Nuclear magnetic resonance (NMR) spectroscopy and imaging can be used to investigate, noninvasively, a wide range of biological processes in systems as diverse as protein solutions, single cells, isolated perfused organs, and tissues in vivo. It is also possible to combine different NMR techniques enabling metabolic, anatomical, and physiological information to be obtained in the same experiment. This review provides a simple overview of the basic principles of NMR and outlines both the advantages and disadvantages of NMR spectroscopy and imaging. A few examples of potential applications of NMR spectroscopy and imaging are presented, which demonstrate the range of questions that can be asked using these techniques. The potential impact of using NMR techniques in a biomedical research program on the total number of animals required for specific investigations, as well as the number of animals used in research, are discussed. The article concludes with a personal perspective on the impact of continuing improvements in NMR technology for future applications in animal research.

Key Words: magnetic resonance imaging; MRI; NMR spectroscopy; nuclear magnetic resonance spectroscopy

Introduction

Nuclear magnetic resonance (NMR) spectroscopy and imaging are arguably the most versatile techniques in use in biomedical research today. The phenomenon of NMR was first discovered in the 1940s and was primarily the domain of physicists (Bloch et al. 1946; Purcell et al. 1946). During the next 50 yr or so, applications of NMR developed rapidly and were used first by chemists. The use of NMR to study the structure of proteins and other biological molecules was markedly improved in the late 1960s with the development of superconducting magnets and the implementation of Fourier transform NMR. However, it was not until the mid-1970s that the first applications of NMR to the study of metabolism in living biological systems were reported (Berden et al. 1974; Burt et al. 1976a,b; Hoult et al. 1974; Moon and Richards 1973; Sequin and Scott 1974). At approximately the same time, it was demonstrated that the use of magnetic field gradients could be used to encode NMR signals spatially (Lauterbur 1973), and thus the concept of magnetic resonance imaging (MRI) was born. Soon thereafter, MR images of the human body were obtained (Andrew et al. 1977; Hinshaw et al. 1977), and as early as 1980, the evaluation of MRI as a clinically useful imaging modality had started.

Currently, with continuing advancements in magnet and gradient technology coupled with increasingly powerful computers, NMR spectroscopy and imaging can be used to investigate a wide range of biological processes in systems as diverse as a single cell, isolated perfused organs, and tissues in vivo. It is also possible to combine different NMR techniques enabling metabolic, anatomical, and physiological information to be obtained in the same experiment. In light of the many potential applications of NMR imaging and spectroscopy in biomedical research, it is impossible to provide a comprehensive review within the context of this article. Therefore, the aim of this article is to provide an overview of the technique and to demonstrate the potential utility of NMR-based methods in biomedical research.

We begin with a brief description of the fundamental principles of nuclear magnetic resonance. This description is followed by a summary of the advantages and disadvantages of NMR spectroscopy and imaging compared with other techniques. We also provide details regarding access to NMR systems. The primary focus of the article is to demonstrate the utility of NMR imaging and spectroscopy. To accomplish this goal, we present a selection of examples that we believe best captures the diversity of questions that can be asked using these techniques. We conclude with a personal perspective regarding the future of NMR spectroscopy and imaging in biomedical research.
Basic Principles of NMR and MRI

The following section is by necessity a general overview of the basic principles of NMR presented from a classical point of view. We provide this broad, albeit simplified, description because we believe it includes enough depth to make the subsequent discussions intelligible without confusing the reader with the details of NMR theory. A more complete classical description as well as a quantum mechanical understanding of NMR and MRI can be found in the following literature: (1) general (Blackband 1995; Gadian 1995); (2) NMR (Abragam 1961; Ernst et al. 1987; Farrar and Becker 1971; Harris 1986; Schlicter 1978); and (3) MRI (Foster and Hutchinson 1987; Mansfield and Morris 1982; Partain et al. 1988; Stark and Bradley 1999; Wehrli et al. 1988).

Nuclear Magnetization

The nuclei in the atoms in materials are spinning and charged and, as such, most form magnetic dipoles (hence the nuclear magnetic in NMR). As a simple explanation, these dipoles are often compared with little bar magnets with north and south poles, and we use this tool herein (Figure 1A). Normally these nuclear bar magnets are randomly oriented. The result is that no net magnetic field arises from them and thus no NMR signal can be generated. However, when placed in a strong magnetic field, Bo, on average some of the nuclei “align” with Bo. The result is a net magnetic moment, M, from the sample (Figure 1B).

The net magnetization, M, may be “pushed” away from alignment with Bo by using a second magnetic field, B1, applied perpendicular to Bo with an excitation coil. When the second magnetic field is turned off, M quickly returns to align with Bo and, as it does so, it generates a signal at radiofrequencies in a detector coil placed around the sample (Figure 1C). It is this signal that forms the basis of MR imaging and spectroscopy. To be effective, the applied magnetic field B1 must oscillate at the same frequency at which the nuclei oscillate in Bo so that there is an efficient energy transfer. The condition that the excitation field must be at the same frequency at which the sample oscillates is called the resonance condition, hence the name nuclear magnetic resonance. Most often the signal excitation and detector coil are the same physical coil, which consists of an array of wires designed to generate B1 and detect the signal homogeneously over the region of interest in the sample.

The nuclear bar magnets oscillate as they realign along B0, and the signal detected thus has a characteristic frequency. The exact frequency depends on which nucleus is examined (e.g., hydrogen, sodium, phosphorus) and the strength of the magnetic field, B0. The signal decays due to energy loss from the excited nuclei to the surroundings, a sort of NMR “friction.” This energy loss is called “relaxation” and is different for differing materials with different surroundings. A full description of the relaxation requires two relaxation rates, T1 and T2, which represent different energy loss mechanisms. T1 represents a loss of energy to the molecular lattice, whereas T2 represents an exchange of energy between neighboring spins. Consequently, T1 and T2 can provide separate information about the nuclear environment. It is here that the unique capabilities of NMR can be appreciated. In other words, by placing a sample in a magnetic field and exciting it with a second magnetic field, information regarding the intrinsic nuclear content and the environment of those nuclei can be gained noninvasively and nondestructively.

NMR Spectroscopy

Nuclei in differing chemical environments (different chemical structures) bond to other nuclei by the sharing of electrons; thus, different chemical groups are subsequently surrounded by different electron shells. These electrons also have associated magnetic fields that have the effect of shielding the nuclei from the applied B0 by slightly differing amounts, depending on the number of electrons in the shell. Consequently, nuclei in different chemical groups will resonate at slightly different frequencies, an effect referred to as the chemical shift. When excited, complex chemical samples thus generate multiple signals at different frequencies corresponding to all of the distinct chemical groups that are detected simultaneously in the signal. A technique called the Fourier transform is used to work out the frequencies of the signal, generating a NMR spectrum. This is the basis of NMR spectroscopy, and the information contained within the NMR spectrum can be used to determine the chemical makeup and structure of materials.

For example, the hydrogen (1H) NMR spectrum of ethanol would contain three frequencies corresponding to the three hydrogen groups, with peak heights proportional to the number of 1H nuclei in each chemical group (Figure 2). Larger molecules such as proteins have much more complicated spectra (literally hundreds of peaks), which can be used to determine its three-dimensional solution structure. Consequently, NMR is now an invaluable tool in the determination of biomolecular structures. Thus, by placing a sample in a magnetic field and exciting a signal, the chemical content and structure of the sample can be determined noninvasively and nondestructively.

Several factors contribute to the line widths of the resonance signals in a NMR spectrum. In the context of biological systems, an important issue relates to the fact that small freely moving molecules typically yield narrow lines, whereas large or relatively immobile molecules tend to result in very broad signals. As a result, NMR spectra of tissues and cells consist predominantly of narrow line-width resonances originating from small nonbound molecules, which are often important metabolites such as adenosine triphosphate (ATP), lactate, and N-acetyl aspartate (NAA). In contrast, the broad signals from membranes, phospholipids, and proteins cannot typically be resolved.
Figure 1 (A) Nuclear dipole equated to a bar magnet. (B) When placed in a magnetic field, $B_0$, the nuclear moments align with $B_0$ and generate a net magnetic moment, $M$. (C) $B_1$ pushes $M$ away from $B_0$. When $B_1$ is removed, $M$ returns to alignment with $B_0$ and generates an oscillating signal.
Distinct chemical groups emit signals at different frequencies. Signals with multiple frequency components can be decoded into component frequencies using the Fourier transform.

Figure 2

MR Imaging

To progress from NMR spectroscopy to NMR imaging, or MRI, required approximately 30 yr. (The nuclear in NMRI is usually dropped to make patients less nervous because no conventional nuclear radiation is employed in NMR.) Imaging is achieved through the inspirational step of applying a linear magnetic field gradient on top of $B_0$.

As illustrated in Figure 3A, a simple sample of water placed in $B_0$ will result in a single $^1\text{H}$ peak from hydrogen atoms in the water molecules. When the linear gradient is applied, different parts of the sample are in different magnetic fields and thus signals at different frequencies are observed. The frequency obtained is linearly proportional to the resultant magnetic field ($B_0 + \text{gradient}$), and the frequency is thus linearly proportional to the position along the sample. In this way, a one-dimensional projection of the object is formed. This is the basis of spatial encoding in one dimension and can be extended to provide two- or three-dimensional imaging techniques.

When an unknown object is placed in the magnet, the gradient may be rotated electronically and a series of projections of the object obtained at different angles (Figure 3B). A mathematical technique, called projection-reconstruction (Herman 1980), can be used to take this series of projections and create an image of the object. There are in fact many ways in which two-dimensional encoding can be accomplished (most being variants of the use of phase encoding), making MR the most versatile of the radiological imaging techniques.

Advantages and Disadvantages of NMR Imaging and Spectroscopy

Advantages

Noninvasiveness and Lack of Ionizing Radiation

The greatest asset of MR techniques is their noninvasiveness, which includes studies of isolated tissues and cells where the MR measurement on that sample is noninvasive. The fact that both biochemical (spectroscopy) and spatial information (imaging) can be obtained without destroying the sample is obviously a great asset for in vivo studies. An additional advantage of NMR methods for both imaging and spectroscopy versus comparable techniques is the lack of ionizing radiation. The majority of non-NMR-based techniques used for imaging or for in vivo studies of metabolism involve ionizing radiation in one form or another. For example, computer-assisted tomography (CT) uses x-rays, and positron emission tomography (PET) involves the administration of radioactive tracers. Even traditional studies of metabolic processes in intact cells and organs use compounds labeled with radioactive isotopes of hydrogen and/or carbon. Therefore, the use of NMR imaging techniques instead of x-ray-based methods avoids the exposure of both the investigators and the subject being studied to x-rays, thus eliminating any exposure to potentially damaging ionizing radiation. The ability to use stable isotopes such as carbon-13 to measure metabolic fluxes with NMR spectroscopy, instead of the traditional radioisotope techniques, not only minimizes the...
Figure 3  (A) Application of a linear field gradient causes different parts of the sample to resonate at different frequencies. If the gradient is linear, then the frequency spread is linear with space. Hence, the spectrum is a one-dimensional projection of the sample. (B) Left, a series of projections are collected at different projection angles, which, at right, are used to reconstruct the image of the sample. Generally, M projections are required to form an M x N digital image, where N is the number of points sampling the signal.
exposure of the investigator to radioactivity but also eliminates the need to dispose of radioactive tissues, carcasses, and other ancillary materials that might be contaminated with radioactivity during an experiment. Thus, the use of NMR techniques can improve the safety of personnel as well as reduce experimental costs due to elimination of the disposal of radioactive material.

**Flexibility**

The most attractive features of NMR techniques are the wide range of biological processes that can be investigated using these methods and the variety and versatility of the specific MR techniques that can be applied. For example, it is possible to study glucose metabolism in isolated neuronal cells in culture, in vivo in the brain of an animal, and in humans using the same basic techniques (Alves et al. 1995; Grutter et al. 1998a,b; Manor et al. 1996; Sibson et al. 1998a,b; Sonnewald et al. 1996). This ability to encompass studies of cells, animals, and humans should greatly enhance the translation of knowledge obtained in basic biomedical research to humans. As another example of flexibility, NMR imaging can provide not only outstanding images, especially of soft tissues, but also physiological information such as blood flow or cardiac function. Furthermore, NMR spectroscopy and imaging can be combined, thus making it possible to obtain metabolic, physiological, and anatomical data in a single experiment. The ability to obtain such a wide range of information in a single experiment is not possible with any other modality. For example, PET can be used to measure metabolism or blood flow noninvasively; however, the spatial resolution of the images is relatively poor so that correlation of the results with anatomy is difficult without the use of other techniques. Similarly, although CT can provide rapid, relatively high-resolution cross-sectional images, it cannot provide metabolic or physiological information.

**Disadvantages**

**Sensitivity**

The greatest disadvantage of NMR spectroscopy and imaging compared with other modalities is the intrinsic insensitivity of the methods. The signal that can be generated in the NMR experiment is small and, for practical purposes, most strongly coupled with the concentration of the nuclei in the sample. For example, the human body is composed of ~70% water, and thus a relatively large signal can be obtained from the 1H nucleus in water that is effectively at a concentration in the tens of molar range. Thus, it is possible to measure signals from cubes (voxels) of tissue as small as ~0.3 mm on a side from the human brain, generating the high-quality images used in clinical MRI. Other nuclei in metabolites found in vivo are typically at much lower concentrations. For example, the 31P nucleus in ATP in tissues is at a concentration ~10^{-4} to 10^{-5} orders of magnitude less than water, which, when combined with lower sensitivity of the 31P nucleus relative to 1H, results in a total decrease in sensitivity of ~10^{6}. Because 1 million is 100 x 100 x 100, the cubic volume needed to obtain the same signal will be 100 times larger on each side, or ~3 cm. This calculation is a broad approximation, and other factors contribute to differences in sensitivity between different nuclei. For example, the use of higher field strengths and small surface coils placed closer to the tissue of interest, as is often the case in animal studies, will make it possible to sample much smaller volumes of tissue. Nevertheless, regardless of the field strengths and size of the coil, the NMR signals from water will always be detectable at resolutions approximately two orders of magnitude greater than those of other NMR-sensitive nuclei. Thus, compounds present in submillimolar and certainly micromolar concentrations cannot practically be detected directly in tissues. (However, it should be noted that exogenous contrast agents can be detected indirectly at much lower concentrations through their effects on the relaxation of the water signal.)

The stronger the magnetic field, B₀, the more nuclei on average that align with this magnetic field. Thus, as B₀ becomes stronger, a larger signal can be generated. This effect is the main reason for the current drive in NMR to build stronger magnets, inasmuch as more signal generally means that higher spatial resolution images and better spectra can be obtained. The strength of the magnetic field that can be generated depends on the open aperture (or bore size) of the magnet required. Smaller bore magnets can be made stronger. Hence, present clinical (human sized, i.e., 1 m diameter bore) magnets range up to 3 T (with 4- to 8-T instruments under evaluation); and smaller bore (as small as 6 cm diameter bore) presently reach more than 20 T, with a range of field strengths and bore sizes available between these extremes. As a result, the sample size generally dictates the choice of magnet and field strength; thus, the smaller the sample, the more sensitive the experiments.

**Working in a High-Magnetic-Field Environment**

An inevitable consequence of carrying out NMR investigations is the need to work in a high-magnetic-field environment. No known intrinsic risks are associated with high magnetic fields; however, the presence of the magnetic field can affect equipment routinely used in animal research. For example, electronic monitors and computer-controlled devices may function improperly or not at all. The handling of standard surgical equipment close to NMR systems can also be difficult and potentially hazardous. One of the very few dangers associated with NMR is the potential for ferromagnetic objects that are not held in place to be attracted to the magnet. Due to the nature of the forces involved, the result can be a scalpel, a pair of scissors, or even a gas cylinder becoming a flying object. Fortunately, an increasing amount of monitoring equipment is now available that is designed to function in relatively close proximity to NMR magnets. There is also a growing number of surgical instru-
ments that can be obtained that are nonferromagnetic, thus reducing the potential difficulties associated with working in a high-magnetic-field environment. Additionally, a steel passive shield or an active shield may be placed around the magnet to reduce the magnet fringe fields and minimize the risks. This reduction can be particularly important when the space available to site the instrument is limited.

Motion Sensitivity

Most MR techniques are motion sensitive, especially when spatial encoding using gradients is employed. This sensitivity leads to signal distortions that are visually most evident in artifacts on images or more subtly in quantitative measurements. Some MR techniques such as functional MRI (fMRI) are particularly sensitive to motion artifact, thus great care must be taken not to minimize the distorting effects of motion and thus minimize misinterpretation of data. In animal studies, anesthesia is usually essential to avoid gross movement of the animal during the study. Imaging of the body often requires specific sequences designed to minimize motion. For most studies of the heart, it is necessary to time image acquisition to the cardiac cycle. This process is normally achieved by recording the electrocardiogram (ECG) signal from the subject, which is then used to trigger the MR console and thus control the timing of the image acquisition. This method is called cardiac gating, and by adjusting the timing of image acquisition relative to the ECG signal, it is possible to obtain images of the heart at any point in the cardiac cycle. Cardiac gating may be necessary even when imaging other organs such as the brain because of motion caused by the pulsatile blood flow. In some cases, such as lung imaging, it may also be necessary to gate to respiratory motion or, alternatively, the subject may be controlled via mechanical ventilation.

Availability and Access to NMR Systems

The purchase of a NMR system is normally beyond the means of a single project or investigator. At the time of this writing, a relatively simple system that can be used for imaging and spectroscopy of small animals such as mice costs approximately $1 million. Costs increase with the size of the magnet, allowing investigations of larger animals and with higher magnetic fields that provide increased sensitivity. A state-of-the-art system that can accommodate animals such as dogs or small pigs will cost at least $1.5 to $3 million. Costs associated with renovation and siting requirements can easily total hundreds of thousands of dollars. Once installed, the basic operating costs will be at least $50,000 to $150,000 per year to cover cryogens required to maintain the superconducting magnet. In most cases, it is also necessary to employ an individual full time to manage and maintain the instrument.

As a result of these expenses, the majority of NMR systems are often purchased by a consortium of investigators via mechanisms such as the Shared Instrumentation Grant Program supported by the National Center for Research Resources (NCRR) at the National Institutes of Health. The objective of this program is to make available to institutions expensive research instruments that can be justified only on a shared-use basis, and it is designed to support investigators to obtain commercially available, technologically sophisticated equipment costing more than $100,000. Users of instruments obtained through such funding mechanisms are typically charged an hourly rate that covers the operating costs of the facility. These rates can range from as little as $10/hour to several hundred dollars per hour depending on the instrument and administrative policies of the institution where the facility is located. Many NMR research facilities are supported by regional resource grants awarded by the NCRR, which help offset some of the operational costs and support research and technical development. Therefore, investigators who are interested in using NMR spectroscopy and imaging in their research program can usually gain access to the necessary equipment without having to purchase their own instrument. A list of NCRR-funded NMR Centers is provided in Table 1.

Examples of NMR Spectroscopy and Imaging

Spectroscopy

Several different NMR-sensitive nuclei can be used in the study of biological systems, and the most common are 31P, 1H, 13C, 23Na, and 19F. 31P, 1H-, and 13C-NMR spectroscopy are typically used to investigate cellular metabolism and bioenergetics, whereas 23Na NMR studies usually focus on issues related to ion transport and regulation of ion pumps (Askenasy et al. 1996; Kohler et al. 1991; Van Emous et al. 1998). Fluorine does not occur naturally in biological systems; however, it is a very sensitive NMR nucleus. Therefore, fluorine-labeled compounds can be introduced into cells and used as an indicator of a cellular process such as calcium concentration (Kusuoka et al. 1991; Marban et al. 1987; Steenbergen et al. 1993; Yanagida et al. 1996). 19F-NMR spectroscopy has also been used to follow the metabolism of drugs, such as 5-fluourouracil (Koutcher et al. 1991; Prior et al. 1990). In addition to the diverse cellular process that can be studied using NMR spectroscopy, another advantage of the technique is the wide range of biological systems that can be investigated. NMR spectroscopic studies are routinely carried out on isolated cells, both in culture (Bhakoo et al. 1998).
Table 1 NMR imaging and spectroscopy research centers funded by the National Center for Research Resources (NCRR)²

<table>
<thead>
<tr>
<th>Research Center</th>
<th>Principal Investigator</th>
<th>TEL/Site/Email/Website</th>
</tr>
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<tr>
<td>Biomedical Magnetic Resonance &amp; Technology Center</td>
<td>Paul C. Lauterbur, Ph.D.</td>
<td>Tel: 217-244-0600; Fax: 217-244-1330; <a href="mailto:Bmrl@bmrl.med.uiuc.edu">Bmrl@bmrl.med.uiuc.edu</a>; Web site: bmrl.med.uiuc.edu:8080/</td>
</tr>
<tr>
<td>Center for Advanced Magnetic Resonance Technology</td>
<td>Gary H. Glover, Ph.D.</td>
<td>Tel: 650-723-7577; Fax: 650-723-5795; <a href="mailto:gary@s-word.stanford.edu">gary@s-word.stanford.edu</a>; Web site: www-radiology.stanford.edu/research/RR.html</td>
</tr>
<tr>
<td>Clinical MR Studies at 4.1T: A Research Resource</td>
<td>Gerald M. Pohost, M.D.</td>
<td>Tel: 205-934-9736; Fax: 205-975-1952; <a href="mailto:nmrcenter@uab.edu">nmrcenter@uab.edu</a>; Web site: <a href="http://www.cnir.uab.edu">www.cnir.uab.edu</a></td>
</tr>
<tr>
<td>The Center for In Vivo Microscopy</td>
<td>G. Allan Johnson, Ph.D.</td>
<td>Tel: 919-684-7755; Fax: 919-684-7122; <a href="mailto:egf@orion.mc.duke.edu">egf@orion.mc.duke.edu</a>; Web site: wvwccvm.mc.duke.edu/</td>
</tr>
<tr>
<td>Pittsburgh NMR Center for Biomedical Research</td>
<td>Chien Ho, Ph.D.</td>
<td>Tel: 412-268-3395; Fax: 412-268-7083; <a href="mailto:chienho@andrew.cmu.edu">chienho@andrew.cmu.edu</a>; Web site: info.bio.cmu/NMR-Center/NMR.html</td>
</tr>
<tr>
<td>The Center for Magnetic Resonance Research</td>
<td>Kamil Ugurbil, Ph.D.</td>
<td>Tel: 612-626-2001; Fax: 612-626-2004; <a href="mailto:kamil@geronimo.drad.umn.edu">kamil@geronimo.drad.umn.edu</a>; Web site: <a href="http://www.cmrr.drad.umn.edu">www.cmrr.drad.umn.edu</a></td>
</tr>
<tr>
<td>Resource for Magnetic Resonance &amp; Optical Research</td>
<td>John S. Leigh, Ph.D.</td>
<td>Tel: 215-898-9357; Fax: 215-573-2113; <a href="mailto:jack@mail.mmrrcc.upenn.edu">jack@mail.mmrrcc.upenn.edu</a>; Web site: <a href="http://www.mmrrcc.upenn.edu">www.mmrrcc.upenn.edu</a></td>
</tr>
<tr>
<td>Center for Magnetic Resonance</td>
<td>Robert G. Griffin, Ph.D.</td>
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</tr>
<tr>
<td>National Magnetic Resonance Facility at Madison</td>
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²From the 1998 Research Resources Directory published by the NCRR. The Directory contains additional information regarding the capabilities of these resources and is available on-line at the NCRR web site: www.ncrr.nih.gov.

1996; Sonnewald et al. 1995, 1996) and under perfused conditions (Gamcsik et al. 1995; Lohmeier-Vogel et al. 1995; Papas et al. 1999); in isolated perfused organs, such as kidney, liver, heart, and vascular smooth muscle (Burns et al. 1999; Chatham et al. 1990; Cohen et al. 1998; Colet et al. 1998; Dowd and Gupta 1993; Freeman et al. 1989; Gupta et al. 1989; Hardin et al. 1995; Leach et al. 1998; Raine et al. 1993); and on tissues and organs in vivo, such as the heart, brain, liver, skeletal muscle, and tumors (Barker et al. 1993, 1994; Bhujwalla et al. 1994, 1996; Chavin et al. 1999; Constantinidis et al. 1991; Cortez-Pinto et al. 1999; Jucker et al. 1999; Kushmerick 1995; Laughlin et al. 1992; Zhang et al. 1999).

The examples described below focus on 31P-, 13C-, and 1H-NMR spectroscopy because these are the most commonly used in the study of biological systems. We have concentrated above on a few examples we believe demonstrate the range of potential questions rather than provide a comprehensive survey of all possible applications. It is worth noting that the limitations of applying NMR techniques to biological systems is more a function of the ability to maintain a physiologically viable preparation for the duration of the experiment than it is related to any limitations inherent in the technique itself.

The isolated perfused heart was one of the first biological systems to be studied in detail using 31P-NMR spectroscopy (Ackerman et al. 1980; Garlick et al. 1977; Hollis et al. 1977; Jacobus et al. 1993). A 31P-NMR spectrum of an isolated perfused mouse heart is shown in Figure 4. The spectrum consists of a total of six peaks or resonances, one each from

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the α-, β-, and γ-phosphates of ATP, one from phosphocreatine (PCr\(^1\)) and two from inorganic phosphate (P\(_i\)). The area under each resonance is directly proportional to the concentration of each of the metabolites; thus, from a single spectrum such as this, it is possible to determine the concentrations of ATP, PCr, and P\(_i\). A unique feature of \(\text{^31}P\)-NMR spectroscopy is indicated by the presence of two resonances from inorganic phosphate, namely, its ability to determine pH. The position of the P\(_i\) resonance, known as its chemical shift, is dependent on pH, as first described by Moon and Richards (1973) in their \(\text{^31}P\)-NMR studies of red blood cells. Thus, the two P\(_i\) resonances in Figure 4 indicate the presence of two compartments containing P\(_i\) with slightly different pH. In this case, the two compartments represent the extracellular medium, which has a pH of approximately 7.4, and the intracellular space, which has a pH of approximately 7.0.

It is important to note that although it is possible to measure the concentrations of ATP and PCr using traditional techniques, these techniques involve destroying the sample under investigation. By necessity, such methods provide information at only a single point in time, in contrast to \(\text{^31}P\)-NMR spectra, which can be collected repeatedly over a prolonged period of time and depend only on the viability of the system under investigation. It is therefore possible to investigate the consequences of interventions on the bioenergetics of the tissue of interest. In studies of the heart, one of the most common interventions is the induction of ischemia followed by reperfusion (e.g., Cave et al. 2000; Portman et al. 1997; Schwartz et al. 1992a,b). In such experiments, the changes in ATP, PCr, P\(_i\), and intracellular pH can be measured as a function of time during ischemic period and after reflow. The time required to obtain a spectrum with adequate signal to noise depends on many factors but can be 5 min or less. Thus, in an experiment in which the protocol consists of 20 min of normal perfusion, 20 min of ischemia, and 20 min of reperfusion, 12 separate measurements of the high-energy phosphate content of a single sample can be obtained. Using traditional biochemical techniques, 12 different samples must be frozen and extracted at each time point. We have used the isolated perfused heart as an example here; however, as noted above, similar studies have been carried out in other isolated perfused organs such as liver (Vidal et al. 1998) and kidney (Dowd and Gupta 1995), and in animal studies in which the organ of interest has been surgically exposed and a NMR coil was placed directly adjacent to the organ (Chavin et al. 1999; Portman and Ning 1990; Schwartz et al. 1992a,b; Sorensen et al. 1998).

There is no doubt that \(\text{^31}P\)-NMR spectroscopy is a very valuable tool for investigating tissue bioenergetics; however, the examples cited above have not exploited the truly noninvasive feature of NMR because they focused on isolated organs or surgically exposed tissues. In many NMR studies of tissue metabolism, it is necessary to harvest samples at the end of the experiment for additional biochemical or histological examination. For this reason, the drive to implement noninvasive localized spectroscopic techniques for animal studies has been relatively limited, and much of the driving force for these methods has been their use in studies of metabolic processes in humans (Bottomley 1989). In animal studies, noninvasive spectroscopic techniques are clearly valuable for investigating the evolution of tissue injury over a prolonged period of time or for determining adaptive or developmental changes in response to a physiological stress. This application is especially valuable when the animals

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**Figure 4** \(\text{^31}P\) nuclear magnetic resonance (NMR) spectrum of an isolated perfused mouse heart collected on a 500-MHz NMR spectrometer. The spectrum required approximately 5 min to acquire. P\(_i\), inorganic phosphate; PCr, phosphocreatine; ATP, adenosine triphosphate; PPM, parts per million.
under investigation are rare or expensive (e.g., primates) (Brownell et al. 1998; Dautry et al. 1999, 2000; Kinoshita et al. 1997; Richards et al. 1995).

With recent growth in the development of transgenic mice models of human disease, there is growing interest in the use of NMR spectroscopy for the noninvasive evaluation of tissue metabolism in mice. Because transgenic mice are expensive to produce, it would be preferable in characterizing a new line of animals to be able to monitor the development of phenotypic changes in individual animals over a period of time. The alternative is to sacrifice animals at arbitrarily selected time points. As a result, there has been interest in applying the techniques of localized NMR spectroscopy to mice. An example of this application is shown in Figure 5, in which a $^{31}$P-NMR spectrum of a mouse heart is shown alongside the accompanying $^1$H-NMR image of the heart and the ECG trace from the same animal. The spectrum is in essence the same as that shown in Figure 4, although due to the lower field strength of the NMR system used for these experiments, only a single P resonance is observed. This limited view is due primarily to the lower field strength of the NMR system used for these experiments. The $^1$H-NMR image of the heart was collected during the same experiment. By collecting multislice images covering the whole heart at both systole and diastole, it is possible to determine cardiac function and cardiac mass (Chacko et al. 2000; Wiesmann et al. 2000; Weiss et al. 2000). Thus, in a single noninvasive study, it is possible to determine function, anatomy, and metabolism. In this example, we have shown data on the mouse heart, which technically is the most challenging of the major organ systems to investigate in this manner because it is moving and the left ventricular free wall is only a few millimeters thick. Thus, similar techniques could be used for the study of other organ systems in mice, such as the liver or brain. Nevertheless, to our knowledge, no investigations of that nature have been published at the time of this writing.

The NMR-sensitive isotope of phosphorus, $^{31}$P, is naturally occurring and represents 100% of the phosphate found in biological systems. Therefore, in $^{31}$P-NMR spectroscopy studies of metabolism, the resonances originate from the phosphorus-containing compounds that naturally occur in the tissues. However, the NMR-sensitive isotope of carbon, $^{13}$C, represents only 1.1% of the carbon atoms that occur in nature. The remainder is carbon-12, which is not NMR sensitive. Thus, a $^{13}$C-NMR spectrum of a biological sample will yield relatively little metabolic information consisting of signals from components such as triglycerides, which have many chemically equivalent carbon atoms. Despite this potential handicap, $^{13}$C-NMR spectroscopy has been used to investigate metabolism in cells and tissues for almost as long as $^{31}$P-NMR spectroscopy. If the sample of interest is pro-

![Figure 5](https://academic.oup.com/ilarjournal/article-abstract/42/3/189/778963/).  

**Figure 5** $^1$H- nuclear magnetic resonance (NMR) image of the thorax of a mouse through the heart at end diastole. Selected $^{31}$P-NMR spectra containing the myocardium (A) and a $^{31}$P standard adjacent to the chest (B). The spectra and images were obtained using electrocardiogram (ECG) gated imaging sequences. (C) ECG tracing of the mouse during the study. Reprinted with permission from Chacko VP, Aresta F, Chacko SM, Weiss RG. 2000. MRI/MRS assessment of in vivo murine cardiac metabolism, morphology, and function at physiological heart rates. Am J Physiol Heart Circ Physiol 279:H2218-H2224.
vided with substrates such as glucose or fatty acids, which are highly enriched with carbon-13. $^{13}$C-NMR spectroscopy can be used to monitor the transfer of the $^{13}$C-labeled carbons from the substrates into a variety of metabolic intermediates. Thus, it is possible to use $^{13}$C-NMR spectroscopy to measure the rate of metabolism through a variety of different metabolic pathways. This process is similar to using traditional radioisotope techniques (i.e., $^{14}$C- or $^3$H-labeled substrates), except that no radioactivity is involved, the measurements can be made without the necessity of separating and isolating the intermediates of interest, and information about several pathways often can be obtained in a single experiment. As with all NMR techniques, the principal limitation is sensitivity; thus, the metabolic intermediates that are of sufficient concentration to be observed in most biological systems are glycogen, lactate, alanine, glutamate, and, to a lesser extent, glutamine and glutathione.

$^{13}$C-NMR spectroscopy has been used to investigate glycogen metabolism in liver (Cohen et al. 1981, 1998), heart (Laughlin et al. 1993, 1994), smooth muscle (Hardin et al. 1995), and skeletal muscle (Jucker et al. 1997, 1999). Although not directly involved in energy metabolism, glutamate is in equilibrium with the tricarboxylic acid (TCA) cycle; thus, the appearance of $^{13}$C-labeling in glutamate from $^{13}$C-labeled glucose or fatty acids can be used as an index of oxidative metabolism and flux through the TCA cycle. In a variety of studies, primarily limited to brain (Hyder et al. 1997; Sibson et al. 1997, 1998) and heart (Chatham et al. 1995; Weiss et al. 1992; Yu et al. 1995), the time course of $^{13}$C-enrichment of glutamate has been used noninvasively to determine TCA cycle flux and oxygen consumption in vivo. One limitation of such studies is the necessity of using mathematical modeling to convert the kinetic NMR data into the required metabolic fluxes. Several different models have been published to date (Chatham et al. 1995; Mason et al. 1995; Weiss et al. 1992; Yu et al. 1995) and, as yet, there is no consensus as to the most appropriate model.

$^{13}$C-NMR glutamate isotopomer analysis, pioneered by Malloy and colleagues at the University of Texas-Southwestern (Malloy et al. 1990), is a powerful method for the study of substrate selection and utilization in biological systems. It is also based on measurements of $^{13}$C-label incorporation from $^{13}$C-labeled substrates into glutamate. However, instead of determining the time course of enrichment, the method is based on analyzing the complex line-splitting pattern of $^{13}$C-glutamate resonances observed in high-resolution $^{13}$C-NMR spectra of tissue or cell extracts. This approach is clearly invasive in that it requires the preparation of tissue extracts; however, it has several advantages over traditional radioisotope methods, including the fact that the contributions of up to four different substrates to energy production can be determined in a single experiment. The analysis of spectra from simple experiments can be carried out by hand readily. The analysis of more complex experiments is simplified by software developed by Mark Jeffrey from the University of Texas-Southwestern and is available free of charge. (For more information, their Web site is listed in Table 1.) This approach has been used to study intermediary metabolism in a variety of systems, including cells in culture (Hall et al. 2001), heart (Chatham and Forder 1997; Chatham et al. 1999a,b; Jeffrey et al. 1995), skeletal muscle (Bertocci and Lujan 1999; Bertocci et al. 1997), and vascular smooth muscle (Allen and Hardin 1998).

Although the focus of many $^{13}$C-NMR studies is on glycolytic and oxidative metabolism, it is possible to investigate other metabolic pathways by the appropriate choice of $^{13}$C-labeled precursors. For example, as shown in Figure 6, glutathione synthesis was measured in a line of adriamycin-resistant human mammary adenocarcinoma cells (MCF7adr). The cells had been grown on a collagen sponge matrix and perfused as previously described (Gamsik et al. 1996). After switching to perfusion medium containing $[3,3'-^{13}$C$_2]$-cystine, resonances from $^{13}$C-labeled glutathione began to appear, which reflected the metabolism of the $^{13}$C-labeled cystine through the gamma-glutamyl cycle and the resulting synthesis of glutathione. From such experiments, it is possible to determine the rate of glutathione synthesis and turnover. Because glutathione is a major intracellular antioxidant that plays a central role in the protection of cells from oxidative stress, it is possible that alterations in glutathione metabolism may contribute to different sensitivities to drug therapy or radiation. Thus, by judiciously choosing $^{13}$C-labeled precursors, it is possible to use $^{13}$C-NMR spectroscopy to determine metabolic fluxes noninvasively through a variety of different metabolic pathways.

The most sensitive NMR nucleus is the proton $^1$H. Given the large number of metabolites that contain protons, one would anticipate that $^1$H-NMR spectroscopy would be widely used to study metabolism in biological samples; however, several factors have limited its widespread use. The main obstacle in the use of $^1$H-NMR spectroscopy is that the resonances from water and lipids are several orders of magnitude greater than that of the potential metabolites of interest. The first step in obtaining a $^1$H-NMR spectrum of metabolites in biological systems is the suppression of the very intense water signal. A variety of methods for achieving this suppression have been documented (Duyn et al. 1993; Ernst and Hennig 1995; Moonen et al. 1989); however, these methods are usually motion sensitive and thus limited to stationary samples. Even with excellent water suppression, the presence of large lipid signals can also obscure much of the spectral region of interest. Consequently, $^1$H-NMR spectroscopy has been applied primarily in the brain (Anderson et al. 1994; Barker et al. 1993, 1994; Monsein et al. 1993) and prostate (Kurhanewicz et al. 1993; Narayan and Kurhanewicz 1992; Narayan et al. 1989), which are relatively stationary and have negligible endogenous lipids. To a lesser extent, $^1$H-NMR spectroscopy has also been used in metabolic studies of perfused cells (Pilatus et al. 1997) and tumors (Bhujwalla et al. 1996; Shungu et al. 1992). Among the few investigations of the heart that have used $^1$H-NMR spectroscopy, most have focused on investigations of lipid metabolism (Balschi et al. 1992, 1997). However, a few reports have documented the ability to measure other
metabolites in the heart with this technique (Bottomley et al. 1997; Ugurbil et al. 1984).

In $^1$H spectra, besides the large water resonance, which is usually suppressed, the major resonances observed in the brain are NAA, creatine, and choline. Lactate is evident in many diseases and can be detected very early in stroke models (Monsein et al. 1993). NAA in the brain is thought to be present predominantly in neuronal cell bodies, where it acts as a neuronal marker; and choline increases are indicative of increased membrane synthesis. Future work may
result in detection of smaller resonances arising from lower-concentration metabolites, and the limits of detection are being explored at increasingly higher level field strengths.

**Imaging**

As with spectroscopy, the range of studies used for MRI is very large. In this section, examples of these capabilities are given in the context of this review. For the most part, the applications of MRI on animals have paralleled similar developments in clinical studies, and both have been intimately tied to technological developments. Magnetic resonance (MR) technology is still evolving and continues to operate with increasingly high levels of field strength. Ancillary hardware, which includes radiofrequency and gradient coils used for spatial encoding, are still undergoing improvements; consequently, the applications of MRI continue to grow and evolve.

**Well-established MRI**

Like clinical MR studies, MR on animal models is immediately effective as a standard radiological technique that, compared with other modalities, provides exquisite anatomical pictures of soft tissues. The application of these images for assessing tissue structure and damage is obvious. The images shown in Figure 7 indicate this potential. It is important to

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**Figure 7** Selection of $^1$H images demonstrating the utility of magnetic resonance imaging for assessing tissue structure. (A) Single neuron from *Aplysia californica* (bright central nucleus surrounded by dark cytoplasm). (B) Isolated perfused rat hippocampal brain slice. (C) Formalin-fixed rat brain (courtesy of Dr. Beneviste, Duke University). (D) Formalin-fixed rabbit heart (courtesy of Dr. Forder, University of Alabama at Birmingham). In vivo (E) axial mouse (courtesy of Dr. Salmon, The University of Florida), (F) sagittal rat, (G) axial cat, and (H) sagittal monkey brain images. Note that the fields of view (size of the image) increase from $\sim$500 μm in (A) to $\sim$16 cm in (H). (I) Live image of a normal 11-day-old rat pup, and (J) a similar animal with hydrocephalus (note enlarged ventricles). (K) Live image of a normal rat brain, with (L) a diffusion-weighted image after inducement of a stroke, which shows hyperintense region on the left.
Restate that the size of the radiofrequency coil used to detect the NMR signal dictates the signal-to-noise ratio that can be attained; thus, higher levels of resolutions are feasible on smaller samples. Additionally, animal studies have often utilized excised tissues to provide improved access and control. The examples in Figure 7 indicate the wide applicability of MRI on samples as small as single cells (spatial resolutions of 10-20 μm) through excised (fixed or perfused) tissues (with resolutions of 20-100 μm), through live whole animal models (resolutions of 100-300 μm). The figure also includes two examples of pathology in rats. The choice of animal model of course is dependent on the biological questions being addressed. Mice, rats, and rabbits are commonly used; however, in principle, any animal can be imaged. This principle has also led to MRI’s use as a diagnostic tool in clinical veterinary medicine; however, this potential is beyond the scope of this article and is therefore not explored.

Signal differences (contrast) observed in images between tissues are caused by variations in tissue water content, T1 and T2. By varying the imaging sequence, the image contrast can be controlled and varied, allowing MRI to discern a wide range of pathologies, including stroke (ischemia) from blood vessel rupture or blockage, cancer, multiple sclerosis, and muscle atrophy. Image contrast can also be improved with the use of injectable contrast agents, mainly Gd-diethylenetriamine penta-acetic acid, which has the effect of changing the local magnetic field (hence, T1 or T2 in the tissue), leading to changes in signal and contrast. Contrast agents, in particular, are especially effective in highlighting regions in the brain where the blood brain barrier has broken down, thus identifying tumors that would otherwise be difficult to see. By using multislice or volume acquisition MRI imaging sequences, three-dimensional information may be obtained. Thus, the size and extent of normal or pathological tissues can be extracted and followed over time in multiple studies.

The amount of information required will dictate the data acquisition time required. The higher the spatial resolution required, the larger the sample volume and number of different sequences with different contrast required, and the longer a single study will be. These choices are modulated by the temporal resolution required and the ability to maintain the animal under anesthesia (which in turn is related to the health of the animal). Although the length of studies can range from minutes to many hours, depending on the specific nature of the experiment, typical total data acquisition times are approximately 1 hr. The sensitivity of the animal to repeated anesthesia will also affect the time difference and frequency of subsequent studies on the same animal.

**Evolving MRI Techniques**

MR signals can be modulated to encode information other than T1, T2, and proton density in many ways, and these methodologies are in various stages of development. Although some are as close to becoming routine on animal systems as they are on clinical instruments, we believe that with MR technology still improving, the extent to which these techniques can be applied is still evolving and is, in fact, the focus of much of the current developmental research. Furthermore, because of commercial issues, the drive to develop animal machines is understandably not as strong as the drive to develop human clinical instrumentation; thus, technology for animal applications lags behind. Although the increase in field strengths helps improve the signal-to-noise ratio, this increase entails new challenges that must also be met. T1 and, to a smaller extent, T2 change with field strength so that the image sequence timings are not the same and must be reevaluated. Artifacts from magnetic field inhomogeneities and sample-induced inhomogeneities (called susceptibility effects) become significant at higher fields and cause image distortions that must be addressed. Radiofrequency coil design and power issues also require much further investigation. In these areas, we are still in a growing phase of MR technology, and we are certain there are surprises yet to come.

MR as described above is rarely quantified in human studies, although some degree of quantification is more prevalent in animal research. Additionally, we do not yet understand the origins of the signals and how they change in tissues—the development of mathematical and physical models is an area of strong research at present. This research, especially using a variety of physical models, will be most effectively pursued on animals systems in which real controls are feasible, and the system may be more accurately perturbed and controlled. It is our long-term hope that MR may to a large extent become quantitative, ultimately leading to improved sensitivity and specificity for the technique. An overview of evolving techniques applied to novel research areas can be found in Gilles (1994), and references to the techniques described briefly below can be found in this and the MRI texts referred to in the Introduction.

As the stability of MR technology has improved, so has the sensitivity of the measurements that can be made. One of the first advances was the realization that moving spins cause the MR signals to change (a spin that moves is not properly rephased by the imaging sequence), which leads to a signal loss that is proportional to the distance the spin has moved. One of the largest (and hence easiest to measure) “movement of spins” arises from blood flow. Images can be sensitized to the blood flow to generate MR angiograms. MR angiography is relatively straightforward, and its clinical use is increasing, particularly as a screening technique. However, it is not yet preferred over the gold standard of invasive x-ray angiography. One of the limiting factors is that spatial resolution restricts the examination to the larger vessels. The method is also sensitive to motion artifacts; thus, application of MR angiography to the heart is problematic. Nevertheless, MR angiography is gaining wider acceptance and is being applied not only to the brain, but also to limbs and the body.

In principle, any moving spin can be encoded in this way; however, the smaller the motion, the less the signal change and the greater the susceptibility to error that will arise from larger confounding motions (such as the animal moving), which could be misinterpreted. Despite these diffi-
culties, the MR signal can be made sensitive to water diffusion. An early application of the technique involved an animal model of stroke in which an ischemic region is clearly well defined in a diffusion-weighted image before any other signal changes can be detected (Lebihan 1995; Mosley et al. 1990). Furthermore, with appropriate encoding, the preferential direction of water diffusion can be quantified, leading to diffusion tensor imaging. These diffusion images are currently being intensively investigated because the anisotropic diffusion thus measured in some way represents the underlying tissue structure. At the time of this writing, the research goal is to identify these structures. However, in more simple cases, the cause of the diffusion anisotropy is clear, leading to the generation of three-dimensional maps of nerve fibers that can be used to track fiber bundles in the central nervous system. Diffusion tensor MRI has also been used to measure fiber organization in the isolated perfused heart and has demonstrated distinct advantages over traditional histological methods for obtaining similar information (Scollan et al. 1998).

MR images also can be sensitized to other physical processes and lead to the generation of, for example, temperature maps (Jolesz et al. 1988; Mulckern et al. 1998; Wang and Plewes 1999), current density maps (Beravs et al. 1997; Bodurka et al. 1999; Joy et al. 1989, 1999), and pH maps (van Sluis et al. 1999). However, great care must be taken in the collection and interpretation of these data. Essentially, they all rely on the same information change in the NMR signal (phase changes); thus, it must be clear that the phase changes result from the variable of interest and not other factors (in particular, motion). For the most part, the utility of these methods is still being evaluated.

The development of so-called functional MRI (fMRI) has generated great interest and has stimulated a flurry of activity in the neurological sciences. During the early 1990s, when MR systems were sufficiently stable and sensitive, it was realized that MR signals were sensitive to local magnetic field changes induced by the change of oxyhemoglobin to deoxyhemoglobin, compounded by blood flow changes when the brain tissue is required to work. Surprisingly, these signal changes can be observed in the brain in local tissues that undergo activation; for example, image contrast is generated in the occipital cortex when the eyes are visually stimulated. Similar localized brain MR signal changes can be observed after a variety of stimulations, including motor (Baudendistel et al. 1996; Deiber et al. 1999; Miezien et al. 2000; Sadato et al. 1997), verbal (Ojemann et al. 1998; Reichle et al. 2000), acoustic (Belin et al. 2000; Giraud et al. 2000), and emotional stimulation (Schmahmann and Sherman 1998; Sprengelmeyer et al. 1998; Teasdale et al. 1999). These observations have ignoted the neurological sciences, offering the potential to visualize brain function directly, noninvasively, and repeatedly. However, the interpretation of the subtle signal changes, a few percentages at best, is fraught with danger; and the development of animal models of fMRI is thus desirable, if not essential. However, the use of animal models in fMRI studies is difficult in terms of developing meaningful stimulation paradigms, especially because the animal most often must be anesthetized, which often negates the activation effects. Consequently, although fMRI development in animal models offers great potential for elucidating the mechanisms of basic brain function, as well as validating fMRI against invasive techniques, it is still a "work in progress" rather than a fully developed technique in routine use.

As stated above, the $^1$H nucleus is most often used in MRI because the relative signal-to-noise ratio is very large (water in molar quantities) compared with signals from other nuclei (millimeters or less). Consequently, the spatial resolution that can be achieved using other nuclei is very poor. Nevertheless, there have been some attempts to use $^{23}$Na, $^{31}$P, and $^{13}$C with imaging techniques. Although we are not yet aware of any significant application of these methodologies, it is still early. Several investigators have speculated that very high fields may have their most significant applications through the studies of nuclei other than the hydrogen nucleus.

Finally, differences between NMR spectroscopy and MRI become less distinct when it is realized that spatially localized spectroscopy is feasible. Although many techniques exist, the most effective techniques involve using imaging methodologies to localize single or multiple regions of tissue spatially and to collect spectra from them. More often it is realized that MR techniques offer the possibility of obtaining structural (MRI), physiological (e.g., diffusion, flow, fMRI), and metabolic (spectroscopy) information on the same tissue, if not simultaneously, at least in an interleaved fashion. The subsequent ability to look at the relations between function, structure, metabolism, and mechanics demonstrates both the flexibility and great potential of MRI.

Impact of NMR Spectroscopy and Imaging on Animal Welfare

Simply stated, the use of NMR spectroscopy and imaging as part of a biomedical research program can significantly reduce the number of animals required for a specific project. For example, consider a study in which the goal is to determine the changes in concentration of ATP every 6 min in an isolated perfused organ in response to a physiological stress such as ischemia during a period of 1 hr. Assume the aim is to investigate some new therapy or treatment designed to minimize the effects of the stress, and there will be two experimental groups—control and treatment. Traditional biochemical methods would require measuring individual samples at each time point. Thus, if we require a sample size of five in each of the two experimental groups, we need a total of 100 animals for the study (2 groups $\times$ 5 samples $\times$ 10 time points $= 100$). In contrast, if we use $^{31}$P-NMR spectroscopy, we require only 10 animals because all of the time points can be measured in each sample (2 groups $\times$ 5 samples $= 10$). This total would represent a reduction of 90% in the number of animals required for that project.
The development of stronger NMR magnets will continue at a program, it should be possible to reduce significantly the number of animals used in research, thereby also decreasing the number of samples required to obtain statistical significance. When using NMR imaging techniques, it is possible to select specific time points that will yield the most information. Currently, ultrasound is the only other method that is routinely used to image mice noninvasively; and although it is a relatively cheap and quick method, it does not provide the detailed anatomical information that can be obtained with MRI.

The examples cited above have focused on the use of isolated organs or small animals; however, NMR imaging and spectroscopy may have even greater impact in studies on larger animals. The cost of purchase and husbandry of larger animals such as dogs and primates has increased dramatically. Clearly, the ability to perform repeated, noninvasive measurements of structure, function, and metabolism in such valuable animals would represent a major cost savings. Furthermore, when using NMR methods, the measurements are made repeatedly in the same sample; thus, the statistical power of the study should also improve. Consequently, in cases in which differences between groups are small, the use of NMR techniques would decrease the number of samples required to obtain statistical significance compared with more traditional methods. Therefore, by including NMR techniques in a biomedical research program, it should be possible to reduce significantly the number of animals used in research, thereby also decreasing experimental costs.

Future Directions

The development of stronger NMR magnets will continue at least in the near future. For example, during 2000, a horizontal 11.7-T, 40-cm bore NMR system (the first in the world) and a 17.6-T, 9-cm bore magnet (one of three in the world) have been installed at the Advanced Magnetic Resonance Imaging and Spectroscopy facility at the University of Florida, Gainesville, Florida. In addition, the National High Magnetic Field Laboratory in Tallahassee, Florida, is presently constructing the world’s first 21.1-T, 11-cm bore magnet. Although the widespread use of such specialized instruments is limited, they will provide a platform for improving our understanding of the fundamental biophysical processes that underlie the characteristics of NMR signals in biological systems. Technological developments, particularly in radio-frequency coil design and high field imaging techniques, will be required to use these instruments effectively so that their potential can be fully realized.

Some potential exists for the development of so-called niche magnets and systems for animal research. Although more of an issue in human studies, real-time interventional studies require easier access to the sample under study than presently feasible with conventional closed cylinder geometry. Open magnet geometry’s (e.g., General Electric’s [Milwaukee, Wisconsin] double doughnut-shaped system,) or other C-shaped magnets afford some degree of open access and may be applied in animal studies. Additionally, Bruker Instruments Ltd. (Billerica, Massachusetts) has determined a need for high-throughput and low-cost MR studies for the rapid MR evaluation of relatively large numbers of animals aimed at the pharmaceutical industry. In response, they have developed a mid-field low cost (approximately $500K) system designed to perform basic MR scans on rats and mice in just a few minutes. Although more sophisticated and time-consuming MR measurements are thus precluded, the hope is that this use will promote the viability of using MR for drug testing. The practicality and utility of this approach are being evaluated.

Probably the largest expansion in the use and utility of MR will be explored through a multimodality approach. Small-scale CT and PET instruments (called microPET; Cherry and Gambhir 2001) have become available, and there is considerable interest in combining these imaging modalities with MR. The superior spatial resolution of MR already provides localization for PET, CT, and radiation therapy through image overlays and stereotactic procedures. Further integration may be warranted through the construction of multimodality instruments.

We believe the development and application of MR techniques will continue along the lines indicated in this review, particularly through efforts to increase the functional information MR can provide and to examine relations between structure, function, and metabolism. However, some of these areas require the following additional comments.

Our knowledge regarding the regulation of gene expression and our ability to manipulate gene expression in cells and animals are increasing rapidly. As a result, there will be an escalating need to evaluate the relation between alterations in gene and protein expression with cellular function. Such investigations will necessitate the quantitative measurement of a wide range of physiological and metabolic processes in intact biological systems. Thus, in the future, the demands for noninvasive, quantitative measurements of anatomy, physiology, and metabolism in transgenic animals will provide additional impetus for the development and application of novel NMR techniques.

We expect that fMRI techniques, which offer great potential for understanding brain function and connectivity, will continue to be investigated. The successful development and exploration of animal models will be key to an understanding of these issues because they can be perturbed in a controlled fashion. Coupled with access to animal brain atlases presently being developed for availability on the Web, the relations between structure and function can be accurately explored.
Targeted agents are being developed for a variety of applications, and the most exciting are those developed to highlight a particular cell or tissue type (Aime et al. 2000; Kayyem JF et al. 1995; Loui et al. 2000; Sipe et al. 2000; Sipkins et al. 2000). These so-called “smart” contrast agents offer great potential for improving the specificity of MR techniques. However, intrinsic difficulties remain in making the agents nontoxic and particularly in the delivery of the agent. Most agents are restricted to the extracellular space that limits the information they can provide. Efforts are under way to construct agents that can enter particular cells, offering many possibilities for assessing function and detecting abnormal cell populations. Aspects of these studies lead to the concept of molecular imaging with MRI in which contrast agents are attached to molecules such as antibodies targeted to specific molecules and receptors (Sipkins et al., 2000). This kind of molecular imaging is analogous to that used in other diagnostic techniques such as PET (Phelps 2000a,b).

Summary

It is clear that MR is still an evolving technology, and a few more surprises are undoubtedly around the corner. Already, MRI is the most versatile and widely applicable imaging modality available today. The combination of MRI and NMR spectroscopy enables us to obtain information from the molecular level through cells, tissues, animal models, and humans. The necessity of animal models in research dictates the development of dedicated animal research systems. With proper use, MR offers the potential for greatly reducing the number of animals that would otherwise be required to move forward in our understanding of intact biological systems.

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Acknowledgments

We thank Drs. Weiss and Gamcsik for providing Figures 5 and 6, respectively, and Drs. Forder, Beveniste, and Salmon for their contributions to Figure 7. This work has been supported in part by National Institutes of Health (NIH) grants HL48789 and HL67464 and an American Heart Association grant-in-aid to J.C.C., and NIH grant NS36992 to S.J.B. Dr. Blackband also gratefully acknowledges the support of the University of Florida Brain Institute and the National High Magnetic Field Laboratory.