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NMR STUDIES OF TISSUE METABOLISM

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CONTENTS

Perspectives and Summary

During the past six or seven years, high resolution nuclear magnetic resonance (NMR) has been established as a powerful tool for the study of cellular metabolism in isolated cells and organs. The non-invasive nature of the measurement offers many advantages over conventional analytical techniques, but limitations on sensitivity restrict the method to the observation of molecules present in the cell at relatively high concentrations (in the order of 0.5–1 mM). Inevitably, many of the initial investigations tended to confirm existing knowledge, but this was important in establishing the validity of the NMR method. In the last few years, NMR has contributed new and interesting information on several aspects of metabolism and metabolic regulation. The majority of reports deal with ^{31}P, ^{13}C, and, to a lesser

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extent, ^{1}H NMR. The particular areas of interest include measurements of metabolic fluxes in the steady state and transport processes, observations of cellular pH and H^+ ion distribution, and compartmentation and spatially resolved NMR of whole animals. The ability to directly observe the intracellular milieu is perhaps the major single advantage of NMR. A very large number of different systems can be studied, but it is important to keep in mind that NMR is simply an additional method for studying metabolism and must always be combined with carefully designed biochemical experiments.

Why Study Metabolism by NMR?

Historically, the study of metabolism is one of the oldest branches of biochemistry, and the well-known "metabolic charts" are unlikely to be changed by the introduction of the relatively recent technique of NMR. But an understanding of metabolism requires considerably more than a description of pathways. We are dealing with cellular dynamics in which the energy-producing and -consuming reactions are controlled and the different forms of energy are tightly coupled. In this article we describe how recent developments in NMR can be used to study some of the problems associated with control, energetics, and dynamics in whole tissue metabolism. We assume a basic knowledge of NMR as applied to cellular metabolism since there have been a number of recent review articles on the subject (1–5).

A Statement of the Problems

Developing a new method is interesting in its own right but the end value of the work must depend on the contributions the technique offers to the solution of specific questions. Some of the outstanding problems in cellular metabolism that can be tackled by NMR are enumerated below.

1. The concentrations of metabolites determined by techniques other than NMR generally rely on measurements of total amounts of metabolites within the tissues. It is essential to evaluate the "free" (i.e. metabolically available) concentrations, which may differ from measured values as a result of tight binding of metabolites to specific macromolecules.

2. The cellular environment has an important role in determining molecular structures, interactions, and functional activities. We need to increase our knowledge about the environment of molecules within the cytoplasm and the various cellular compartments.

3. Compartmentation of small molecules is in some cases known, and is invoked in others to explain deviations from expected behavior. We need to devise experiments to evaluate the distribution of molecules within the cell.

4. The knowledge of in vivo fluxes is a prerequisite for the definition of cellular dynamics. Both rate-limiting reactions and those operating close to equilibrium must be followed in vivo.

5. A central hypothesis of metabolic control is the presence of "regulatory enzymes." Their regulatory role is normally inferred by studying the properties of the isolated enzyme and by showing, from measurements of mass-action ratios, that the appropriate substrates are far from equilibrium in vivo. There are, however, several notable anomalies (e.g. pyruvate kinase) based on this approach. It is therefore important to measure interactions with regulatory ligands and enzyme activities in vivo.

6. Most tissues and organs are composed of a heterogeneous assembly of cells. In some cases, careful dissection of an organ (e.g. kidney) can be used to prepare different cell types. It is, however, necessary to determine to what extent cell heterogeneity has a role in the function of the whole intact organ.

The NMR Observation

High resolution NMR of tissues and cells essentially detects only mobile molecules, because highly immobilized molecules give signals that are too broad to observe. Thus signals are observed from metabolites, but not, in general, from macromolecular structures such as membrane phospholipids and DNA. However, special techniques are available for observing immobile molecules, as illustrated by some ^{31}P NMR studies of bone (6). The NMR spectrum is characterized by the position (frequency) of the absorption (which yields chemical identification), by the intensity of the signal (which gives quantitative analytical information), and by the two relaxation times (which give information about molecular motion and interactions).

The most suitable nuclei for high resolution studies of organelles, cells, and whole organs are ^{1}H, ^{13}C, and ^{31}P, and we restrict our discussion to these nuclei. We concentrate on studies of whole tissues, although some reference is made to work on isolated cells and subcellular organelles.

Identification and Quantification

^{31}P NMR spectra have been recorded from a variety of cells and perfused organs (e.g. 1, 5). In general, assignment of ^{31}P resonances is relatively simple, since the chemical shift range for biologically occurring phosphates is large (~40 ppm), and only components present in a mobile form in concentration larger than about 0.5 mM are detected. The major signals are from ATP (3 resonances), ADP (2 resonances, but generally obscured by ATP), phosphocreatine, P_i, sugar phosphates (mainly glucose 6-phosphate), 2,3-diphosphoglycerate (2 peaks in blood and red blood cells), and AMP (e.g. in kidney). In addition, hitherto unsuspected metabolites are detected by their signals in the phosphodiester frequency range in spectra from rabbit

muscle, frog gastrocnemius (7–9), and dystrophic chicken pectoralis (10). On the basis of chromatographic separation and chemical analysis, these compounds were identified as glycero-3-phosphorylcholine in mammalian muscle (7) and as L-serine-ethanolamine phosphodiester in dystrophic chicken muscle (10). The presence of several such compounds in toad muscle (9, 11), kidney (12), brain (13), and rabbit heart (14) was also reported, though they have not yet been chemically identified. It was also shown that the concentration of glycero-3-phosphorylcholine in Duchenne dystrophic quadriceps was significantly lower than that in normal human quadriceps, whereas elevated levels were detected for muscles with Werdnig-Hoffman syndrome (15). The search for the function of these compounds continues (16).

We note the very low levels of phosphocreatine in liver (17) and kidney (18), a fact that is used in the spatial selection of signals from in vivo organs (see below).

Quantification of the metabolites is based on the fact that the NMR signal area, or intensity, is proportional to the total amount of the detectable species. The derivation of an absolute concentration is, however, not simple and has been achieved only in a few instances [for a detailed discussion of the problems see (4)]. The concentrations of ATP and phosphocreatine determined by NMR and freeze extraction are in broad agreement for frog sartorii (11) and perfused rat heart (19). This implies that NMR detects the full intracellular content, within ~20%, of these compounds.

In contrast, the ^{31}P spectra from muscle and brain of live rats show that the analytically determined ADP and P_i levels are considerably higher than those measured by NMR (20). This is also true for P_i in perfused heart [see, for example, spectra in (21)]. The suggestion (20) that estimates of the phosphorylation potentials in intact tissues derived from freeze extraction experiments are considerably lower than the real values has important consequences in relation to the thermodynamic considerations of oxidative phosphorylation.

Measurement of the upper limit to the concentration of mobile ADP can be obtained directly from NMR spectra. However, even if the ADP signals are not detectable, the concentration of free ADP can sometimes be evaluated if the creatine kinase reaction is close to equilibrium within the tissue. This is because it is possible to estimate, primarily by NMR, the concentrations of all the substrates of this reaction other than ADP. In resting skeletal muscle, the creatine kinase reaction is close to equilibrium (see below), and ~20 μM is obtained for the free ADP concentration in anaerobic frog muscle at 4°C (22). This very low value has profound implications with regard to theories of metabolic control. Furthermore, if the adenylate kinase reaction is also shown to be at equilibrium, the level of free AMP deter-

mined from this reaction would be about 0.1 μM. This value is orders of magnitude lower than the value normally quoted for AMP, and again this would have most interesting effects on our ideas about metabolic control.

The discrepancy in the ADP concentrations measured by NMR and by other means is still unexplained. In muscle, the explanation is straightforward, for it has been assumed for some time that a large percentage of the ADP is tightly bound to the proteins of the myofilaments. Presumably this ADP is too immobilized to generate detectable signals. In brain, there may be unavoidable breakdown of high energy phosphates in the extraction procedures used; this would explain the relatively high levels of both ADP and P_i measured in this way. Alternatively, a significant fraction of the intracellular content of these compounds could be sequestered in such a way [e.g. in the mitochondria (23)] that it generates no detectable signal. Unfortunately, the NMR signals from metabolites within the mitochondria have yet to be fully characterized. Ogawa et al, in their studies of purified rat liver mitochondria, have observed signals that can be assigned to intramitochondrial ATP and P_i (24). However, their experiments were done at 0°C and the nature of the signals from the mitochondrial compartment requires further quantitative study.

The assignment and quantification of ^{13}C resonances is not as simple as for ^{31}P NMR spectra. Using ^{13}C-enriched substrates, Shulman and his collaborators have, however, identified a large number of resonances in isolated cells (3) [e.g. liver cells (25)] and in perfused liver (26). In general, only relative concentrations have been reported.

Because of the large number of overlapping peaks and underlying broad signals, observation of proton resonances from mobile components requires special pulse sequences (27, 28). Using these procedures in studies on red cells (27, 29) and a variety of gland storage systems (28, 30), signals from small molecules (e.g. glutathione, lactate in red cells, and adrenaline in the adrenal medulla), and from some parts of proteins (e.g. the histidine resonance of hemoglobin, chromogranin) have been assigned. The redox state of the red cells can be deduced from the nature of the gluthathione signals (27).

Cellular Environment

MOLECULAR INTERACTIONS The NMR signals obtained from intracellular components depend on the cellular environment and on specific interactions within the cell. In general, the resonances are broader and the relaxation times shorter than in aqueous solutions of similar ionic strength to the intracellular milieu. An attempt has been made using HeLa cells (31), to analyze the numerous factors responsible, but only tentative conclusions

could be drawn. Detailed relaxation studies in storage granules have yielded more information (see below). In an interesting series of papers, Fossel & Solomon (32, 33) presented results that were interpreted in terms of a network of enzymes involving the membrane of the red blood cell and phosphorylase kinase, phosphoglucomutase, and glyceraldehyde 3-phosphate dehydrogenase. The experiments were based on observations of very small chemical shifts in the ^{31}P spectra of intracellular 2,3-diphosphoglycerate under different conditions (e.g. on addition of oubain outside the cell). The validity of these experiments however, has been seriously questioned (34).

The fact that over 90% of cellular ATP is complexed with Mg^{2+} was deduced from the first study on muscle, as the three ^{31}P resonances of ATP are shifted in a characteristic manner (35). Similar observations have been made on other tissues (18, 36) and cells (37). Recently, measurements on the extent of ATP complexing with Mg^{2+} have been used to derive the free Mg^{2+} concentration in erythrocytes (38) and frog skeletal muscle (39). The value of 0.6 mM for the latter system is very low in comparison to the 4.4 mM (amended to 3mM), estimated from the effect of Mg^{2+} on the T_2 relaxation times of the phosphocreatine signal in frog gastrocnemius (40). Both of these values rely on assumptions that may not be totally valid. For example, the value of 0.6 mM was obtained by deducing from the chemical shifts of the ATP resonances that 93% of the intracellular ATP is complexed to Mg^{2+}. The latter figure relies on very precise measurements of chemical shifts and on the assumption that no intracellular features other than Mg^{2+}, pH, and ionic strength have any detectable effect on the ATP chemical shifts. This assumption, if proved, would be surprising in view of the very specific way in which (Mg)ATP binds to a variety of macromolecules. In our opinion, the chemical shifts observed in frog skeletal muscle suggest a lower limit of about 0.5 mM for the free Mg^{2+} concentration, but cannot provide an accurate upper limit.

Since the first report by Moon & Richards on erythrocytes (41), ^{31}P NMR has been widely used to measure the intracellular pH in a variety of systems (1–5). pH can be measured from the frequency of ^{31}P NMR signals of phosphate groups of pK values close to the pH. The two ionic species are in "fast exchange," and the frequency of the observed resonance is therefore determined by the relative amounts of the two species concerned.

The validity of the pH measurements requires some assumptions about intracellular environment (e.g. ionic strength and composition) and about the lack of effects (e.g. binding to protein) that might shift the resonances in an unpredictable way. Nevertheless, there is now general agreement that cytoplasmic pH in muscle (11), heart (42, 43), kidney (18), and other tissues can be reliably measured. Some recent important developments include the

recognition that contributions from extracellular P_i must and can be excluded (42, 43), and that the use of more than one pH marker is an advantage (44). For example, the uptake of deoxyglucose into perfused rat hearts results in the accumulation of deoxyglucose 6-phosphate in the cytoplasm. From the resonances of this molecule, pH values were obtained identical to those measured from the P_i resonance. This confirms that both P_i and the sugar phosphate measure cytoplasmic pH, and the criticisms that the possible sequestering of P_i into the mitochondria distorts the pH measurement (45, 46) can be discounted. Further support for the validity of the pH measurement by NMR comes from a comparison the values derived by NMR and microelectrodes in barnacle muscle fibers [C. R. Bagshaw, R. D. Vaughan-Jones, D. G. Gadian, and G. K. Radda, unpublished observations; see also (4)].

In a study of acute renal acidosis it was demonstrated that the intracellular pH in perfused rat kidneys decreased by only 0.3 units when the extracellular pH is changed by 0.6 units. This fall in total intrarenal pH is insufficient to explain the changes observed in the metabolites of the glutamate dehydrogenase reaction in acute metabolic acidosis (47).

COMPARTMENTATION It was noted above that the observed linewidth of the P_i signal from skeletal muscle is often considerably greater than that for phosphocreatine (8, 48). This was particularly noticeable during the metabolic rundown of anoxic muscle (8), which suggests that P_i experiences several pH environments (ΔpH $\sim$0.2) in the cell. Perturbations of the pH in muscle by acetate resulted in the detection of two P_i and sugar phosphate signals (48). Studies on frog muscle supported the tentative interpretation that this represents compartmentation of these molecules between the cytoplasm and sarcoplasmic reticulum. When the muscle was treated with 2,4-dinitro-1-fluorobenzene, a new "acidic" component appeared, which was not detected when the muscle was pretreated with glycerol (to disrupt the transverse tubules) (49). pH gradients were clearly seen between the cytoplasmic and mitochondrial compartments in isolated liver cells (50); such gradients have not yet been observed in perfused organs. In perfused kidneys the width of the signal for P_i corresponds to a pH heterogeneity of 0.4 units (47, 51), although this is most likely to be a result of cellular heterogeneity and not of intracellular distribution.

More convincing and readily identifiable compartmentation is seen in various storage systems. For example, the ^{31}P NMR spectrum of the adrenomedullary chromaffin granules shows ATP resonances at frequencies different from those of cytoplasmic ATP, as a result of the low pH (5.7) and interaction with catecholamine inside the vesicles (52–54). The internal pH drops by 0.4–0.5 units when external ATP is hydrolyzed by the membrane-

bound ATPase (52, 53). Cytoplasmic and intragranular ATP can be observed as distinct signals in isolated chromaffin cells, perfused adrenal gland (A. Bevington, R. W. Briggs, G. K. Radda, and K. R. Thulborn, unpublished observations), and platelets (55, 56). Chromaffin granules, adrenal medulla, and many other storage systems have been examined by ^{1}H, ^{13}C, and ^{19}F NMR and important information about the nature of the "packaged" materials is emerging (28, 57–59). In a recent report, ^{31}P NMR studies on maize root tips have shown that plant vacuoles also have a low pH environment (60).

Reaction Rates

CHANGES IN METABOLIC STATE In vivo reaction rates have been measured by NMR in several ways. Relatively slow metabolic transition can be monitored by successive accumulations of the spectra, the time resolution being determined by the time required for signal averaging (typically 0.5–1 min for ^{31}P spectra). Thus metabolic changes during global ischemia, and recovery of perfused hearts (42), kidney (61), liver (62), and brain (63) have been followed. In perfused rat hearts and kidney the decrease in pH (in heart from 7.05 to 6.4) is considerably diminished by the use of buffers like bis-tris-propane in the perfusion medium, and this appears to have a significant protective effect on the ischemic tissue (42, 64). Quantification of the production of H^+ in rat hearts led to the conclusion that all the protons produced during ischemia come from the anaerobic breakdown of glycogen (42). The rate of glycogenolysis can therefore also be measured in a nondestructive way. Using this information, and the fact that accumulated deoxyglucose 6-phosphate inhibits phosphorylase *b*, the extent and rate of *b* to *a* conversion and phosphorylase *b* activation in rat heart during global ischemia have been estimated to show significant (~50%) phosphorylase *b* activity after about 1 min (44).

Several of the ^{31}P resonances in the spectra of perfused and in vivo liver have unusually short spin-lattice relaxation times [about 100 ms for liver ATP (62) in contrast with about 1–2 s for ATP in other tissues]. This considerably enhances the time resolution of kinetic studies of the liver, as illustrated by the studies of McLaughlin et al (62) on oxygen supply and Iles et al on fructose metabolism (65).

GATED NMR In situations where rapid and repetitive changes occur, time resolution can be greatly improved by synchronizing NMR data collection with different phases of the cycle. For example, electrical stimulation of frog sartorious muscles has been used to gate ^{31}P NMR measurements

of the metabolic events associated with contraction (11). In more recent experiments on frog gastrocnemius muscles, synchronization of data collection with electrical stimulation has enabled detailed kinetic analysis to be performed of the reactions taking place during contraction (66) (see below). Experiments on the fluctuations of PCr and ATP concentrations during the contraction-relaxation cycle of perfused hearts have been reported (67).

SATURATION TRANSFER The measurement of reaction rates in the steady-state or equilibrium conditions in functional organs can be achieved using the technique of saturation-transfer NMR. In this approach the spin magnetization of one chemical species is perturbed from its thermal equilibrium value, and the rate of appearance of the nonequilibrium spin magnetization of the second species is monitored to determine the reaction rate (68). The first in vivo application was for *Escherichia coli,* where the unimolecular exchange rates between P_i and ATP, catalyzed by the coupling ATPase, were measured (69). The role of the creatine kinase–catalyzed exchange between phosphocreatine and ATP in the energetics of frog muscle and perfused rat hearts was studied by ^{31}P saturation-transfer NMR (70).

In anaerobic frog muscle at rest at 4°C, the forward- (PCr to ATP) and reverse-reaction fluxes are about 1.6×10^{-3} M sec^{-1}, which shows that, as expected, the creatine kinase is at equilibrium (71). We note that the measured fluxes do show a small discrepancy (reverse "flux" is 1.2×10^{-3} M sec^{-1}) perhaps because the adenylate kinase reaction also contributes to the observed effects. When this reaction is taken into account, the two derived fluxes for the creatine kinase reaction become closer. By gating the saturation-transfer measurements with respect to 3-sec contractions, it was found that the forward-reaction flux remained at 1.6×10^{-3} M sec^{-1}, but the reverse flux decreased to 0.85×10^{-3} M sec^{-1}. The net rate of phosphocreatine breakdown during such a contraction was 0.75×10^{-3} M sec^{-1}. From these observations the following conclusions were reached (71):

1. During contraction, the creatine kinase reaction is no longer close to equilibrium; contrary to expectation, the creatine kinase reaction is not considerably faster than the rate of ATP utilization during contraction.
2. Nevertheless, the well-known observation that during contraction the ATP concentration remains constant can be accounted for by the measured rates.
3. That the forward flux does not increase during contraction and that the backward flux does decrease can be explained on the basis of competition of reactants for a limited amount of enzyme.

4. The formation of the non-productive enzyme-creatine-ADP complex could have an important regulatory role.

The situation in perfused beating hearts is more complex than in resting muscle, partly because of the relatively high rate of ATP utilization. Even though the system is in a steady state, the flux derived by NMR for the forward reaction of creatine kinase was measured to be larger than the reverse flux (61, 70, 72), yet, of course, they must be the same. This discrepancy may be a result either of competing reactions [e.g. ATP hydrolysis and snythesis or adenylate kinase activity (61, 70)] or of compartmentation (70). In spite of arguments in favor of compartmentation (72) we still believe that the alternative hypothesis cannot be discounted (73). There are indications that the adenylate kinase reaction can also be followed in this way (71).

ISOTOPE EXCHANGE AND INCORPORATION The exchange of the C-2 and C-3 protons of lactate and pyruvate with solvent ^2H_2O has been followed by "spin-echo" proton NMR in red cells (27). From the exchange of the C-3 protons, the equilibrium rates across lactate dehydrogenase can be measured (29). The C-2 exchange of lactate depends on several reactions; the distinct differences between the exchange rates in normal blood and blood from patients with a pyruvate kinase deficiency may have metabolic importance.

Shulman and his collaborators have carried out extensive ^{13}C NMR studies of isolated cells (3 and references therein) examining the relative rates of utilization of the α- and β-anomers of glucose and the degree of disequilibrium of the reactions catalyzed by aldolase and triose phosphate isomerase (74, 75). They have also examined perfused mouse liver by ^{13}C NMR. Using isotopically enriched [^{13}C]alanine and [^{13}C]ethanol as substrates, they measured the enrichment of specific carbons of glucose, glutamate, glutamine, aspartate, acetate, acetoacetate, β-hydroxybutyrate, and lactate (26). The authors state that "the implications of the experimental results presented here are so extensive that only limited interpretations can be presented." Their significant findings, simply stated, are that isotopic scrambling, distribution, and incorporation can be used to follow individual enzyme activities, partitioning between different pathway fluxes, and substrate selection (26). Detailed interpretation and consolidation of this work are awaited with interest.

TRANSPORT If the intracellular and extracellular signals for a given compound can be distinguished, or the uptake of the molecule results in a change of some NMR signal from a given cell, the rate of transport can be

followed. The few reports available include measurements on the influx of alanine into red cells (76) and of deoxyglucose into perfused hearts (44), and a brief mention of the uptake of Mn^{2+} into the heart (77, 78).

Relationship Between the Biochemical and the Physiological States

An attractive feature of NMR is that metabolism, monitored continuously, can be related to physiological function. Dawson, Gadian & Wilkie (22, 79, 80) have studied the biochemical basis of fatigue in anaerobic frog gastrocnemius muscles at 4°C. In muscles subjected to various patterns of electrical stimulation, force development was found to be closely correlated with metabolite levels, rather than with changes in excitatory conduction. During fatigue, the force developed remained proportional to the rate of ATP hydrolysis during contraction. In addition, the decline in the rate of mechanical relaxation correlated with the change in the free energy for ATP hydrolysis in vivo. The latter change, which accompanies fatigue, may be responsible for a reduction in the rate of Ca^{2+} uptake into the sarcoplasmic reticulum, which in turn slows down the rate of mechanical relaxation.

Several brief reports on the relation between cardiac function (e.g. left ventricular pressure) and metabolism have appeared, including studies on acidosis (81), heart rate (61), and the effect of inotropic agents (82). Renal function (e.g. glomerular filtration rate, Na^+ reabsorption, etc) following ischemic periods, has also been measured in relation to NMR observations on blood-perfused rabbit kidney (61) and in vivo rat kidney (64). The evaluation of these important observations awaits the appearance of detailed reports.

Spatially Resolved NMR

Over the last ten years, there has been increasing interest in an NMR technique known as "spin-imaging" or "zeugmatography," which involves the use of magnetic field gradients to provide information about the spatial distribution of molecules within a sample. Various ingenious methods of spin-imaging have been devised. [For contributions from many laboratories see (83).] An underlying theme of the method is to obtain two- or three-dimensional images of the proton signals from water within animals and human beings. The images are a function not only of proton density, but also of the proton relaxation times, which reflect the average mobility of the water molecules. Consequently, high intensity in an image generally reflects a region containing a large concentration of mobile water molecules. Remarkably clear discrimination can be obtained between different tissues, as

illustrated by the image of a human wrist obtained by Hinshaw et al, which has a resolution of about 0.4 mm (84).

There are indications that protons in tumorous regions have longer spin-lattice relaxation times than in the corresponding healthy tissues (85 and references therein). This raises the possibility that NMR can provide a way of screening for cancer. A wide range of other disorders associated with water concentration, diffusion, and flow may also be amenable to study. Clinical trials on this method are imminent.

For the biochemist, it would be of great interest to combine a spin-imaging method with high resolution ^{31}P NMR, in order to evaluate the metabolic state of different tissues within an intact animal. There are, however, severe problems in sensitivity associated with ^{31}P spin-imaging (86).

Fortunately, there are several simpler methods for obtaining high resolution information from a defined region of a live specimen. The simplest (but clearly restricted to animal experiments, as it requires surgery) is to place the radio frequency coil around the organ to be investigated, which has been done for rat heart (21) and kidney (47, 64). In rat heart the ratio of PCr to ATP in vivo is higher than in perfused heart (21), which relfects the efficiency of O_2 delivery by blood in comparison to that by conventional buffers.

In recent ^{31}P NMR experiments, use has been made of a simple, unconventional "surface coil" (20), a circular loop of copper wire. When placed, for example, against the leg of a rat, the copper loop will detect a signal from an approximately disc-shaped region of the muscle in front of the coil. The signal can be obtained very rapidly, and by placing the coil at various positions on the leg, one can map out variations of the metabolic state throughout different regions of the leg muscle. For example, ischemic regions can readily be distinguished from healthy regions. Similar studies on brain metabolism can be done by placing a surface coil against the head of a rat (20).

Investigations of internal organs by this approach are rather difficult and inefficient, and require the use of a further method of localization. One method, topical magnetic resonance (TMR), utilizes magnetic field gradients designed so that the field is only homogeneous over a selected central volume. It is only from this volume that high resolution signals are detected. This technique has been successfully used to investigate the metabolic state of the liver within anaesthetized intact rats (87), and more recently ^{31}P NMR spectra were recorded from a selected region of human arm muscle (88). In those experiments a surface coil was used in combination with the TMR method. The time course of changes in phosphocreatine and P_i following the application of a tourniquet were observed.

In summary, high resolution NMR has already proved to be a useful tool in metabolic studies and we expect that it may well have direct clinical applications in human investigations and diagnosis. The method seems particularly suitable for studying tissue energetics in normal, pathological, and stress conditions. Insufficiencies in oxygen delivery (resulting, for example, from vascular disease), metabolic recovery following therapy, and other diseased states that result in an imbalance in energy metabolism, should all be amenable to investigation. Before such uses further detailed studies on animal models and isolated organs are necessary.

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