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Six Decades of Progress in Magnetic Resonance: The Contributions of James S. Hyde

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Abstract The development of electron paramagnetic resonance (EPR) and magnetic resonance imaging (MRI) over six decades is sketched with an emphasis on the contributions of James S. Hyde. For twenty years starting three years after the first commercial EPR spectrometer was shipped by Varian, he led commercial EPR developments, and then for more than forty years, he led development of instrumentation and biomedical applications of EPR at the Medical College of Wisconsin. It was there that he also made major contributions to MRI, and especially functional MRI.

1 Comments by Gareth Eaton

James S. Hyde (known to all of us as Jim, so in this essay we will say "Jim did xyz") joined Varian in 1959, about 3 years after the first commercial Varian EPR spectrometer was shipped. Since then, he has contributed to a wide range of magnetic resonance. To EPR spectroscopists, he may be best known for developing resonators, but as the following comments will illustrate, the scope of his contributions is much broader. Although starting out with a physics degree, he has strongly supported chemical applications, established a biophysics institute, and has in the last couple decades morphed into a leading developer of medical MRI. This essay will focus more strongly on the spin physics and technology development efforts.

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References with numbers between [1] and [432] in this introduction to the special issue of *Applied Magnetic Resonance* dedicated to J. S. Hyde are in his autobiography [433]. That list includes most of Jim's papers (he omitted some where he felt that his contribution was not very significant). Rather than a simple chronological list, the papers are gathered by topic, so the user can quickly find the paper, regardless of title, that would be considered development of saturation transfer, MRI, basic EPR, etc. Included also are two lists of patents, one of EPR technology development and the other MRI development.

In [2], Jim reviewed the development of EPR at Varian from 1954 to 1974, when he left to establish with Harold M. Swartz the first NIH-funded EPR Center, at the Medical College of Wisconsin (MCW), where he has been since then. An appreciation by Swartz [434] emphasizes Jim's contributions to the founding of the MCW EPR Center and surveys some of the EPR and MRI science conducted by Jim there.

During Jim's time at Varian, there were major efforts to tell the world about the utility of EPR. The "EPR at Work" series of advertisements were one-page tutorials about EPR. Jim and I brought this series together, with updated references and explanations, in a special issue of Concepts in Magnetic Resonance [3]. In this, as in so many efforts of the MCW lab, Jim's wife Karen played such a large role in making it happen that we offered her co-authorship, but, as usual, she declined recognition.

When Jim turned his attention to MRI, he quickly realized that MRI could be enriched by applying there the type of resonator technology that he had developed jointly with Wojciech Froncisz in 1982 [33]. Some early versions of the MRI resonators were constructed on plastic sewer pipe. Given signal-to-noise limitations inherent with in vivo MR studies, a particularly important innovation was the quadrature surface coil [311–313]. Coils designed for specific body structures and innovative schemes for decoupling (eliminating) the mutual inductance between multiple coil arrays—and schemes for parallel data acquisition and analysis from such arrays—were critical advances from the Hyde lab. A company (Medical Advances) was formed to market MRI resonators, EPR loop-gap resonators (LGR), and low-frequency EPR bridges. One of the students from the Eaton lab in Denver joined the company, and the Denver EPR Center tested many prototypes. It did not take long for Jim to explore innovative applications of resonators [304].

A truly seminal pioneering application was to detection of increases in the intensity of T_2*-weighted MRI signal in regions of increased brain activity. Regional brain activation (task activation, e.g., thinking) results in a marked blood flow increase localized within the activated region. This results, somewhat paradoxically, in a regional increase in blood oxyhemoglobin content (concomitant decrease in deoxyhemoglobin) and a reduction in endogenous magnetic susceptibility-induced magnetic field gradients. The initial 1.5-T "finger tapping" experiments from the MCW lab detected activation in the primary motor cortex in 1992 [370]. Three other groups, at Harvard University, at University of Minnesota, and at Yale University, also published human brain activation MR images in 1992, closely followed in 1993 by groups at Göttingen and NIH, and the field of "functional MRI" (now fMRI) exploded. Although the Hyde group formally published first, in

the journal *Magnetic Resonance in Medicine*, the development at Harvard [reported orally at the August 1991 Society of Magnetic Resonance in Medicine (now ISMRM) meeting] received more recognition. However, I recall a presentation on the subject by an MCW student shortly after the first demonstration, during one of the annual meetings of the scientific advisory board of the MCW EPR Center.

Jim has perplexed us somewhat using the term "pulsed EPR" to mean strictly saturation recovery, while many researchers would think first of spin echo when hearing pulse. Jim's 1972, 1974, and 1975 papers [144–147] established the principles of instrumentation for performing saturation recovery, including the value of the three-arm bridge. During my visit to Varian in early 1972, Jim was describing his saturation recovery spectrometer to me. In an amusing confusion, the salesman was eager to get me out of the room, since he found Jim incomprehensible and the spectrometer was not a product, and I was eager to stay a few more minutes hoping to learn a few more details of how it was built. The Denver lab eventually built one [435].

The significance of Jim's 1960 paper on rapid passage in LiF [84] was largely overlooked. This built on the 1955 Portis paper [436], and together these papers provide the intellectual foundation for a wide range of applications of passage phenomena in magnetic resonance, including such experiments as performing ENDOR on rapid passage dispersion spectra at cryogenic temperatures [437]. The development of rapid scan EPR in the Denver lab [438, 439] exploits passage effects in some applications, and Jim followed with what is called non-adiabatic rapid scan (NARS) in his papers [17, 74, 76, 190]. Since he had a 95 GHz spectrometer, where a resonator easily has a 1 GHz bandwidth, he was able to demonstrate rapid frequency scan [73], which is nowhere near as feasible at low microwave frequencies, except for very narrow spectra. Another outgrowth of thinking about relaxation rates relative to passage rates was the development of saturation transfer spectroscopy [79] and its application to molecular motion, especially of nitroxide spin labels [85].

The 1982 Froncisz and Hyde paper introducing the loop-gap resonator (LGR) to the EPR community [33] is deservedly famous. Once it was published, it changed the mindset of the magnetic resonance community away from the old idea of fitting a sample to the resonator to designing a resonator to optimize an available sample. It is a bit ironic that long ago, engineering books thought that the lumped-circuit resonator was the obvious way to describe a resonator and used it as the base from which to show a student that one could morph it into a cavity. The early TE_{102} X-band cavity became such an inherent part of EPR by 1982, that it was a new idea to replace a cavity with a lumped-circuit resonator. The bimodal resonator that Jim made as an exercise in microwave design long ago [269], has now been replaced by crossed-loop and other lumped-circuit bimodal resonators [41, 43, 440].

The interplay between g- and a-anisotropy was recognized as creating special opportunities to find frequencies at which one or another parameter was highly resolved. While many labs sought higher frequencies to resolve differences in g, the Hyde lab developed S-band to enhance the resolution of nuclear, especially nitrogen, couplings in the g-parallel region of Cu(II) complexes [191, 192], which are of importance in many biomolecules. The development of the theory, and the

design and construction of resonators and bridges to demonstrate the effect popularized S-band EPR [193–211].

Similarly, recognizing that g-anisotropy is minimized at low microwave frequencies, and that this would lead to narrow EPR lines for nitroxide radicals at L-band, stimulated development of methods to measure nitroxide–nitroxide distances via dipolar broadening of the central line in spectra of slowly tumbling spin-labeled molecules [190].

Tradeoff between resolution and signal-to-noise dominates selection of experimental EPR parameters, and selection of post-acquisition filtering. The Hyde lab made an important contribution in this area with the "pseudo-modulation" program for enhancing resolution in EPR spectra [15]. The program has been widely distributed. It inspired inclusion of analogous mathematical approaches in other data analysis programs.

Although magnetic field modulation and phase-sensitive detection were crucial to making EPR sensitive enough to apply to low-concentration samples in the early days of EPR, field modulation also caused many problems of signal distortion, eddy currents, etc. Jim delivered the Bruker lecture [80] (published in 1989) on alternatives to field modulation. Multi-quantum EPR developed with his student Hassan Mchaourab is one alternative [173, 174, 176–183]. Continued struggle to minimize the negative impacts of field modulation led to several papers on optimizing slits in loop-gap resonators [48, 55].

Jim is almost as well known for developing saturation recovery as for resonator development (see his reviews in 1974, 1979, and 1998 [141–143]). The instrumentation papers are foundational [144–147]. The interpretation of recovery signals required consideration of many possible contributions, which could be sorted out only when there was sufficient signal-to-noise in the recovery signal to permit fitting with three exponentials. Most of the applications in the MCW lab have been to spin labels. Measurement of T_1 at S-, X-, and Q-bands stimulated analysis [441] and tests [442] of the mechanisms of relaxation of nitroxides as a function of microwave frequency. One of the lessons Jim taught was that for many EPR problems, you need at least three microwave frequencies, and that for some problems a particular frequency provides more information than do other frequencies because of various frequency-dependent terms. The MCW center consequently developed and applied L-band and S-band to a range of spin label and Cu(II) problems. The lab also made important contributions to EPR spectrometers above X-band, especially K-band (19 GHz) [40, 62], and Q-band [61]. Jim had been a major contributor to the Varian Q-band spectrometer. He was surprised to find that technology improvements in both source and detectors made possible a substantial improvement in signal-to-noise. His lab designed an improved Q-band source, starting at the chip level [61, 213].

A W-band system was designed by mixing up from the Q-band spectrometer [71, 73], and using a superconducting magnet that allowed inserting the resonator from the side rather than from the top. This provided many conveniences regarding resonator and sample placement. Both mechanical and microwave innovations in this spectrometer illustrate the range of talents in the Hyde lab. At 95 GHz, a

resonator Q of about 100 provided ca. 1 GHz bandwidth, which was exploited in a demonstration of frequency-swept EPR [73].

Many features of loop-gap resonator (LGR) technology made feasible experiments that were very difficult to achieve with cavity resonator technology. The size of a cavity at L-band and S-band would severely limit applications, but the LGR can be as small at these frequencies as at X-band. The EPR signal is proportional to ηQ, the filling factor times the resonator Q. The filling factor can be very large for an LGR relative to the filling factor of a cavity at the same frequency. Although the Q of an LGR is inherently lower than that of a cavity, the ηQ of an LGR can exceed that of a cavity. The low Q is also useful for pulsed EPR and for dispersion EPR [10].

Many spin systems need to be studied in aqueous samples near room temperature. These are challenging at X-band. One way to handle such samples is to use a flat cell to locate the aqueous sample close to the nodal plane of the E field in a TE_{102} cavity. These flat cells are expensive and difficult for many experimenters to use. A TM_{110} cavity was developed to improve sensitivity for aqueous samples in flat cells [274] (patented in 1975). In 1972, Jim reported that several labs had noted that rotating the cell 90°, which violated the concept of putting the water in the node, gave high-quality EPR spectra. He analyzed this in terms of surface charges and achieved very good improvement in S/N with a custom Rexolite cell [5]. We showed in 1977 that stacking multiple flat cells in this perpendicular orientation provided further improvement [443]. Many years later, the Hyde lab analyzed the aqueous sample problem with modern electromagnetic simulation software and gave solid basis for these empirical phenomena [16, 49]. Bruker now markets an aqueous sample holder (the AquaX) that takes advantage of the benefit of separating the lossy sample into multiple layers or tubes.

The standard TE_{102} cavity with small modulation coils built into the sidewalls emphasizes the EPR signal from about 1 cm of the sample because of the spatial pattern of the microwave B_1 and the modulation field. Many of the improvements in sensitivity over the years have involved efforts to detect more of the length of the sample in the resonator. Several papers by Jim's lab have calculated methods of creating axially uniform resonant cavity modes [44–47]. Inspired by efforts in the Swartz lab at Dartmouth to measure radiation defects in fingernails, Jim and his colleagues designed a resonator to limit depth sensitivity [54], the reverse of the more common goals.

Melanin proved to be a challenging spin system [217], whose investigation led in several directions, including interaction of transition metals with free radicals [216], a field that was then investigated in its own right [233]. The most common paramagnetic material to affect the EPR spectra of organic radicals is O_2. Starting in 1981, Popp and Hyde [240] and continuing to the present, Jim and his colleagues published extensively on measuring O_2 by its effect on nitroxides [238–259], with a special focus on diffusion in membranes, e.g., [246].

Multiple frequencies reveal aspects of spin systems hidden in normal EPR. Early development of ENDOR and its application to a wide range of systems involved improvements in cavities, methodology, including multi-frequency, and selection of samples. Jim's 1964 paper with Maki was the first ENDOR study of an organic

radical in fluid solution [105]. This stimulated a vast new field of study. Applications of ENDOR by Jim's lab included amorphous solids, proteins, and catalysis as well as organic radicals in fluid solution [90–125]. Electron–electron double resonance (ELDOR) techniques developed include CW and pulse techniques, frequency sweep, and applications to many dynamical problems [126–138].

One of Jim's papers that particularly pleased him is the one coauthored with his son about interpreting the wings of inhomogeneously broadened spectra [9].

Jim's parallel work in MRI and EPR enriched EPR when his lab recognized a data acquisition mode being used in MRI that Jim called time-locked subsampling [67]. With the speed of modern digitizers, some of these type mathematical methods can be applied now in EPR that previously were limited to the slower time scale of NMR.

Beginning with the application of the LGR concept to problems in MRI in the mid-1980s, Jim quickly learned the MRI field and became a leader in many areas, especially in fMRI, where issues of noise are crucial, analogous to EPR. His publications in MRI are as varied and extensive as in EPR. The journal *Brain Connectivity*, volume 4, number 9, 2014, published personal reflections on Jim by 16 of his colleagues.

NMR was described as an "evergreen" [444], and in the decades since then the enhancements are even more impressive than when it properly was so described. Recent developments show that EPR is also an evergreen. It is hoped that a major message of this essay about Jim's contributions will be that when some technologies appear to be "mature", a new and better way to look at the same spins comes along.

2 Comments by Wayne L. Hubbell

Although Jim is a physicist by passion and practice, many of his seminal contributions to EPR spectroscopy have had, or are poised to have, a major impact in the biological sciences. The CW EPR method of saturation transfer spectroscopy for measuring slow molecular rotation must be mentioned as one of the most cited EPR methods in biophysics. The LGR has enabled remarkable advances in understanding the molecular basis of protein function. For example, the small-sample capability of the LGR made possible the development of site-directed spin labeling (SDSL) which relies on very small amounts of genetically engineered proteins expressed in tissue culture. The SDSL technology that evolved as a result of the LGR is now in routine use in many laboratories around the world. The high-filling factor and low Q of the LGR also enabled pulse SR and pulse ELDOR to be applied to nitroxide spin-labeled biomolecules. Among other things, these methods provide a window into the functionally important time domain of μs, where the density of protein dynamic modes is high and difficult to access by other techniques. The effective time base for these methods is the nitroxide spin–lattice relaxation time T_1 (order of μs). The applications of these T_1-based methods to protein dynamics is only beginning. Curiously, MQ spectroscopy, which provides direct information on T_1, has largely escaped attention as a tool to explore protein fluctuations via T_1 information. Through the benchmark instrumentation

developments of the LGR, pulse SR, pulse ELDOR and MQ spectroscopy, Jim has provided serious entertainment for the next generation of biologically oriented EPR spectroscopists… EPR is definitely "evergreen".

3 Comments by Wojciech Froncisz

The beginning of my academic career correlates with Jim Hyde's visit to Moscow in 1967. After 2 years of studying physics at the Warsaw University, I had the opportunity to continue my studies at Lomonosov Moscow State University in plasma physics. Upon arriving in Moscow in 1964, I learned that at the Faculty of Physics of the Moscow University, the world's first Department of Biophysics was established, which began to train biophysical specialists from physicists. Professor L. A. Blumenfeld was the founder of the new department and its first head. This triggered me to change my interests from plasma physics to biophysics. This was of great importance to my further research career.

In 1955, L. A. Blumenfeld together with A. E. Kalmanson finished the manufacture, installation and commissioning of the EPR X-band spectrometer with their own hands. They were able to register the first EPR signals of some biological objects [445]. In this way, quite accidentally, I entered the environment of pioneering research on biological objects with the help of EPR. In 1966, I received the proposal to start research on free-radical products of the reaction of ninhydrin with peptides using EPR. I conducted the research at the Department of Biophysics at the Institute of Virology led by A. E. Kalmanson using a Russian RE-1301 radio spectrometer. Unfortunately, that spectrometer was not designed to study aqueous samples. Fortunately, in 1967, an exhibition of scientific equipment made by Varian was organized at the Institute of Virology. Jim Hyde was a member of the Varian team serving the exhibition. One of the presented instruments was a small E-3 spectrometer equipped with a rectangular TE_{102} cavity, which, using a flat quartz cell, was well suited for water samples. I got permission to use the spectrometer for the whole week of the exhibition. I worked days and nights collecting EPR spectra. The results obtained allowed me to write a master thesis which I defended in the autumn of 1967. Some of the results were also presented in two publications [446, 447]. One of them expressed a thank-you note "The authors are grateful to the staff of the exhibition of scientific instruments of the Varian company in Moscow in 1967 for the opportunity to work on a E-3 radio spectrometer". It is interesting that in both publications, my name is misspelled which was a consequence of its Cyrillic writing in the university documents and then transcription of Cyrillic to Latin alphabet without looking at the original Polish documents.

From that Moscow event, I remember a seminar where Jim Hyde presented new instruments of Varian and talked about the low-phase noise of the Varian klystron. This was the first time I heard about the phase noise problem in the context of EPR which was helpful in a later study in Milwaukee in collaboration with Jim.

To sum up, that period of my stay in Moscow, where I met Jim for the first time, shaped my scientific interests, which I could develop successfully in later years in

close cooperation with Jim. This was possible because in 1975 Jim decided to leave Varian and join the faculty of the Medical College of Wisconsin in Milwaukee.

My real long-term cooperation with Jim started in 1977 when, at the invitation of Harold Swartz, I came to the National Biomedical EPR Center at the Medical College of Wisconsin as a Post-Doctoral Fellow. My first research was about copper ion binding sites of synthetic and natural melanin using EPR spectroscopy at X-band [223, 224]. This work made us conscious of the need for improved resolution when using Cu^{2+} as a probe, since very often there was severe overlap of spectra from various sites. Preliminary analysis led to the conclusion that resolution can be improved by studying copper ion complexes at lower microwave frequencies. In the summer of 1978, Jim gave me an assignment to build an S-band microwave bridge. Being a physicist, I was not too happy to receive such an engineering task but Jim knew what he was doing. I was lucky to have found a complete S-band radar station at a local military surplus store. We bought it for 10 dollars! Many S-band microwave parts from that station could be used for the new bridge and they operate reliably to the present day. The only major part which had to be purchased was the mechanically tunable transistor oscillator. It took me 2 months to put together an octave bandwidth microwave bridge operating between 2 and 4 GHz. Using a piece of waveguide from a radar station, I constructed the rectangular cavity that could excite TE_{102} and TE_{104} modes at frequencies of 2.6 and 3.8 GHz, respectively. The bridge was coupled to a standard Varian E-line console employing 100 kHz field modulation. The results were astonishing, we could observe resolution improvements for many copper ion complexes, e.g., [191, 192, 448, 449]. It was found, using the new system, that a good EPR spectrum of copper could be obtained using a 1 mL icicle 7–8 mm o. d. and 20 mm long at a concentration of 1 mM. The problem was the large size of the rectangular cavity and the associated sample size. One day, when Jim was away, something tempted me to try a different approach to the resonator design to decrease its dimensions. Instead of using a distributed element resonator, which is a rectangular cavity, I used a lumped-element resonator which consisted of an inductor and capacitor. This was a novel approach to the microwave resonator design for EPR spectroscopy allowing considerable reduction of its size. Making a loop of silver-plated copper wire soldered to a piece of a double-sided printed circuit board created a small resonant circuit that showed astonishingly high sensitivity. After returning to the lab, Jim immediately recognized the potential of such structures for EPR spectroscopy. He named it the loop-gap resonator [33] written with the hyphen. And here comes the story of the hyphen in English. My wife being a student of English grammar class at UWM in Milwaukee got an assignment to write an essay about the rules of using the hyphen between words in English. At lunch time, I asked Jim if he remembered those rules. The result of the test was very impressive. After a while, Jim cited all 11 rules. Jim told me that he remembered it from high school. I asked the same question to my other American colleagues and no one knew how to respond correctly. This event showed where his exceptional skills of writing any scientific texts, grant proposals, etc. in a transparent and efficient way came from.

Jim is a very productive scientist. Traveling down his scientific road has resulted in more than 400 scientific articles cited nearly 25,000 times. I was very lucky that

my road often ran parallel to Jim's. As a result, among these 400 articles, almost one-fourth was established with my participation and they were cited almost 3000 times. These numbers are relative because Jim's single article [399] was cited almost 4000 times. Nevertheless, I am very proud that our paper on the loop-gap resonator [33] resulted in more than 400 citations, many new papers, patents, grants and so on.

There are very few scientists in the world who could be proud of such a long and successful career in science, particularly in EPR and MRI research. The Jagiellonian University, where I have been working for almost 50 years, is proud to have given Jim the highest title of honorary doctorate in 1989. Jim is, therefore, among such notables as Pope John Paul II and Nobel Prize winners: Maria Skłodowska-Curie, Linus Pauling, Ilia Prigogine, Paul Lauterbur, Czeslaw Milosz, Peter Mansfield and others.

Just a few personal remarks. Adding up all my fifty visits to Milwaukee, I spent nearly 10 years of my life there. I can say that Milwaukee was my second home over the last four decades. I recall these visits with a large dose of nostalgia. I miss our conversations during lunch time about not only spin physics and new scientific experiments but also about the basis of the use of the hyphen in English, about making wooden cabinets, etc.

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