Biological Magnetic Resonance
Volume 14

Spin Labeling
The Next Millennium

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Edited by

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To Harden M. McConnell and Eduard G. Rozantsev

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Preface

We present here the second issue devoted entirely to the spin-labeling technique as part of *Biological Magnetic Resonance.* Volume 14 commemorates a modification in our editorial policy with the retirement of my esteemed coeditor, Jacques Reuben. From this juncture into the future, each issue will focus on some special topic in magnetic resonance. Each volume will be organized in most cases by guest editors, for example forthcoming issues will address the following topics:

^{13}C *in vivo* magnetic resonance (P. Robitaille and L. J. Berliner, eds.)
Modern techniques in proton NMR of proteins (R. Krishna and L. J. Berliner, eds.)
Instrumental techniques of EPR (C. Bender and L. J. Berliner, eds.)

The current volume, *Spin Labeling: The Next Millennium*, presents an excellent collection of techniques and applications that evolved during the past decade since the last volume, volume 8 (1989). Some obvious omissions, such as multiquantum EPR and very high-frequency FT-ESR were unfortunately not possible for this volume. Perhaps they will appear in *Spin Labeling: 2001.*

Lastly it is a pleasure to honor two scientists whose contributions were both pioneering and pivotal to the spin label technique: Professor Eduard G. Rozantsev (Moscow), whose synthetic feats in nitroxyl chemistry set the broad stage for a versatile catalog of labels; and Professor Harden M. McConnell, last year's International ESR (EPR) Society Gold Medalist, who conceived and developed the spin label technique to address many biological problems (proteins, enzymes, membranes, cells, immune response, etc.).

Lawrence J. Berliner
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Introduction

Reflections on the Beginning of the Spin Labeling Technique

Lawrence J. Berliner

This volume is dedicated to the two pioneers who contributed seminally to the beginnings of the spin label technique, Eduard G. Rozantsev, who developed a broad range of nitroxyl compounds and reagents; and Harden M. McConnell, whose foresight into the areas of biological structure and function brought the world the broad leadership that guided this technique to where it is today.

As Harden McConnell's first graduate student assigned to spin labeling, I take great pride in reflecting on the early days and subsequent development of the technique from the early- to mid-60s when it first began. McConnell's lab was located in the Physical Chemistry Laboratories of the Department of Chemistry at Stanford University, where his research to that point had been heavily involved in areas of chemical physics, solid-state physics, and theoretical aspects of spin physics. Rozantsev's lab, then at the Institute of Chemical Physics, USSR Academy of Sciences, Moscow, was publishing extensively on the chemistry, synthesis and physical chemistry of several nitroxyl compounds (coined iminoxyl at the time); this research was published mainly in Russian academy journals. Since it was difficult to obtain samples from Moscow at that time, a major synthetic effort was launched in the McConnell lab to reproduce compounds synthesized in the Rozantsev

lab and to develop analogues of protein modification reagents. In fact early during this period, the first *in vivo* related experiment was done in the McConnell lab to assess toxicity of nitroxyl compounds to cells or living systems. Several grams of di-t-butyl nitroxide were dissolved in an aquarium where a goldfish swam. The goldfish survived the nitroxyl environment, but when someone allowed hot water to drip slowly into the aquarium, the animal died either from heat exposure or by falling out of the aquarium when the water level reached the top and overflowed into the sink! Another effort involved analyzing EPR lineshape and motional characteristics of the tumbling nitroxyl group. The first paper (Stone *et al.*, 1965) reflected the effort of three postdoctorates, two of whom were trained as organic chemists; the latter two authors (including McConnell) were experts in lineshape analysis. The major efforts in the McConnell laboratory from 1966–67 focused predominantly on protein and enzyme problems. There was another massive effort by a team of postdoctorates who attempted to apply, in a blanket fashion, a new maleimide spin label to a variety of enzymes and proteins (Griffith and McConnell, 1966).

The common attributes of McConnell and Rozantsev is that they were great scientists, interested purely in the joy of pursuing important fundamental problems (instead of what was currently politically in favor.) To the best of my knowledge, McConnell's conception of the spin-labeling technique was based heavily on the pioneering fluorescence work of Gregorio Weber (University of Illinois), who studied fluorescence depolarization, and other steady-state techniques with extrinsic fluorophores introduced into specific sites in proteins and enzymes. At the same time Stanford hired Lubert Stryer in the medical school (Department of Biochemistry), who was a consultant in protein structure/function. Nonetheless, it is fair to say that most of the driving force, motivation, and discovery came from within the McConnell group through the foresight and leadership of its mentor. This of course led to the applications of spin labeling in several other areas of biochemistry and medicine: lipids and membranes, cellular machinery (such as the complement system), studies of diffusion phenomena within lipid bilayers. Most recently there were impressive *in vivo* applications in animals, as outlined in the chapter by Swartz and Halpern. McConnell also applied nitroxyl groups as paramagnetic relaxation agents to probe protein structure by NMR. This was elegantly described in volume 8 of this series by Anglister (in Berliner and Reuben, 1989). McConnell was literally the father, grandfather, and great-grandfather of spin labeling. Rarely a publication on the subject does not cite at least one or two of his fundamental papers. It is certainly an honor and a tribute to recognize his contributions in this book.

Eduard Rozantsev was a prolific chemist who was also well-versed in physical chemistry and biochemistry as well. Under the tutelage of M. B. Neiman at the Institute of Chemical Physics, he grew to be an international giant in this field, collaborating frequently with other physical and biophysical chemists at the Institute on applications of nitroxyl compounds, some modeled after McConnell's early articles. Unfortunately during Soviet times the Institute of Chemical Physics was

not always headed by extremely benevolent directors. For reasons (all of which are not totally known to me) Rozantsev fell into disfavor with Director V. Buchachenko, who banished him from the Institute in the later 1970s. Rozantsev took a position in the Institute for Applied Biotechnology in Moscow, where he continued a research and teaching program, albeit much smaller than that at the Institute of Chemical Physics. During the time period of Buchachenko's reign, Rozantsev was frequently denied travel; for example he did not attend the First International Symposium on Nitroxyl Compounds in Pećs, Hungary nor the Second International Symposium in Novosibirsk in 1989. Nonetheless this editor had the pleasure and honor of meeting him twice in Moscow, once in 1981 on a National Academy of Sciences exchange visit to the Shemyakin Institute of Organic Chemistry, where we were allowed to have a private meeting. This was shortly after Rozantsev and Renat Zhdanov undertook the massive effort of translating the first volume of *Spin Labeling: Theory and Applications* (Berliner, 1976) into Russian (Rozantsev, 1979) for Mir Press. The second visit was in 1994 when I met him and one of his postdoctoral students (an excellent physical chemist who carried on the tradition of high-quality organic synthesis, characterization, and new insights into novel structures as a result of Rozantsev's excellent mentoring). Rozantsev was also a true giant in his field. His book, *Free Nitroxyl Radicals* (Rozantsev, 1970) still serves as a useful guide. In fact, it is noteworthy that we continue the tradition in every spin-labeling volume of including a complete chapter on new aspects of organic synthesis of nitroxyl compounds, each containing detailed methods and materials sections enabling a chemist to carry out synthesis without referring to the original publication.

The spin-labeling field blossoms even more as we head into the next millennium. As exemplified by the chapters here, it has been applied to systems in food chemistry, nucleic acid and nucleotide biochemistry, to cell applications, *in vivo* applications with small animals, and most recently as a tool for specifically incorporating spin labels at unique amino acid residue positions in mutated proteins. As described in other chapters, new instrumental advances combined with other techniques, such as NMR (ENDOR), allow us to probe detailed motional and structural characteristics, especially at very high frequencies (e.g., 95 GHz). No doubt there are many other techniques yet to come. In conclusion, speaking for the other authors as well, we hope that the subject matter in this volume and continuing contributions from the two honored scientists and their research groups inspire into even more applications and techniques in the millennium to come!

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