Biological Magnetic Resonance
Volume 5

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Preface

Judging from the articles published in *Biochemistry*, magnetic resonance techniques (NMR and ESR) are now among the most popular methods in biochemical research. The series *Biological Magnetic Resonance*, the fifth volume of which we are proudly presenting, is intended to provide authoritative coverage of topics of current interest. Previous volumes have covered a number of aspects in a thorough and pedagogical fashion rarely found in other publications in this field.

Continuing to fulfill the mission of the series, this volume presents a chapter by Baxter, Mackenzie, and Scott on the applications of carbon-13 NMR spectroscopy in investigations of methabolic pathways *in vivo*. Blomberg and Ruterjans give a comprehensive summary of the use of nitrogen-15 NMR in studies of systems of biological interest. Phosphorus-31 NMR investigations of enzyme systems are described by Rao. Tsai and Bruzik outline the principles of and summarize the state-of-the-art advances in the use of oxygen isotopes (^{17}O and ^{18}O) in phosphorus-31 and oxygen-17 NMR studies of biophosphates. Lipid–protein interactions as reflected in ESR and NMR data are discussed by Devaux.

We wish to thank the authors for their cooperation in maintaining the philosophy and continued high standards of the series.

Lawrence J. Berliner
Jacques Reuben

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CMR as a Probe for Metabolic Pathways *in vivo*

R. L. Baxter, N. E. Mackenzie, and A. I. Scott

1. INTRODUCTION

The application of NMR methods for the investigation of metabolic processes in living systems is now well established. In recent years ^{31}P-NMR in particular has provided a wealth of information regarding the flux of phosphorylated metabolites under physiological conditions in whole cells, isolated organs, and within intact organisms. These studies have been extensively reviewed (Burt *et al.*, 1979; Radda and Seeley, 1979; Gadian and Radda, 1981). Despite this success in the elucidation of cellular energetic processes ^{31}P-NMR suffers intrinsic limitations as a metabolic probe. Firstly, many processes of interest do not directly involve net changes of concentration of phosphorylated species, and secondly since the ^{31}P nucleus is present at 100% natural abundance the fate of a particular atom cannot be monitored. Although a phosphorylated metabolite may be undergoing rapid turnover this may not be readily apparent since the method is sensitive only to changes in overall concentration. An exception to this is the application of saturation transfer methods which have provided valuable kinetic information on the exchange rates between ATP and Pi and phosphocreatine and ATP *in vivo* (Brown *et al.*, 1977; Gadian *et al.*, 1981).

While the ^{13}C nucleus has a receptivity of less than 0.25 $\times$ that of ^{31}P and the experimental conditions required for obtaining well-resolved CMR

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spectra of biological materials are more stringent, CMR spectroscopy offers significant advantages as a general method for the investigation of metabolic processes. These stem from the ubiquitous position of carbon-containing molecules in nature and the low natural abundance of the ^{13}C nucleus (1.1%) coupled with the sensitivity of chemical shift to molecular environment exhibited by the ^{13}C nucleus. Thus the fate of a particular ^{13}C atom in an artificially enriched precursor, which may only be present at $0.1\times$ the natural concentration, can be followed with time. This is directly analogous to the traditional use of ^{13}C and ^{32}P isotopes as tracers in biochemistry but possesses a unique advantage in that direct observation of enriched species avoids much of the subjectivity involved in the design of analytical protocols for "expected" intermediates. Apart from the direct measurement of rates of intracellular processes and the detection of metabolic intermediates, examination of ^{13}C distribution in metabolites enables the degree of randomization of label to be determined as a function of time, hence allowing direct evaluation of "cycling" and pathway convergence phenomena.

2. CONSTRAINTS

Lack of sensitivity to low concentrations of metabolites is the major failing of the CMR method. The advent of high field superconducting magnets and recent advances in probe and coil design have dramatically improved sensitivity, but it is unlikely that the sensitivity of CMR can ever approach that of radiochemical methods, a finite limit being reached as the concentration of enriched species approaches $0.01\times$ the concentration of the unenriched metabolite. With presently available spectrometers the minimum detectable amount of a small transient intermediate molecule (mol wt. 150, $t_{1/2} \sim 1$ min) in a 10-mm sample tube appears to be about 0.5 μMol. The threshold of detection may be lowered by increasing sample volume.

The requirement for ^{1}H decoupling to enhance ^{13}C signals gives rise to dielectric heating, a phenomenon particularly acute in high field magnets with large heterogeneous samples where the low efficiency of heat transfer results in steep thermal gradients (Led and Petersen, 1978). In general, low buffer concentrations (≤ 0.1 *M*) and gated decoupling techniques (with consequent increase of the time required to achieve acceptable signal to noise) have been employed to minimize this, but recently significant reduction of dielectric heating has been achieved using a slotted resonator decoupling coil (Alderman and Grant, 1979). The use of berylium oxide tubes to improve the efficiency of heat transfer has recently been suggested (McNair, 1981).

Field distortion by differences of magnetic susceptibility within the

sample is a major cause of poor resolution and sensitivity in biological NMR. This is exacerbated in the case of intact organs and oxygenated cell suspensions where spinning the sample becomes impossible. In the latter case electronic synchronization of data collection and bubbling rate (Navon *et al.*, 1977) and addition of H_2O_2 to peroxidase (Ogawa *et al.*, 1978) have been used to circumvent the additional loss of homogeneity due to gas bubbles, but in large-diameter sample tubes (> 20 mm) slow streams of fine air bubbles do not appear to affect resolution markedly while noticeably improving heat transfer. Maintenance of the integrity of the intracellular environment makes broadening of signals by interaction with paramagnetic ions an unavoidable hazard. Fortunately the effect is not so pronounced for ^{13}C as it is for ^{31}P-NMR. This effect has been usefully exploited by using paramagnetic species, which do not penetrate the cell, to minimize the signals due to extracellular compounds (Campbell *et al.*, 1973; de Kruijff *et al.*, 1979; van den Besselaar *et al.*, 1979).

3. CMR STUDIES OF METABOLIC PATHWAYS

Only in special cases where large amounts of specific metabolites are produced can natural abundance CMR be used to monitor metabolism. For example, Kainosho *et al.* (1977) have studied the fermentation of glucose by acetone-treated yeast cells and have followed the extra cellular accumulation of ethanol, fructose-1,6-diphosphate (FDP), ATP, AMP, trehalose, and glycerol by CMR at 25.2 MHz over a period of 30 hr. It is significant that the concentrations of phosphorylated metabolites, although much lower than the other fermentation products, could be measured by ^{31}P-NMR with much shorter data accumulation times. More recently the changes in cellular metabolite pools during development of avian and reptile (Kainosho, 1978) embryos have been followed by CMR.

While such experiments exemplify the usefulness of CMR as an analytical tool they add little to our knowledge of biochemical processes. The power of CMR as a probe in the analysis of metabolic pathways can best be appreciated in those studies in which the fates of ^{13}C-enriched precursors have been followed as a function of time. We have elected to classify these on the basis of the biochemical pathways involved, rather than by the instrumentation and techniques employed. Examples are outlined in the following sections.

3.1. Glycolysis and Gluconeogenesis

The catabolism of ^{13}C-labeled glucose by *E. coli*, yeasts, and mammalian erythrocytes have been examined by several investigators. Ugurbil *et al.*