was suggested to me by George Radda. He proposed that I carry out research on the glycolytic enzyme phosphoglucomutase. This is the enzyme that interconverts glucose 1-phosphate and glucose 6-phosphate. I must say that this seemed to be one of the duller of the glycolytic enzymes (unlike, for example phosphorylase and phosphofructokinase, which have strong regulatory roles within the glycolytic pathway) which may perhaps explain why I, a mere physicist, was given access to it. If I were to make mistakes, who would notice, and would it matter too much? One of the notions was that I would examine aspects of the structure of phosphoglucomutase by using NMR to measure distances between a manganese (Mn^{2+}**)** binding site and the substrate binding site. This type of NMR study, namely the use of paramagnetic ions to probe molecular structure, was very much in vogue at the time. George also suggested that I might assay the enzyme by using NMR to monitor the time-course of conversion of glucose 1-phosphate to glucose 6-phosphate. Actually, this seemed a rather daft thing to do, as NMR was so much more expensive and less sensitive than the standard spectrophotometry that was routinely used for enzyme assays. And yet, in the end, it didn't turn out to be quite so daft …

I should add here that the team had decided – serendipitously as it would transpire - to focus primarily on ^{31}P NMR rather than on the more prevalent approaches using ^{1}H NMR. This was partly because of the biological relevance of phosphorus-containing compounds, but to a large

extent because at the time the field homogeneity of the two spectrometers in Rex's lab was inadequate for state-of-the-art high-resolution ^{1}H NMR spectroscopy.

My DPhil thesis

I shall use my DPhil thesis as the template for my story. The thesis was submitted in November 1974, and begins with a dedication to my parents and to Ro. Ro was my girl-friend – we had met a couple of years earlier when she joined Rex's group as a technician during her gap year between school and entry to Oxford as a biochemistry student. We are still together 50 years later!

NMR sensitivity and field homogeneity

My thesis had a general introduction followed by the experimental chapters (Chapters 1-5) and three appendices. Chapter 1 and Appendices 1 and 2 provide accounts of my physics work overseen by David Hoult. I learned hugely from this work, and it occupied a large portion of my time as a DPhil student. There were two main components to this work. The first, to quote from my thesis, was to use David Hoult's principle of reciprocity 'to design r.f. coils suitable for superconducting magnet systems which would optimise the signal to noise ratio, while maintaining the resolution and the amplitude of the spinning sidebands at a manageable level.' On the basis of computer simulations, we concluded that saddle-shaped coils provide an effective and highly satisfactory means of transmitting power into a sample and receiving the resulting signal. Among the results, it was found that the B1 field homogeneity goes through a sharp maximum at coil angular widths of 120^o, in excellent agreement with theoretical predictions. One of the key conclusions when designing saddle-shaped coils was therefore that they should have an angular width of 120^o.

The second section of Chapter 1 was involved with analyses of the effectiveness of a new type of B_0 field-correcting coils that David had designed. Following a not inconsiderable amount of coil-winding, the design was put into practice and operated successfully on *Mark 2*. Nevertheless, my recollection is that, for much of my time as a DPhil student, *Mark 2* was pretty unreliable if one needed good field homogeneity. It transpired that events over Christmas 1973 would reveal a possible source of this unreliability.

I won't dwell any further on the physics/technical aspects of my thesis, other than to say that the words 'field homogeneity' will remain forever etched in my memory. Instead, I'll move on to the subsequent chapters of my thesis, which deal with the biochemical components of my research.

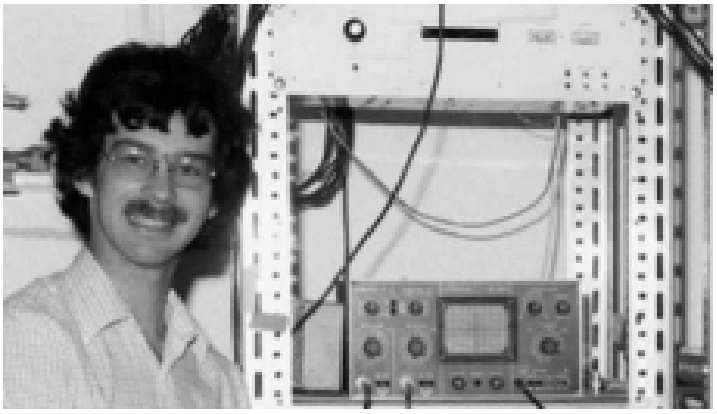

Figure 2. In the lab.

Phosphoglucomutase and enzyme-bound phosphate groups

I will skip Chapter 2 for now, as chronologically it fits in rather better with a later part of the story. Instead, I will write a little about the biochemistry work I carried out prior to the end of 1973. Most of this involved the enzyme phosphoglucomutase. This enzyme catalyses the inter-conversion of glucose 1-phosphate and glucose 6-phosphate and requires bivalent metal ions for activity. The most effective metal ion is Mg^{2+}, but among the others that provide some activity is Mn^{2+}. My initial objective was to examine the relaxation that Mn^{2+} confers upon the substrate, in an attempt to determine the relative positions of the metal and substrate binding sites and hence to obtain a better understanding of the role of the metal ion in the reaction mechanism. My findings were described in Chapter 3. They were published in 1974 (5), but it is fair to say that, outside the somewhat modest group of phosphoglucomutase enthusiasts (and possibly even within this small group), the paper drew relatively little attention worldwide, with 13 citations to date according to Google Scholar. This is a shame, for included within this paper were some additional observations, namely the use of ^{1}H and ^{31}P NMR to monitor, in real time, the conversion of glucose 1-phosphate to glucose 6-phosphate (see Figs. 3 and 4). The paper was submitted in January 1974, and includes the following statements:

‘The NMR assay requires about ten times the quantity of enzyme and substrate as the coupled and colorimetric assays, but it does have several advantages over the conventional assays. Firstly, no chemical modification is required, so the assay is totally non-destructive. Secondly, since the NMR assay does not perturb the reaction, the reaction may be followed as it proceeds. This can be done by the coupled assay, but not by the colorimetric method. Finally, whereas the colorimetric and coupled assays distinguish indirectly between glucose l-phosphate and glucose 6-phosphate, and measure the concentration of only one substrate, the NMR method gives a direct, completely unambiguous way of observing both substrates simultaneously.

‘Since the NMR assay is capable of distinguishing between many substrates, it could be very useful in studying multienzyme systems, where it may be impossible by any other means to observe all the substrates.’

I mention this because it is indicative of the way we had been thinking about metabolic studies, some time before the first muscle spectra were envisaged.

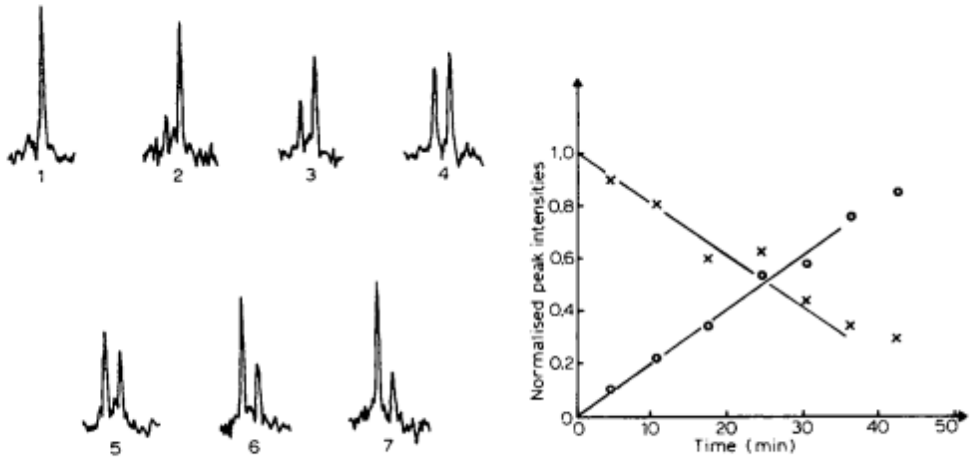

Figure 3. Assay of phosphoglucomutase by phosphorus NMR. The left-hand or low-field peak in each spectrum is the glucose 6-phosphate resonance, the upfield peak the glucose l-phosphate resonance. The enzyme was preincubated in 2 mM $MgCl_2$, 1 mM EDTA, 100 mM imidazole, 100 mM Tris, pH 7.5, and added, at a final concentration of 6.2 µg/ml to a solution containing 100 mM glucose l-phosphate, 100pM glucose 1,6-bisphosphate, 5 mM $MgCl_2$, 1 mM EDTA, 50 mM imidazole in 100 mM Tris, pH 7.5. Temperature 19.5 °C. x, glucose 1-phosphate; o, glucose 6-phosphate. Reproduced with permission from ref (5), Copyright Elsevier.

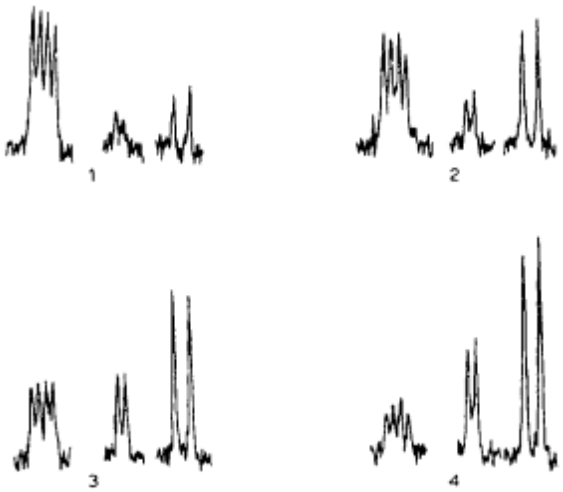

Figure 4. Various stages in the conversion of glucose 1-phosphate to glucose 6-phosphate as observed by proton NMR. Each spectrum shows the quartet of glucose 1-phosphate, and the two doublets of glucose 6-phosphate. Reproduced with permission from ref (5), Copyright Elsevier.

As an aside, it is worth noting that the ^{31}P spectra were recorded on *Mark 1*; *Mark 2* was evidently still unreliable at the time. The ^{1}H spectra were recorded on the Oxford Enzyme Group's Bruker 270 MHz system, which had the necessary spectral resolution.

NMR studies of enzyme-bound phosphate groups

In Chapter 4 of my thesis, I went on to describe NMR studies of enzyme-bound phosphate groups. For this work, we were able to use *Mark 2*, which was fine for broad signals, such as those of enzyme-bound phosphate groups, because it offered higher sensitivity than *Mark 1* and its homogeneity issues were not relevant. Observations were carried out of three glycolytic enzymes, namely phosphoglucomutase, phosphorylase and glyceraldehyde 3-phosphate dehydrogenase. These studies did not prove particularly conclusive, but I mention them for completeness and because these studies cemented my collaborations with Steve Busby, who was working on phosphorylase and glycogen particles, and with John Seeley, who was working on glyceraldehyde 3-phosphate dehydrogenase. Steve and John were two extremely bright students working in George Radda's lab. Both had Natural Sciences degrees from Cambridge, which evidently prepared them well for interdisciplinary research such as ours. Our collaboration was crucial to the next phase of our research.

Christmas vacation 1973

Before moving on to this next phase, I must recount what happened over Christmas 1973. I will draw on my recollections as told at a Witness Seminar held at the Wellcome Institute for the History of Medicine, London, on 2 July 1996. The following is taken from the transcript.

*'**Professor David Gadian**: Just another anecdote about Oxford around 1973– 1974. It was, I believe, Christmas 1973 when I was looking after the magnet. It had to be filled with helium every three or four days in those days and we had awful problems with the field homogeneity. I remember coming in over the Christmas vacation and trying to shim the magnet yet again and having big problems. I was adjusting the superconducting shims, because we weren't making very much progress with the room temperature shims. It was Christmas and I went off somewhere, came back on the following day and saw a touch of ice and whatnot around the magnet. What I had done was to forget to switch off one of the superconducting shim coils and the magnet had quenched; it had run out of helium. I was somewhat embarrassed about all of this, in fact more than embarrassed, I was desolate, I was quite young. Anyway, I was embarrassed to phone Rex [Richards] over the Christmas holidays, but I did so, and I remember him coming in, I think late 30 December or 1 January. Whenever it was, he took the magnet to bits, found that in fact the reason why we'd been having problems shimming the magnets was that one of the superconducting shims wasn't working. We got it working, connected it all up, and shortly afterwards as a result of this the spectroscopy was much improved because of the rewiring of the superconducting shim. I think that was the start of a rather golden period in the development of the phosphorus spectroscopy at Oxford.*

***Richards**: I think it was Christmas day actually!*

***Gadian**: It was pretty awful anyway.'*

(Transcript reproduced from ref 4, pp28-29:)

1974 and the first muscle spectra

The events over Christmas 1973 proved to be transformational, for *Mark 2* now had the capabilities we had been hoping for, and this heralded the start of a new phase of our research. We realised that we could use ^{31}P NMR to monitor metabolism in multienzyme systems, we had set up all the appropriate collaborations among us as physicists, chemists and biochemists, and *Mark 2* – our high-field (7.5T) spectrometer - had the sensitivity and spectral resolution needed for NMR work of this type. In addition, a seminal ^{31}P NMR paper had been published from Fred Richards' lab in Yale. This paper (9) provided a foretaste of things to come by reporting on 2,3-diphosphoglycerate metabolism in intact erythrocytes and showing that the chemical shifts of this metabolite could be used to monitor the intracellular pH.

At the time, Steve was working with John Griffiths, another of George's talented DPhil students, on mechanisms underlying activation of the enzyme phosphorylase which, incidentally, is the enzyme that generates the substrate for phosphoglucomutase. In muscle cells, most phosphorylase is bound to the glucose-storage polymer glycogen in structures known as glycogen particles, and so Steve and John had chosen to investigate phosphorylase in the glycogen particle environment. John's main focus was on the use of ESR as a probe of phosphorylase activation, and he had noticed that, in order to interpret the ESR signal of spin-labelled phosphorylase in the presence of the particles, he needed information about the concentrations of a number of metabolites that influenced phosphorylase activity. And so we embarked on a three-way collaboration; Steve prepared the particles and measured phosphorylase activity, John made the ESR measurements and I carried out ^{31}P NMR experiments to look at how various metabolite signals evolved during transient activation of the phosphorylase (ref 10; see also John's reminiscences in ref. 4). Figure 5 shows a typical timecourse of metabolic changes that we were able to detect by ^{31}P NMR.

In parallel with these studies, John Seeley and I carried out ^{31}P NMR experiments on crude extracts of homogenised muscle. These were preparations that included the glycolytic enzymes and, in an analogous manner to the glycogen particle studies, we were able to show how glycolytic activity could be monitored in real time (Fig. 6). To aid spectral assignments and to permit measurement of properties such as sample pH and binding of Mg^{2+} to ATP, the two of us also worked feverishly on characterising the chemical shifts of the relevant ^{31}P resonances, looking in detail at their dependence on a number of factors including pH, ionic strength, tem-

perature and metal ion binding (Fig. 7). This characterisation work is described in Chapter 2 and Appendix 3 of my thesis.

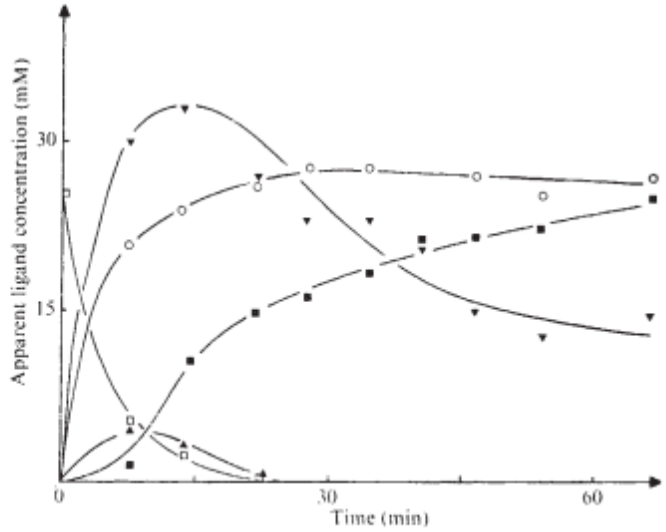

Figure 5. Variations in concentrations of □, ATP; ▲ ADP; ○, AMP and IMP; ■, glucose 6-phosphate; and ▼, inorganic phosphate during flash activation of phosphorylase in glycogen particles (27 mM ATP, 25 mM $MgCl_2$, 1mM $CaCl_2$, 50 mM triethanolamine, 100 mM potassium chloride, 1 mM EDTA, pH 6.9). ATP was added at time zero. Sweep width 5 kHz, pulse interval 3 s, 50 scans. Spectrum recorded without proton irradiation. Differential saturation of the resonances can lead to small errors in the relative concentrations of ligands as measured from the areas under the peaks. The measured concentration of any ligand, however, is always a fixed proportion of its true concentration. Reproduced from ref (1), Springer Nature.

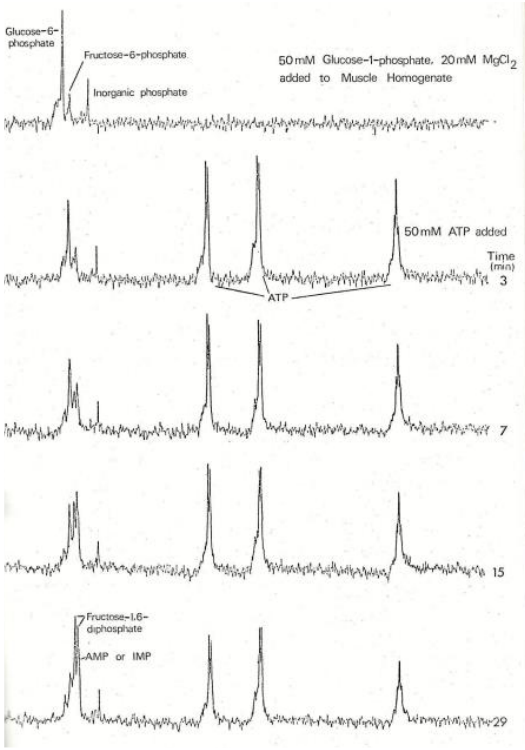

Figure 6. Spectra showing glycolysis in a crude muscle extract. Glycolysis was initiated by the addition of 50mM glucose 1-phosphate and 20mM $MgCl_2$. The first spectrum shows the formation of glucose 6-phosphate and fructose 6-phosphate as a result of phosphoglucomutase and phosphoglucoseisomerase activity. Following the addition of 50mM ATP, the next four spectra show the gradual formation of fructose 1-6-diphosphate and AMP or IMP. Reproduced from ref (2).

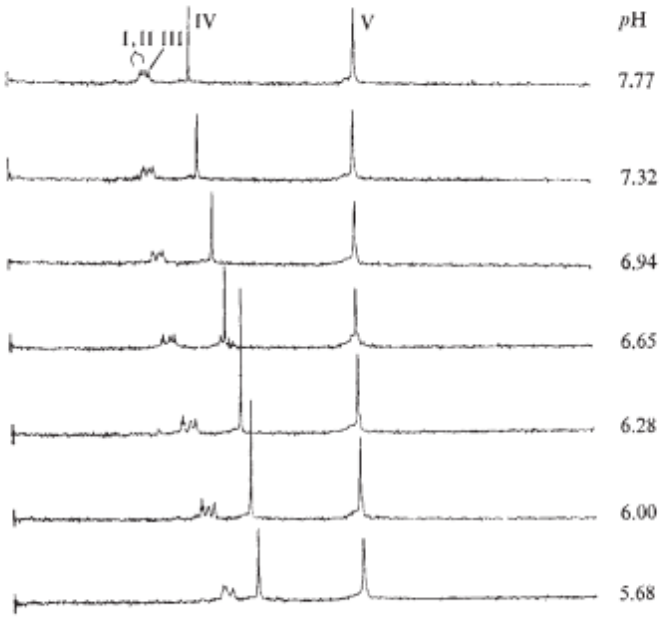

Figure 7. ^{31}P NMR spectra of a mixture of fructose 1- 6-diphosphate (I, II), IMP (III), inorganic phosphate (IV), creatine phosphate (V) at various pH values, recorded at 129 MHz. No buffer present; total phosphate concentration 60 mM. Sweep width 5 kHz, pulse interval 2 s, 200 scans. Spectrum recorded without proton irradiation. Reproduced from ref (1), Springer Nature.

The experiments on glycogen particle preparations and crude muscle extracts proved very fruitful. However, it was not an obvious step to extend this work, which was carried out on relatively conventional liquid samples, to studies of intact tissue. Why was it not obvious? Perhaps it was more of a step for an NMR specialist than for a biochemist. From an NMR perspective, a major conceptual difference is that a muscle is more solid and less homogeneous than a liquid sample, and as such might be expected to give broader signals, thus limiting spectral resolution. From a biochemical standpoint, the wide range of metabolites present within a muscle tissue might make it difficult to separate out the metabolites of interest. Nevertheless, one of us evidently suggested having a go. Who was it? George Radda says it was Steve (4,11), Steve doesn't remember, I don't remember, John Seeley doesn't remember, while David Hoult (12) defers to George's view on the matter and adds that he (i.e. David) disagreed that field homogeneity would be compromised.

Putting all of the accounts together, I come to the following plausible view as to what happened. A number of us were in the tea-room of the Biochemistry Department when Steve Busby made the throw-away remark that we should try looking at an intact muscle. I suspect he

felt that if we wanted to look at the combined effects of lots of muscle enzymes, why bother with preparing an extract? Why not try the whole muscle? There was a varying level of scepticism among us, but it was decided to have a go. So shortly afterwards (in March 1974 according to Steve's thesis), when Steve was preparing rabbit muscle extracts and glycogen particles for other experiments with John Griffiths, he carefully excised intact muscle tissue. We loaded it into an NMR tube, and it revealed the now familiar signals of inorganic phosphate, creatine phosphate (phosphocreatine), and the three phosphates of ATP.

This first experiment is described in Chapter 5 of my thesis as follows: 'During the preparation of a crude rabbit muscle extract for use in experiments on glycolysis, it was decided to put aside an intact muscle in the hope that it might produce a ^{31}P spectrum of interest. While the rest of the excised muscle was being homogenised, the intact muscle was cajoled into an NMR tube and an accumulation was started. Somewhat surprisingly, after a couple of minutes a free induction decay appeared above the noise, and on Fourier transformation five resonances were observed, one of them fairly sharp. Clearly, there were exciting prospects of being able to study intact tissue by ^{31}P NMR …'.

We subsequently decided to switch from rabbit muscle to muscle taken from the hind leg of the rat. Quoting from my thesis again: 'The reasons for studying this particular muscle are that it can be easily excised without damage, that it fits snugly into the NMR sample tubes, and that it produces very good NMR spectra….The muscles were excised from rats killed by etheration, bathed in Locke's saline, and placed as rapidly as possible into an NMR tube, care being taken to ensure that they were not damaged and were maintained in the relaxed state throughout'.

These experiments showed that we could use ^{31}P NMR to detect signals from ATP, phosphocreatine and inorganic phosphate in intact muscle (Fig. 8), and that we could follow time-dependent changes in these metabolites associated with ageing of the muscle (Figs. 8 and 9). We were also able to monitor changes in pH via the chemical shift of the inorganic phosphate signal, and to conclude that the ATP was predominantly complexed to Mg^{2+}. Our findings opened up a new area of research, namely the study of tissue metabolism *in vivo* using NMR spectroscopy (nowadays commonly referred to as MRS) - research that continues to this day.

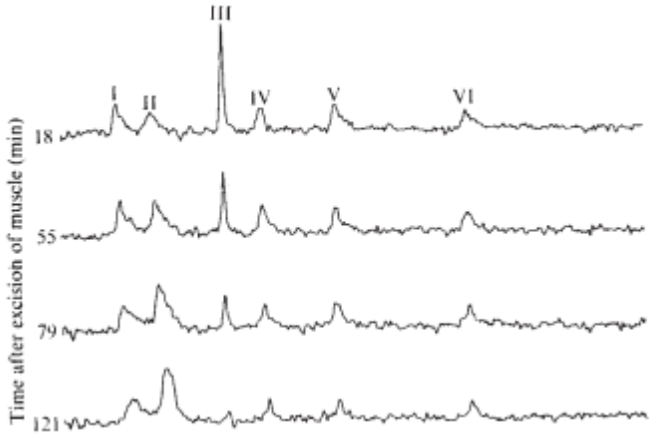

Figure 8. ^{31}P NMR spectrum of an intact muscle from the hind leg of the rat recorded at 129 MHz, without proton irradiation. Temperature 20° C and pulse interval 16 s. Peak assignments: I, sugar phosphate and phospholipid; II, inorganic phosphate; III, creatine phosphate; IV, γ ATP; V, α ATP; VI, β ATP. The times are midpoints of the 50 scan spectral accumulations (referred to excision time as zero). The muscle was bathed in a minimum volume of calcium-free Locke ringer. Reproduced from ref (1), Springer Nature.

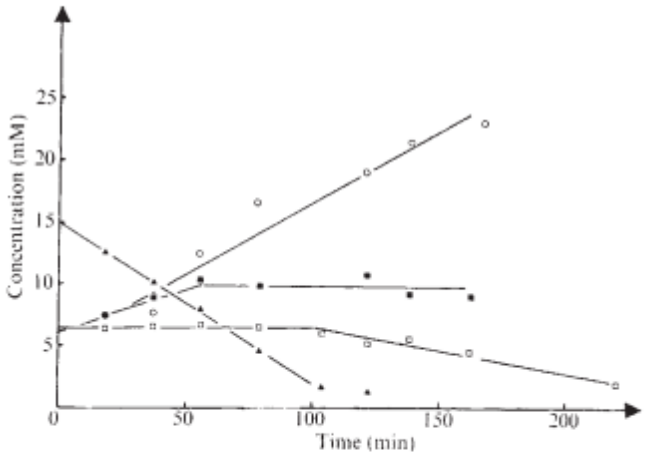

Figure 9. Variation of phosphate metabolite levels in an intact rat muscle with time after excision. The integrals of spectra shown in Fig. 8 are plotted in this graph. ○, inorganic phosphate; ▲, creatine phosphate; ■, sugar phosphate and phospholipids; □ ATP. An absolute concentration scale was established by running a standard sample of 10 mM inorganic phosphate in the same conditions as the muscle. Reproduced from ref (1), Springer Nature.

What was our reaction on seeing these initial spectra? In my thesis, I say 'somewhat surprisingly…a free induction decay appeared… and five resonances were observed'. Steve refers to it as an 'unexpected observation'. No doubt there was an element of understatement here. But it was only later that we realised how fortunate we were with this very first attempt, for a number of subsequent preparations revealed a single inorganic phosphate signal, presumably reflecting loss of high energy phosphates as a result of muscle damage at some stage in the process. If the first experiment had yielded just the inorganic phosphate signal, what would we have done? Would we have continued? Or would we have just added this brief venture to the pile of bright ideas that came to nothing?

Postscript

Our *Nature* paper was submitted early in July 1974, just six months after the serendipitous transformation of Mark 2's capabilities. There were six co-authors – David Hoult, Steve Busby, myself, George Radda, Rex Richards and John Seeley. We were placed in alphabetical order, except for David Hoult, who was designated first author in recognition of all his contributions on the physics side, including spectrometer design and construction. It was a dramatic time for us, not least in the emotional ups and downs we experienced. There were downs because not everyone was enthusiastic about our findings. One local expert in cellular metabolism perceived NMR as a very expensive and unwieldy way of finding out about intracellular metabolism, adding little to what could be discovered far more cheaply and easily using standard (destructive) freeze-clamping techniques. *Nature* too had to be persuaded that our paper was worthy of publication, following an initial negative review. But others of course, including numerous enthusiasts who went on to join or collaborate with us, proved to be much more excited by the notion that tissue metabolism could be studied non-invasively. Working together with a number of these colleagues, our own initial developments included the incorporation of techniques for maintaining tissues viable and functioning within the NMR spectrometer (13,14). These will form the starting point for the next phase of my recollections.

Looking back at my time as a DPhil student, a number of things stand out. One was the extent to which serendipity played a role in so many of the developments that took place. Another was that we had a great deal of freedom to try out new things, regardless of whether or not they might prove to be useful or impactful. The notion that I might use NMR to assay an enzyme could well have been dismissed as a rather wasteful use of expensive technology, but it was one

of the factors that led us to think about using NMR to follow metabolic processes in real time. Some experiments proved disappointingly fruitless - others proved surprisingly fruitful. Perhaps most important was the collaborative spirit amongst the research students that led us, on a day-to-day basis, to think about stuff that we would not otherwise have thought about, and to learn and become engaged with ideas that would take us in new directions.

Acknowledgements

My thanks to Steve Busby, John Seeley, David Hoult and John Griffiths for their kind thoughts and comments; it has been a pleasure to share recollections with them. Thanks also to Colin Fraser for Fig. 1. Steve Busby's DPhil thesis was made available via the SOLO (Search Oxford Libraries Online) resource.

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