APPLICATIONS OF NUCLEAR MAGNETIC RESONANCE TO CARDIAC STRUCTURE AND FUNCTION

MICHAEL B. SMITH*, K. M. A. WELCH and PAUL D. STEIN

Department of Neurology and Medicine, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, Michigan 48202

(Received February 1988)

INTRODUCTION

Many techniques have been applied to assess cardiac structure and function. Most of the imaging techniques used in cardiac diagnosis utilize ionizing radiation with the exception of ultrasound. Nuclear Magnetic Resonance (NMR) has now been introduced into medicine and offers a benign and non-destructive method to characterize anatomical structure and metabolic state.

HISTORICAL DETAILS

The origins of our earliest understandings of NMR began with the experiments of Stern and Gerlach [1] who used the deflection of an atomic beam in a magnetic field to measure nuclear magnetic moments. This experiment was further improved by Rabi and colleagues [2] in 1938 to observe the phenomena of nuclear magnetic resonance. Nuclear magnetic resonance was demonstrated in solids or liquids in 1945 when Edwin Purcell [3] at Harvard University and Felix Block [4] at Stanford University simultaneously observed nuclear magnetic absorption and subsequently shared the Nobel prize for this discovery in 1952. Shortly thereafter, chemists recognized the capability of NMR to define the structure of molecules. In recent years, with the development of higher field magnets and Fourier transform NMR, a large number of nuclei are routinely examined.

The application of NMR to living systems began shortly after its conception in 1946. Block is said to have detected a proton signal from water by placing his finger in the radiofrequency coil of the spectrometer [5]. Perhaps the earliest study of intracellular metabolites was shown in 1972 by Eakon, Matwiyoff and coworkers [6] who showed that $^{13}$C NMR can be used to follow metabolism of specifically labelled substrate ($1^{-13}$C-glucose) directly within a living cell. Further, in 1973, Moon and Richards [7] demonstrated how the chemical shift of certain resonances can be used in determining the intracellular pH using $^{31}$P NMR. In 1974, Hoult et al. [8] produced the first $^{31}$P spectra of intact muscle which contained resonances identified as ATP, inorganic phosphate, phosphocreatine and sugar phosphate.

*Present address: Department of Radiology, The Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033
Figure 1  Schematical representation of a hydrogen nucleus containing a single proton spinning on its axis. This spin produces a net magnitude moment along its axis and can be thought of as a timing bar magnet. Its orientation with the static magnetic field $B_0$ is not that expected for a bar magnet but is described by quantum mechanics.

THEORY

The basic postulates of NMR are relatively straightforward. Particles of matter possess properties of mass, charge, and spin or angular momentum. Elements, therefore, possess magnetic properties as a result of their composition of these characteristics. When an element contains an even number of protons and neutrons, it possesses no net magnetic moment and thus cannot be used in the NMR experiment. Therefore, elements which contain odd numbers of protons and/or neutrons are detectable by NMR (Figure 1). Classically, the relationship between the magnetic moment, $\mu$, and the angular momentum, $L$, can be shown to be:

$$\mu = \frac{e}{2mc} L,$$

where $c$ is the velocity of light, $e$ is charge and $m$ is mass. When the nucleus is placed in a magnetic field, $B_0$, the observable magnetic moment or the projection of $\mu$ on the magnetic field is given by the formula:

$$\mu = \gamma L h,$$

where $\gamma$ is equal to $ge/2mc$ and is a constant for a given nucleus called the gyromagnetic ratio, $Z$ is equal to the spin or the magnitude of the spin of the nucleus and $h$ is Planck’s constant. Thus, there exists $2Z+1$ energy levels (Figure 2) whose magnitude of energy between levels is given by:

$$AE = h\nu_0,$$
where $\Delta E$ is the separation of energy levels. Transitions between these two energy levels may be produced by the application of a radiofrequency magnetic field whose frequency, $\nu_0$, is proportional to the static magnetic field, $B_0$, as:

$$\nu_0 = \frac{\gamma B_0}{2\pi}.$$

Figure 2 depicts a schematic representation of a nucleus precessing in a magnetic field. It becomes a simple matter to classify nuclei according to their spin quantum numbers. As we have noted for nuclei in which $I=0$, there is no net angular momentum and thus resonance absorption cannot occur for this nucleus. Nuclei in which $I>0$ possess values of $I$ in integral or half integral magnitude. Nuclei with $I=1/2$ have a spherical symmetry of electron charge distribution around their central core. When $I \geq 1$, however, an electron distribution results which is nonspherical or asymmetric in its symmetry. Electric field gradients result from this charge asymmetry and spectral lines are widened due to more efficient relaxation.

Normally, when nuclei align themselves with the magnetic field, they drop to the lowest energy state. This behavior is opposed by the thermomotion of the molecules and atoms which tend to equalize the populations in the $2I+1$ energy levels. The distribution of the population is predicted by the Boltzmann equation:

$$\frac{n^+}{n^-} = \exp \left( \frac{\Delta E}{kT} \right),$$

with $k$ being the Boltzman constant, $n^+$ and $n^-$ represent the upper and lower energy level respectively, and $T$ is the absolute temperature. From the equation, it can be seen that the population ratio is dependent on the absolute temperature of
the system and the magnetic field strength. At the magnetic fields normally employed for NMR experiments, only a difference of $10^{-5}$ or $10^{-6}$ exists between the populations at room temperature. However, when one considers the large number of nuclei involved, a small microscopic magnetic moment may be measured directly along the static magnetic field. This microscopic magnetization complies with the Larmour equation and precesses about the static magnetic field at a rate which is predicted by the individual nuclear moment and static magnetic field strength.

![Figure 3](image3.png)

**Figure 3.** Larmor precession of the nuclear magnetic moment, $\mu$, at an angular momentum, $L$, in the magnetic field $B_0$.

![Figure 4](image4.png)

**Figure 4** NMR signal or free induction decay (FID) showing the progressive decrease of the precessing magnetization (left) and its Fourier transform to a Lorentzian line.
During the NMR experiment, energy from a radiofrequency magnetic field at the frequency $v_0$ will cause the microscopic magnetization to tilt away from the static field axis. The precession of the nuclei as they decay to their ground state induces a tiny alternating voltage which is measured by an appropriate receiver coil. The decaying sinusoidal signal (called the free induction decay or FID) can be converted from a time to a frequency domain by using Fourier transform analysis (Figure 4). The signal intensity is dependent on the position to which the applied radiofrequency field has rotated the microscopic magnetization. A signal maximum will occur when the magnetization lies in the $xy$ plane orthogonal to the static magnetic field.

![Schematic representation of the orbital motion of an electron around its nucleus induced by the static field $B_0$.](image)

The movement of this electron creates an opposing and smaller magnetic field $B$ (Top). The opposing magnetic field decreases the intensity of total magnitude field experience by the nuclei. Since the observation frequency of a nucleus is proportional to the magnetic field strength, the frequency of nuclei shielded by electron motion will always be less (Bottom). The magnitude of the decrease in frequency is dependent on the local electron density around the nucleus. Changes of the resonance frequency of nuclei of this type are called chemical shift.

**CHEMICAL SHIFT AND IDENTIFICATION**

Traditionally, chemical shift has been one of the more important features of chemical applications of NMR. In 1951, the observations by Arnold et al. [9] observed that elements of the same molecule possess slightly different resonance frequencies. The origin of this chemical shift is the alteration of the magnetic field due to the screening or shielding of electrons surrounding the nucleus (Figure 5).
The motion of electrons produces magnetic fields which oppose the static magnetic field such that the resulting field, $B_{\text{eff}}$, that the nucleus experiences is equal to:

$$B_{\text{eff}} = B_0(1 - \sigma),$$

where $\sigma$ is the screening factor or shielding factor of a particular nucleus whose magnitude is dependent on the orientation of the molecule relative to the applied field. The resonance frequency is field-dependent, thereby the chemical shift is also field-dependent and often measured in Hertz. Since the chemical shift in Hertz can vary depending on the applied field in which it is measured, a convenient system has been developed to eliminate the field dependents of resonance shifts. This is expressed in the dimensionless units of parts per million, $\delta$, as given by the formula:

$$\delta = \frac{\nu - \nu_0}{\nu_0} \times 10^6,$$

where $\delta$ is the frequency in parts per million, $\nu$ is the absolute frequency in Hertz of the sample and $\nu_0$ is the absolute frequency of the reference. Measuring chemical shift in this way has the advantage that frequencies can be compared regardless of the applied static magnetic field. This allows the frequency to be expressed relative to a standard in which the downfield shifts are positive and upfield shifts are negative on the frequency scale.

**QUANTITATION**

NMR is a form of spectroscopy in which energy in the form of radiofrequency radiation is absorbed by nuclei possessing the magnetic moment. The intensity of the resonance signal arises from a particular nucleus in the applied static magnetic field. Its position along the frequency axis will be determined by the degree of electron shielding around the nucleus itself. The intensity of the resonance line is proportional to the concentration of the absorbing nuclei in the sample. The shape of the resonance line is affected by a number of parameters which may include $\pH$, spin lattice ($T_1$) and spin–spin ($T_2$) relaxation, nuclear overhauser enhancement and chemical exchange.

In the application of high resolution NMR to tissue and cells, only the mobile molecules are observed. The signals from immobile molecules are present, but are often too broadened to observe due to enhanced $T_2$ relaxation. Thus, in general, signals from macromolecules such as membrane phospholipids, DNA and RNA are not seen or seen as very broad components of the resonance line. A good example of signal from immobile structures in $^{31}\text{P}$ NMR is that signal which arises from bone, although broad, can be seen due to its high phosphorus concentration.

The relaxation processes, $T_1$ and $T_2$, are described in Figure 6. In order to accurately quantitate the concentrations of metabolites, nuclei must be allowed to fully relax, that is, to return to their lowest energy state before the next radiofrequency pulse is applied. When accumulated in this way, the intensities or the areas of the individual resonances are proportional to the total number of nuclei within the sample. To perform an experiment of this kind, a nominal 90° radiofrequency pulse would be given and spectra accumulated, and a waiting time of at least five times the spin lattice relaxation time, $T_1$, is allowed before the
Figure 6  (Top) Following a 90° pulse, the vector of magnetization will return to its equilibrium value. The rate at which this occurs is described by the time constant $T_1$ or spin-lattice relaxation time. The $T_1$ is affected by any phenomenon which facilitates the exchange of energy to bring the nuclear spins to thermal equilibrium with the lattice of the molecular structure. (Bottom) Additionally, following a 90° pulse the vector of magnetization in the xy plane will begin to dephase to its equilibrium value. The rate of this decay is described by the time constant $T_2$ defined as spin–spin or transverse relaxation time. The $T_2$ is affected by energy exchange between nearby nuclei which return the net component of magnetization in the xy plane to zero. The $T_2$ decay can be significantly increased by inhomogeneity of the static field $B_0$.

Figure 7  Effect of $T_1$ recovery of the longitudinal magnetization (i.e., signal intensity). In substances which have molecular motions too rapid to allow efficient energy exchange between spinning proton and the lattice, long $T_1$'s are seen. In substances which have shown rotational and translational motion or other efficient energy transfer mechanisms, $T_1$ or spin–lattice energy/transfer time is short.
sequence is repeated. Corrections can be employed when using more rapid waiting periods if the \( T_1 \) for each resonance is known. “Saturation factors” can be determined from the ratios of the intensities of the fully relaxed spectra as compared with a more rapid sampling rate (Figure 7). The attraction of the faster repetition rates is that signal to noise may be significantly improved.

It is also necessary to calibrate the concentrations in some way, either by the use of an internal standard or by using the concentrations from freeze-clamping extraction [10]. These methods assume that the volume of the internal standard is very accurately known, the freeze-clamping procedure does not change the concentration of free metabolites, and/or the spectra possess adequate resolution to correctly evaluate the peak intensities or areas. A direct method of measuring absolute concentrations using a second nuclei as an internal standard has been developed by Thulborn and Ackerman [11]. The method has been specifically applied to surface coils, in which quantitation is sometimes difficult due to its inhomogeneous \( B_1 \) field. All methods involving quantitation of surface coils, whether relative or absolute, assume that the sample beneath the coil is homogeneous and often it is not. Although the technique assumes tissue homogeneity, presence of skin, subcutaneous fat, blood vessels, bone and other physical structures produce inaccuracy.

Finally, the cytosolic volume or intracellular space should be considered for truly accurate absolute concentration within a cell. In addition, inaccuracy due to the bound or compartmentalized state of metabolites should not be ignored. Bound metabolites are NMR invisible due to the decrease over their \( T_2 \) relaxation times (Figure 8) brought about by their binding to larger molecules.

![Figure 8](image_url)

**Figure 8** Effect of transverse magnetization relaxation or \( T_2 \) decay on signal intensity. Substances which contain the potential for localized internal fields effect a loss of coherence of the transverse magnetization. This results in a rapid decay of the signal intensity due to the short \( T_2 \). Substances free of internal field gradients preserve their transverse magnetization with time. These substances are said to have a long \( T_2 \). Generally, long \( T_2 \)’s exhibit narrow line-widths while short \( T_2 \)’s are indicative of broadened spectral lines.
SENSITIVITY AND RESOLUTION

One of the major disadvantages of the NMR technique is its low sensitivity. The observation of metabolites \textit{in vivo} is presently limited to concentrations of roughly $10^{-3}$M or higher. The current level of sensitivity has been enhanced to its present state by the use of Fourier transform techniques and the development of superconducting magnets. An obvious way to increase sensitivity is to increase the applied magnetic field strength since the signal noise would be proportional to $B_0^{1/2}$. There is clearly both a structural and possible safety limitation to further increases of the static field for whole-body magnetics. The lower sensitivity is occasionally fortuitous since the observable nuclei are usually limited to small mobile molecules of high concentration. This results in the simplification of spectra due to fewer numbers of compounds present and often less overlap between resonance lines.

\textbf{pH AND METAL ION BINDING}

One of the more important aspects of NMR has been in its use for the evaluation of intracellular pH (Figure 9). The technique exploits the chemical shift dependence due to fast exchange of such molecules as inorganic phosphate with change in pH. The chemical shift arising from inorganic phosphate and certain phosphomonoesters (i.e., glucose 6-phosphate) are often employed due to their high pKa value which is in the range of physiological pH. In rare circumstances, the chemical shift of the gamma phosphate of ATP is used in tissues or organelles exhibiting more acidic pH. The method is routinely calibrated by titration of the appropriate phosphorus compound in an isotonic environment similar to that of the tissue of interest (Figure 10). Certainly, errors can occur from an improper evaluation of the constituency of the cellular fluid. Extensive investigation has been performed on factors other than pH which will influence local shift behavior. Subsequently, phosphates have been shown to interact with proteins, phospholipids and nucleic acids as well as metal ions such as magnesium, potassium and calcium and to have strong interactions with changes in ionic strength [12]. The accuracies in the measurement of pH may therefore be in the order from 0.2 to 0.5 pH units or higher, if cellular ionic strength and environment is not well defined. It is clear that care should be taken in the application of this technique.

\textit{In Vivo} MEASUREMENT OF ENZYME KINETICS

Possibly one of the more interesting and potentially useful applications of NMR may be found in the area of the noninvasive assay of cell energy metabolism and kinetics in a number of body tissues. The non-destructive facet of this application of NMR offers an undisturbed look at tissue physiology which in the past has not been available. Although we now take invasive procedures almost for granted, there is significant uncertainty introduced by the sampling procedure.

The saturation transfer experiment was developed by Forsen and Hoffman [13, 14] based on the derivation of the Bloch equations for exchange by McConnell and Thompson [15]. The saturation transfer experiments allow for the determination of the unidirectional exchange rate of nuclei experiencing a dissimilar magnetic field. In the reaction catalyzed by creatine kinase,
\[ \text{pH} = \text{pK}_a + \log \left( \frac{f - f_a}{f_b - f} \right) \]

Figure 9. Measurement of pH by \(^{31}\text{P}\) NMR. At pH = 7 inorganic phosphate is present in two forms as \(\text{HPO}_4^{2-}\) and \(\text{H}_2\text{PO}_4^{-1}\). In the absence of chemical exchange processes two individual resonances would be seen corresponding to the two forms at frequencies \(f_b\) and \(f_a\). However, the two are in fast chemical exchange and only a single resonance results. The position or frequency of the resonance is determined by the quantity of each of the two forms of inorganic phosphate. Therefore, pH is predicted by the Henderson–Hosselfbalch equation where \(f\) is the measure frequency of inorganic phosphate. The figure shows the position of inorganic phosphate at basic, neutral and acidic pHs.

Figure 10. Resonance shifts in the \textit{in vitro} titration of Pi and y-phosphate of ATP. (a) (left) sodium phosphate (5 mM) in 100 mM NaCl and 5 mM KCl (○) and with the additional presence of 5 mM MgCl\(_2\) (■). The pH was adjusted with NaOH or HCl. (b) (right) sodium ATP (5 mM) in the above medium in the absence (■) or presence (○) of 5 mM MgCl\(_2\). Also shown in a calculated curve for 0.90 molar ratio of Mg:ATP. (Smith \textit{et al.}, \textit{Biol. Reprod.}, used with permission).
phosphoryl exchange occurs between PCr and the terminal phosphate of AW. The individual forward and reverse rate constants, $k_f$ and $k_r$, are shown for the enzyme catalyzed reaction. The forward flux of the reaction describes the concentration of phosphoryl groups from PCr relocated to ADP to become ATP with time. If it is assumed that the reaction is pseudo first order, magnetization is not leaked via other side reactions, and there is no compartmentation which affects the substrate concentration. The dependence of the PCr magnetization with time is given by the equation:

$$\frac{dM_{PCr}}{dt} = \frac{M_{PCr}^0 - M_{PCr}}{T_{1PCr}} - k_f M_{PCr} + k_r M_{ATP},$$

where $M_{PCr}^0$ and $M_{PCr}$ are the maximum magnetization and the magnetization at any time ($t$), respectively. The intrinsic $T_1$ of PCr in absence of exchange is given by $T_{1PCr}$. If a secondary frequency, $f_2$, is used to saturate the magnetization of the $\gamma$-phosphate of ATP ($M_{ATP}$), the $M_{ATP}$ is forced to a value equal to zero. If the spin lattice relaxation, $T_{1PCr}$, is measured in the presence of this secondary irradiation, it may be said that:

$$\frac{1}{T_{1PCr}^*} = \frac{1}{T_{1PCr}} + k_f \text{ and } \frac{M_{PCr}}{M_{PCr}^0} = \frac{T_{1PCr}^*}{T_{1PCr}}.$$ 

In this way both the magnitude of $T_{1PCr}$ and $k_f$ may be calculated. The resulting forward flux of the creatine kinase reaction will be equal to $k_f \{PCr\}$. The concentration of PCr can be measured from a fully relaxed spectrum. Additional experiments may be performed to measure the reverse rate for creatine kinase as well as the rate for ATPase and adenylate kinase. Figure 11 demonstrates saturation transfer in muscle.

APPLICATIONS TO CARDIAC TISSUE

Identification and Concentration of Compounds

The ability of NMR to delineate between chemical species has been utilized by chemists for identification for some time. It is not surprising that these powers of identification have subsequently been utilized in biochemical systems. In biological systems, however, identification and quantitation are limited to the more sensitive NMR nuclei. Normally, this kind of NMR detection is possible for those metabolites whose concentrations are at least 1 mM or greater.

The insensitivity of the NMR technique used to observe the phosphorus containing compounds which occur in the heart is sometimes an advantage in that the resulting spectra are simplified. Spectra are further limited not only by insensitivity of NMR spectroscopy, but also by NMR’s inability to detect immobile molecules. With regard to the heart, nucleic acids, phosphoproteins and certain phospholipids are often immobile enough to escape detection by excessive line broadening. Thus, the mobile phosphorus metabolites at high enough concentrations to be detected by NMR in cardiac tissue are phosphocreatine, AW,
Figure 11  Schematic representation of a saturation transfer experiment measuring the forward rate constant of the creatine kinase (ATP:creatinine phosphotransferase, EC 2.7.3.2) reaction. (Top) $^{31}$P NMR spectrum showing resonance lines for inorganic phosphate, PCr, $\gamma$-phosphate of ATP, $\beta$-phosphate of ATP, $\gamma$-phosphate of ATP from left to right. The arrow indicates the position of a saturating narrow band radiofrequency $f_2$. This irradiation is used at frequency equal to the distance between PCr and ATP on the opposite side of PCr as a control. (Middle) $^{31}$P NMR spectra shows $f_2$ irradiation (arrow) at ATP$_\gamma$ and the subsequent “transfer” of its saturation to PCr. (Bottom) Difference spectrum of the above indicating the magnitude of saturated ATP and PCr.

Inorganic phosphate, nicotininide adenine dinucleotide (NAD), phosphadiesters (phospholipids), phosphomonoesters (sugar phosphates, etc). Phosphorus metabolites, such as AMP, cyclic-AMP, ADP, and many other phosphorus intermediates are either not mobile enough or in a high enough concentration to be detected by $^{31}$P NMR in routine \textit{in vivo} spectroscopy. A fully relaxed spectra of the left ventricle of a rabbit heart showing some of the more common phosphorus metabolites is shown in Figure 12.

Since \textit{in vivo} spectroscopy is routinely performed at relatively low magnetic field strengths and with the sample not spinning, the resulting resonance lines of the spectra are often broad. In the absence of gating the acquisition of the spectrometer with the heartbeat, excessive movement of the organ is responsible for a portion of this increased linewidth. Also, there are a number of magnetic susceptibility effects due to the air/water interface which results from the heart’s position between the lungs in the body cavity of the animal. Additional broadness in the spectra can also

\begin{figure}[h]  
\centering  
\includegraphics[width=\textwidth]{figure11.png}  
\caption{Schematic representation of a saturation transfer experiment measuring the forward rate constant of the creatine kinase (ATP:creatinine phosphotransferase, EC 2.7.3.2) reaction. (Top) $^{31}$P NMR spectrum showing resonance lines for inorganic phosphate, PCr, $\gamma$-phosphate of ATP, $\beta$-phosphate of ATP, $\gamma$-phosphate of ATP from left to right. The arrow indicates the position of a saturating narrow band radiofrequency $f_2$. This irradiation is used at frequency equal to the distance between PCr and ATP on the opposite side of PCr as a control. (Middle) $^{31}$P NMR spectra shows $f_2$ irradiation (arrow) at ATP$_\gamma$ and the subsequent “transfer” of its saturation to PCr. (Bottom) Difference spectrum of the above indicating the magnitude of saturated ATP and PCr.}  
\end{figure}
be a result of overlapping resonances (i.e., α-ATP and NAD), homonuclear or heteronuclear coupling (i.e., β-ATP), or contamination of paramagnetic ions (i.e., Mn$^{+2}$ or Fe$^{+2}$).

Several reports indicate the ratio in perfused heart or septum of [PCr]/[ATP] is near 1.1[16, 17]. 

**In vivo** characterizations of the value of these [PCr]/[ATP] ratios are a factor of two higher than those of perfused hearts. These differences may be due to artifacts introduced in the perfused preparation caused by inadequate delivery of oxygen, damage to the organ by removal and handling, and/or differences in substrate composition and delivery [18,19].

Measurements of the relative concentrations of the phosphorus metabolites in cardiac tissue in situ state are important because they reflect the state of the tissue metabolism. The [PCr]/[ATP] and [PCr]/[Pi] ratios are important because they can estimate the values of phosphorylation potential and energy charge to the cell. Thus, it would be expected that measurements of phosphorus metabolites would reflect the changing energy metabolism of the tissue during conditions of excessive work, ischemia and reduced availability of substrate and oxygen. Furthermore, dynamic changes in phosphorus metabolites can monitor pharmacological intervention.

---

**Figure 12** Typical

Typical **in vivo**$^{31}$P NMR spectrum of the rabbit left ventricular myocardium. This spectrum is the sum of 100 free induction decays accumulated in about 13 minutes with an eight second interpulse delay. Resonances are assigned as follows: (a) 3-phosphate of blood, 2,3-diphosphoglycerate plus myocardial phosphomonoesters; (b) 2-phosphate of blood, 2,3-diphosphoglycerate plus myocardial inorganic phosphate; (c) uncharacterized phosphodiester compounds; (d) phosphocreatine; (e) γ-phosphate of ATP plus γ-phosphate of ADP; (f) α-phosphate of ATP, γ-phosphate of ADP and phosphates of NAD[H]; and (g) γ-phosphate of ATP. The scale below represents the chemical shift in parts per million referenced to phosphocreatine at 0 ppm. Because the spectrum was accumulated under nonsaturating conditions, the peak areas of the #2-phosphate of ATP and phosphocreatine reflect their relative intracellular concentrations. (C.R. Malloy, P.M. Matthews, M.B. Smith and G.K. Radda, unpublished data).
An interesting property of certain nuclei is that the chemical shift of the resonance frequency is dependent on the environment of the solution in which it resides. Thus, the frequency of the nuclei is sensitive to a number of properties of the media which include pH, ionic strength, dielectric constant, and metal ion concentration. Given the provision that these nuclei’s possess appropriate pKa, they may be used as indicators of pH within the cell. Inorganic phosphate and sugar phosphates have been used as indicators of pH in physiological systems because they possess pKa’s near neutrality. The resonant frequency of the γ-phosphate of ATP is also pH sensitive but since its pKa is around 5.5, its use is confined to acidic conditions.

Inorganic phosphate has been routinely used to calculate intracellular pH in a number of different tissues [20]. Generally the value of these pH’s is near neutrality within a range of 7.0 to 7.2. The \(^{31}\)P NMR measured pH in Langendorff perfused rabbit and guinea pig heart have fallen within this range [21, 22]. The pH of rat heart in vivo has been found to be 7.35 ± 0.05 which may reflect the pH of the blood in the heart rather than the cytosolic tissue pH [23]. The difficulty found in making the measurement of pH in vivo is that the small inorganic phosphate resonance signal is usually obscured by the signal from 2,3-diphosphoglycerate, 2,3-DPG, present in the blood.

APPLIED STUDIES USING THE NMR TECHNIQUE

Ischemia

The earliest NMR observations of heart were performed on ischemic cardiac tissue, where blood flow or perfusion was partially or completely obstructed. The heart was dissected from the animal, and the changes in the metabolite levels were monitored as ischemia developed. In experiments performed on rat heart, rapidly cooled after its dissection, energy requirements of the tissue were substantially reduced and the pH of the resting muscle was found to be near neutrality. Subsequently, as the temperature was raised to 30°, sporadic contractions of the heart began and increases in the inorganic phosphate concentration were noted as phosphocreatine and ATP were reduced. Concomitant with the changes in metabolite levels was a progressive increase in the intracellular acidity of tissue which decreased to approximately pH 6.0. Subsequent experiments using perfused hearts in which the left anterior descending coronary artery was ligated, revealed changes from an initial starting point of pH 7.4 to an ischemic pH of 6.6 [24, 25]. When reperfusion was allowed, phosphorus metabolite levels and pH returned to their normal values.

Other studies [26] have recorded that during ischemia, contraction of the heart stopped and the high energy phosphates, phosphocreatine and ATP could not be detected by NMR. Upon reperfusion, the cardiac tissue regained its ability to contract and a limited resynthesis of ATP and phosphocreatine was observed. In total heart ischemia, increased concentrations of signal phosphates were observed [27], similar to that seen after extreme contraction in skeletal muscle[28].

Creatine phosphate in ischemic heart muscle measured by NMR results showed more rapid rate and magnitude of change than adenosine triphosphate [29]. These changes observed in vivo correspond well with the temporal changes observed in isolated rat [30] and guinea pig hearts [31, 32]. An approximately 80% reduction of
Creatine phosphate occurs after five to ten minutes post-ischemia [30, 31]. Creatine phosphate values in biopsies of in situ dog hearts, showed an 83% reduction by three minutes, a level sustained for 30 minutes [33]. Following 20 min of ischemia, some investigators found levels of creatine phosphate that were 5% or less of control values [30, 32, 34].

The level of myocardial adenosine triphosphate has been thought to be a good indicator of the severity of myocardial ischemic injury and possibly a major determinant of the ability of the tissue to survive [34, 35]. Recently, however, a dissociation of adenosine triphosphate levels and recovery of myocardial function was demonstrated [36]. Stein et al. demonstrated a progressive reduction of adenosine triphosphate, the trends being noticeable ten minutes after ischemia and reductions of 39% noticeable 30 min after ischemia [28]. Previous observations after ischemia in isolated heart were variable. Some investigators reported similar findings of 30% reduction in ATP by 14–25 min after ischemia [32, 37] and maximal reduction of 40% after one hour of ischemia [32], but these isolated rat hearts were observed during hypothermia. Others reported a greater rate of ATP reduction [31, 34]. A 26% reduction was shown 12 min after ischemia by Nakazawa et al. [31]. A 60% reduction at 15 min and a 78% reduction at one hour was described by Flaherty et al. in isolated hypothermic rabbit hearts [34]. Observations within the first few minutes of ischemia have been made by biopsy of in situ dog hearts [33]. Adenosine triphosphate was reduced by 13% at 30 s and one minute. By three minutes, ten minutes and 30 min, it decreased 42%, 55% and 75%, respectively. These reductions were more prominent than we and most others observed by 31-P NMR [31, 32, 37], although the late changes were similar to those reported by Flaherty et al. [34] in hypothermic isolated rabbit hearts.

In the ischemic myocardium, there was a rapid decrease of creatine phosphate before a marked reduction of adenosine triphosphate took place. A reduction of contractile activity in the ischemic region also took place rapidly. Changes of regional function occur at a time when the levels of adenosine triphosphate are still relatively high. In a previous study, it was shown that systolic wall thickening, for example, decreased following five to ten seconds of complete occlusion of the left anterior descending coronary artery [36]. Cardiac muscle stops contracting when adenosine triphosphate levels reach 4.5–4.9 μmole/g, when only about 20% of the metabolite is depleted [33]. Non-ischemic muscle can maintain contraction and survive at adenosine triphosphate levels as low as 1.5–2.0 μmole/g [33]. This indicates that it is not solely the lack of this metabolite in the cardiac muscle that is responsible for the rapid reduction of myocardial contractility [33]. A deficient transfer of energy-rich phosphates from the site of synthesis in mitochondria to the site of utilization for muscle contraction may contribute to the early reduction of contractile activity [33].

A number of experiments have been performed by Garlick et al. [38] to establish the origin of the acidosis seen during ischemia. The phosphorus metabolism and pH of hearts were monitored during periods of ischemia up to 30 min, whereupon reperfusion was introduced. Features of the ischemic period included the metabolism and pH change previously noted as well as a 25% decrease in the intracellular ATP pool. In hearts of fed rats, the cardiac tissue pH decreased to 6.2 during 13 min of ischemia while in hearts of glycogen-depleted animals perfused with glucose-free media, pH fell only to 6.6 within the same time period. The initial pre-ischemia hydrogen ion concentrations of the tissue were the same in both the treated and untreated hearts. The suggestion is made that the major pathway produced in hydrogen ions in ischemia is glycogenolysis. This assumption is further
supported by the data in glycogen-depleted animals which indicates that the time when ATP disappears (when glycogen is exhausted) correlates to the time when the pH drops no further in its acidity. This effect is not observed in the normal hearts where glycogen concentration would be high. In the same experiment, hearts that would receive a preperfused treatment with 100 mm Hepes in Krebs–Henseleit buffer, displayed a smaller drop in the intracellular pH during ischemia at pH 6.7. If these buffer preperfused hearts were allowed to reperfuse, they showed a much more rapid recovery of phosphocreatine than those which were not treated with buffer.

The mechanism of glycogen involvement in the acidosis of ischemic cardiac tissue was examined in more detail by Bailey et al. [39]. In this experiment, rat hearts were perfused for approximately two hours in the presence or absence of 2-deoxyglucose and subjected to global ischemia. Those hearts receiving the perfusion with deoxyglucose displayed a decreased rate of acidosis which reached the value of pH 6.4 and 6.13 for treated and untreated hearts, respectively. The molecule, 2-deoxyglucose, cannot be fully metabolized in cardiac tissue and accumulates 2-deoxyglucose-6-phosphate which was shown to be an inhibitor of glycogen phosphorylase. Therefore, the use of 2-deoxyglucose inhibits glycogen phosphorylase and therefore reduces intracellular acidosis by inhibiting the pathway from glycogen to lactate.

The mechanism of glycogen involvement in the acidosis of ischemic cardiac tissue was examined in more detail by Bailey et al. [39]. In this experiment, rat hearts were perfused for approximately two hours in the presence or absence of 2-deoxyglucose and subjected to global ischemia. Those hearts receiving the perfusion with deoxyglucose displayed a decreased rate of acidosis which reached the value of pH 6.4 and 6.13 for treated and untreated hearts, respectively. The molecule, 2-deoxyglucose, cannot be fully metabolized in cardiac tissue and accumulates 2-deoxyglucose-6-phosphate which was shown to be an inhibitor of glycogen phosphorylase. Therefore, the use of 2-deoxyglucose inhibits glycogen phosphorylase and therefore reduces intracellular acidosis by inhibiting the pathway from glycogen to lactate.

The values reported for intracellular acidosis are reasonably consistent between pH 6.15–6.6 for global or regional ischemia in rat, rabbit and guinea pig myocardium [22, 25, 34, 40]. During in vivo ischemia, the pH may decrease to values as low as pH 5.8 [41]. Regarding the development of acidosis, Stein et al. noted a prompt reduction of intracellular pH within the first five minutes of ischemia, during which time pH fell from 7.39 to 6.71 units [28]. Further reductions to 6.07 units required 30 minutes of ischemia. Hollis et al. [25], on the other hand, described no change at one minute but a reduction from 7.4 to 6.9 at six minutes. By 40 min, the intracellular pH fell to 6.4 units. Comparable results were shown by others [34]. A greater reduction was noted by Ruigrok et al. [29]. The magnitude of the intracellular acidosis may be influenced by substrate [35, 36], increased with insulin treatment [35], and decreased after propanolol treatment [32]. The increase in ischemia-induced acidosis in vivo results from a number of contributing factors.

Grove et al. [23] were able to monitor the time course of changes in creatine phosphate ATP in inorganic phosphate when the heart was subjected to respiratory and global or focal ischemia introducing a surface coil by an incision in the thorax of a rat. In similar experiments, mechanical ventilation was turned off and the heart was subjected to normoxia, hypoxia and finally anoxia. Subsequently as the heart failed, phosphocreatine was found to decrease rapidly to an undetectable level, ATP was unaffected until the phosphocreatine supply was exhausted and ATP also decreased to an undetectable level. Nunnally and Bottomley [40] have employed the surface coil technique to follow ischemia in the left ventricle by occlusion of the left anterior descending coronary artery in rabbit hearts. Using verapamil (a calcium ion influx inhibitor), and chloropromazine, significant improvements in myocardial viability as judged by higher concentrations of phosphocreatine and ATP were shown.

Ingwall et al. have observed significant increases in ATP synthesis after ischemic insult with the addition of the purine inosin [42]. The treatment was based on the theory that ATP resynthesis following ischemia may be limited to the availability of purine nucleotide precursors. If during the breakdown of ATP during ischemia, purine nucleotides are lost from the cell, ATP resynthesis will occur at a greatly
reduced rate. In another laboratory, Hollis et al. [43] have found that potassium chloride-arrested hearts are preserved against loss of ATP concentration and maintain a pH near neutrality after long periods of ischemia.

In a study in open chest dogs using proximal LAD occlusion, the [PCr] was reduced minimally to levels equal to [ATP] after 2.5 h of ischemia [44]. The maintenance of [PCr] may reflect a measurement of the transmural nature of cardiac ischemia. This is due to the receptive characteristics of the surface coil whose sampling is weighted heavily to nuclei nearest the coil. Earlier reports of transmural gradients of high energy phosphates in cardiac tissue of dogs have indicated highest preservation in the epicardial surface [45]. It is obvious that the use of surface coils in systems where the sample is inhomogeneous, such as in transmural ischemia, must be approached cautiously to avoid artifacts.

Figure 13  Time course of 31P NMR spectra of the ischemic region of the left ventricle accumulated after occlusion of the LAD in a propanolol-treated rabbit. Each spectrum represents the sum of 180 free induction decays accumulated over three minutes with an interpulse delay of one second. The reduction in the phosphocreatine/ATP resonance area ratio in the “control” spectrum relative to that in Figure 12 is due to partial saturation of phosphocreatine (see text for details). The time noted for each spectrum refers to the midpoint of each three minute accumulation after occlusion of the LAD at 0 min. Peak assignments are shown in Figure 12. The resonance “shoulder” that appears to the right of 2,3-diphosphoglycerate arises from myocardial P1. This resonance, which increases in intensity at 15 minutes with time, shifts upfield during ischemia, indicating increasing cellular acidosis. (C. R. Malloy, P. M. Matthews, M. B. Smith and G. K. Radda, unpublished data).
In a recent study, Malloy et al. [41] placed a surface coil over the anterior lateral left ventricle and continuously measured the phosphorus metabolite concentrations and intracellular p\(\text{H}\) in an ischemic region of the rabbit heart while monitoring the hemodynamic parameters by conventional methods (see Figure 13). Since catecholamines stimulate ATP consumption and glycogenolysis in isolated hearts, it might be expected that a protective effect of \(\beta\)-blockers may reduce the ATP consumption or attenuate the intracellular acidosis in ischemic tissue. Two groups of animals were studied, one in which the animals were treated with propranolol prior to the onset of ischemia and the other in which no pretreatment was provided. In the control group, the ATP decreased at a rate of approximately 0.5 mM/g dry weight per minute and the intracellular p\(\text{H}\) decreased approximately linearly to a final p\(\text{H}\) of 5.8. The animals treated with propranolol displayed a rate pressure product significantly reduced for and during the ischemic period and cessation of premature beats and rhythm disturbance after the onset of ischemia. There were no apparent differences between the control animals and treated group, but the rate of decrease in ATP concentration was reduced in the propranolol-treated animals. The observation was made that since the relationship between ATP concentration and glycolytic rate was not consistent in each group, intracellular acidification may occur by differences in regulatory mechanisms. Finally, the study points out that the protective effect of propranolol on cardiac function in vivo is not due to a reduction in intracellular acidosis.

Reperfusion effects of ischemic myocardium were studied by Bailey and Seymour [46]. Consequences of reperfusion are important since it may cause structural damage to the cell effecting metabolism as well as loss of contractile function. Myocardium recovery from an ischemic insult shows a rapid response of [PCr] to levels far above initial preischemic concentrations. This may be due to a rapid decrease of [ADP] which changes the equilibrium of creatine kinase to produce PCr. Further creatine kinase binds to the mitochondria during ischemia possibly providing more available access for mitochondrial ATP. Also cytoplasmic PCr aids ATP transport from the mitochondria to the myofibrals [47].

Recently it has become possible to obtain spectra from a number of human organs including the heart. Figure 14 shows a representative spectrum from skeletal muscle, heart, brain and liver. It is interesting that each time has a characteristic spectral signature which offers interesting diagnosis potential.

A number of reviews covering spectroscopy of cardiac tissue are listed for the interested reader [5, 28, 48–50, 55–57].

**NMR IMAGING**

In 1973, Lauterbur [51] demonstrated the first crude NMR images of two glass tubes containing water. Lauterbur suggested that when a strong field gradient is applied across the sample, each nuclei will have its frequency altered corresponding to its position in the field gradient (Figure 15). Resulting NMR spectrum is a one-dimensional projection of the coincidence of the nuclei along the direction of the gradient. If the gradient is applied many times in different directions across the sample, a set of one-dimensional image projections can be obtained. When these projections are processed in the same way as CT X-ray scans, images may be obtained. Perhaps the earliest NMR “imaging” experiment was performed by Gabillard [52], who studied the dynamics of glass and liquid structures using field gradients to produce one dimensional NMR projections. In
1971, Damadian observed differences in the NMR relaxation times of cancerous and normal tissue and suggested that the human body might be examined by NMR for clinical diagnosis.

Several methods [64] have been employed to generate two and three dimensional image information. These methods include the projection reconstruction method [51], the two-dimensional Fourier transform method [53], and the echo planner technique [54]. It should be noted that most of the major clinical techniques, excluding ultrasound, utilize ionizing radiation. The NMR technique is non-invasive and does not present any known hazard at the static magnetic fields currently being employed for imaging and spectroscopy. Unlike many other imaging modalities, a three-dimensional NMR imaging data set can provide slices in any orientation.

![Figure 14](image)

**Figure 14** Comparison of the $^{31}$P NMR spectra in different human tissues. Results are shown for (a) skeletal muscle over the heart, (b) heart, (c) brain and (d) liver. The downfield resonance is the MDP standard attached to the backside of the coil. Resonance assignments: (1) MDP (external standard). (2) 2,3-diphosphoglycerate, (3) phosphomonoesters, (4) inorganic phosphate, (5) phosphodiesters, (6) phosphocreatine, (7) γ-phosphorus of ATP, (8) α-phosphorus of ATP (plus small amounts of NAD+, NADH), (9) β-phosphorus of ATP. The MDP signals are not to scale in each spectrum. (Unpublished results from M. B. Smith, M. Rajajoplan and G. K. Radda).
In 1976, Mansfield and Maudsley reported the first NMR images in a human from a live finger [55]. Since that time, images have been produced in improving quality of most of the major structures in the body [56]. To date, the highest magnetic field used for human images have been taken at a field strength of 4.0 Tesla, which is undoubtedly a momentary ceiling. Much emphasis is now being placed on the development of clinical NMR systems which will have the capability of not only whole-body imaging but broad-band spectroscopy. For additional information on the technique and theory, the reader is directed to a number of excellent books and reviews [56, 57].

The first proton NMR heart images were demonstrated in a study by Damadian [58]. Since that time, cardiac images have improved dramatically in quality with technical advances which include gating of the NMR acquisition to the patients ECG. This allows the NMR image to be reasonably independent of movement artifacts by receiving signal only when the heart resides in the same position. This is accomplished by synchronizing the data collection of the spectrometer to be triggered by the R-wave of the ECG. When the data is collected in this way, late systolic or early diastolic images may be produced. When no gating is employed to produce cardiac images, the average image produced will be a high percentage diastolic since a large portion of the cardiac cycle is monopolized by diastole. An example of an NMR image of human a heart is shown in Figure 16.

In initial studies of $T_1$ enhanced cardiac images, a contrast difference was seen between the blood in the left and right ventricles [59]. It was suggested that paramagnetic oxygen absorbed in the blood was exerting paramagnetic relaxation effects and thus changing the relaxation time. Further, it was also shown that blood,
when it is moving rapidly, appears as a white region on the NMR image, and when flow is reduced, blood appears as a dark grey contrast. Therefore, in cross-sectional images, it is possible to observe changes in blood flow in structures such as inferior vena cava, the root of the aorta and pulmonary artery, media stinal structures, left main coronary artery, and left anterior descending and circumflex branches. When the appropriate cardiac gating is used, there is considerable diagnostic potentials in the examination of these structures,

During myocardial infarction, the tissue undergoes a degree of edema and hemorrhaging. Williams et al. [60] have observed an increase in the $T_1$ relaxation time of infarcted cardiac tissue in dogs. Other investigators have shown the presence of myocardial infarction in dogs using the imaging techniques dependent on proton density in the ratio of $T_2/T_1$. Some additional enhancement of the infarct area can be produced by the use of paramagnetic contrast reagents such as magnesium. In a well executed study, Steiner and colleagues [61] have performed myocardial infarction in dogs by occluding the left anterior descending coronary artery. Subsequently, the ischemic area in the left ventricle was observed without the use of contrast reagents. These kinds of studies have currently been extended to show myocardial infarction in humans. Go et al. [62] have been able to show regions of myocardial ischemia in patients with acute myocardial infarction as a function of the decreased $T_1$ relaxation time.

Overall, it would be premature to conclude that NMR imaging of the heart will become a routine diagnostic tool. There is sufficient data to warrant further investigation in this area. Currently, there is a great emphasis being placed on the use of paramagnetic contrast reagents which involve the chelation of iron, manganese, and gadolinium or some other lanthanide ion to a larger molecule.
Currently some of these paramagnetic substances are being coupled to tissue, specific substrates, monoclonal antibodies and polymers. Paramagnetic imaging enhancement has also been achieved by the use of stable nitroxide free radicals.

The use of NMR has now been extended from the study of chemical and physical systems to its current new application as a probe of structure and function in living organisms. The implications concerning its role in diagnostic medicine are attractive. While the practicality of NMR in several areas of medicine have been realized, it is premature to suggest that the information it may provide for cardiac diagnosis will supplement or exceed existing techniques. However, the future of NMR in cardiac medicine may certainly be viewed with guarded optimism.

REFERENCES