PROCEEDINGS OF THE NUTRITION SOCIETY

The Four Hundred and Forty-fourth Scientific Meeting (One Hundred and Seventy-fourth Scottish Meeting) was held at the School of Agriculture, Aberdeen on 29/30 September 1987

SYMPOSIUM ON 'TECHNIQUES FOR THE MEASUREMENT OF METABOLIC REGULATION AND ORGAN FUNCTION'

Metabolic studies using localized magnetic resonance spectroscopy

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Nuclear magnetic resonance (NMR) spectroscopy provides a non-invasive method of studying metabolism in vivo. The nucleus that has been most widely used for metabolic studies is ^{31}P, which is 100% naturally abundant. ^{31}P spectra include signals from ATP, phosphocreatine (PCr) (in brain and muscle), and inorganic phosphate (P_i), and in addition to measuring the relative concentrations of these metabolites, it is also possible to determine the intracellular pH. In view of the key role of PCr, ATP and P_i in energy metabolism, together with the intracellular pH changes that may be associated with lactic acid production, ^{31}P NMR can be used to study the metabolic changes that occur in response to large decreases in energy supply or increases in energy demand, as in ischaemic disease or muscular exercise (Radda *et al.* 1984; Radda, 1986). The ability to distinguish different energy states is also valuable in monitoring cancer metabolism and response to therapy (Evanochko *et al.* 1984).

Of the other nuclei that are available for metabolic studies, there is increasing interest in the use of ^{1}H NMR. ^{1}H NMR has the advantages of high sensitivity and potential accessibility to a wide range of metabolites, but is technically more difficult than ^{31}P NMR because of the need to suppress the water and fat signals, and also because of spectral overlap between different metabolite signals. The non-invasive monitoring of lactate is of particular interest, not only because it can provide further information about the biochemical processes that occur during and following ischaemia, but also because of its possible impact on spatial resolution in localized NMR studies of disease (see p. 352).

In the present paper, a few examples are given of the applications of ^{1}H and ^{31}P NMR spectroscopy in biomedical research. These include studies of body fluids and isolated tissues, for which no localization procedures are required, and studies of whole animals and humans, for which localization techniques of varying degrees of sophistication are necessary. These examples provide an indication of the range of studies that can be performed; several recent reviews cover the field much more extensively (Evanochko *et al.* 1984; Radda *et al.* 1984; Avison *et al.* 1986; Radda, 1986; Williams & Gadian, 1986). Finally a brief discussion is given of the scope and technical limitations of localized spectroscopy.

^{1}H studies of body fluids

^{1}H NMR has been used for many years as a routine method of chemical analysis, and as a method of studying the structures of molecules in solution. Typically, the spectra are

obtained from about 0·5 ml of solution. As an extension of this type of study, it has been shown that ^{1}H NMR can also be used for the study of body fluids. For example, ^{1}H NMR studies have been described of human serum, plasma and urine (Nicholson *et al.* 1984; Iles *et al.* 1985). Signals from the ketone bodies can be observed in serum, plasma and urine samples from fasting and diabetic subjects (Nicholson *et al.* 1984), and unusual metabolites can be detected in urine samples from patients with inborn errors of metabolism (Iles *et al.* 1985). Although NMR lacks the sensitivity of other methods of analysis of body fluids, it does have the advantages that no pretreatment of the sample is required, and that information about a range of metabolites can be obtained in a single experiment, within minutes of collecting the sample.

Metabolite levels and metabolic control

The simplicity of the ^{31}P spectra observed from intact tissue reflects the fact that narrow signals are observed only from mobile P-containing compounds that are present at concentrations of above about 0·2–0·5 mM; highly-immobilized compounds produce very broad signals which often show up as a sloping baseline, while compounds that are present at concentrations below about 0·2 mM produce weak signals that may be lost in the noise.

Provided that appropriate controls are performed, the concentrations of the various NMR-visible metabolites are proportional to the areas of their respective signals. Therefore relative concentrations of metabolites can be determined fairly easily, and metabolic processes can be followed simply by monitoring how the signal areas vary with time. The measurement of absolute concentrations is more difficult, but they can often be deduced by comparing the relative concentrations as measured by NMR with the total metabolite levels determined from freeze-clamping studies. Fortunately, the measurement of relative concentrations usually provides a sufficient basis for interpretation.

In some cases, concentrations may be deduced of compounds that do not produce detectable signals. ADP provides a good example of this, for the concentration of free ADP may often be calculated from the measured concentrations of the reactants of the creatine kinase (*EC* 2.7.3.2) reaction, provided that this reaction is close to equilibrium.

An observation of considerable interest is that the concentrations of P_i and ADP as measured or calculated by NMR are generally much lower than values obtained using more traditional invasive methods. One possible explanation for this discrepancy is that other methods involve some breakdown of high-energy phosphates. Alternatively, there is increasing evidence to suggest that significant quantities of P_i and ADP are bound or sequestered in such a way that they produce no detectable signal. Regardless of the reason for the discrepancy, the low levels of NMR-visible P_i and ADP have important implications in relation to metabolic control, the free energy of ATP hydrolysis, and the possible compartmentation of intracellular metabolites (for relevant references, see Williams & Gadian, 1986).

Studies of organ preservation

^{31}P NMR provides non-invasive sequential measurements of the energy status of tissue during preservation, and can therefore assist in evaluating the relative efficacy of different preservation procedures. For example, in recent studies using isolated rabbit hearts, it was found that a solution (designated CP5) containing glucose and a lower concentration of calcium than that in St Thomas's Hospital cardioplegic solution (which is used in the Papworth Hospital clinical cardiac transplantation programme for storage of hearts) gave better preservation as indicated by the measurement of contraction

amplitude and perfusate flow-rate during subsequent normothermic Langendorff perfusion. ^{31}P NMR studies of the rates of changes of high-energy phosphates in stored rabbit hearts have added support to the conclusion that CP5 may be preferable to St Thomas's solution for hypothermic preservation of cardiac grafts (English *et al.* 1988). However, they do not provide proof of this; further information is required from transplantation studies.

^{1}H and ^{31}P studies of an animal stroke model

The combined use of ^{1}H and ^{31}P NMR can provide a detailed picture of energy metabolism, particularly when used in conjunction with additional techniques. For example, methodology has recently been described whereby ^{1}H and ^{31}P NMR spectra and regional cerebral blood flow can be measured concurrently and repeatedly in the anaesthetized gerbil (Gadian *et al.* 1987). The NMR measurements were made with two surface coils, one on each hemisphere, each coil being doubly tuned to the ^{1}H and ^{31}P frequencies; the flow measurements were made using the hydrogen-clearance technique. Changes in metabolic state and in regional flow were monitored during and following 60 min of unilateral carotid occlusion or 30 min of bilateral carotid occlusion. During ischaemia, no changes were detected in the ^{1}H and ^{31}P spectra provided that the regional cerebral blood flow remained above a threshold value of 20 ml/100 g per min (Crockard *et al.* 1987). Below this level, the characteristic metabolic features of energy failure were detected, i.e. there was a decrease in high-energy phosphates and intracellular pH, and an increase in P_i and lactate. The threshold flow value is similar to that at which, in a variety of species, electrical activity ceases, and is also similar to the flow level at which water accumulates in the brain. These results therefore provide evidence suggesting that the thresholds for electrical function and oedema are a direct consequence of energy failure.

Human studies

Extensive ^{31}P NMR studies have been performed on skeletal muscle at rest, during exercise and during recovery, both in control subjects and in patients, as reviewed elsewhere (Radda *et al.* 1984; Radda, 1986). ^{31}P NMR has also been used to investigate the brains of new-born infants who have suffered birth asphyxia (Hamilton *et al.* 1986). In the asphyxiated infants, information obtained on the first day of life showed no differences from those in normal infants, but by the 2nd to 9th days there were significant reductions in the $PCr:P_i$ ratios. This latency suggests the possibility of effective early treatment before irreversible metabolic damage sets in. $PCr:P_i$ ratios below 0·80 were associated with a poor prognosis for survival and early neurodevelopmental outcome.

A major application for NMR spectroscopy will be in the monitoring of tumour metabolism and its response to therapy (Evanochko *et al.* 1984), and this area will undoubtedly receive further interest with the development of improved methods of localization.

Localization

In the animal and human studies described previously localization was achieved simply by taking advantage of the radiofrequency field distribution of a surface coil. In its simplest form, a surface coil is a circular loop of wire, of one or more turns. When such a coil is placed adjacent to a sample, then under normal circumstances most of the signal detected by the coil will originate from an approximately disc-shaped region of the object immediately in front of the coil, of radius and thickness approximately equal to the radius of the coil. The use of surface coils therefore provides a very simple method of

localizing on superficial regions, and they have been extensively used for studies of muscle and brain metabolism. However, the volume that is observed is not precisely defined, and does not have sharp boundaries. More powerful techniques therefore need to be developed, particularly for investigation of internal regions. Although increasingly sophisticated methods using radiofrequency field gradients have produced interesting results, it seems likely that the most powerful and versatile methods will rely on the use of pulsed field gradients superimposed on the static magnetic field, in an analogous manner to their use in ^{1}H imaging. Particularly promising results have recently been obtained in humans using phase-encoding techniques (Bailes *et al.* 1988). In these studies, multiple simultaneous spectra have been obtained from an array of volume elements within the body, with spatial resolution of 20–30 mm.

Localized spectroscopy is therefore becoming increasingly dependent on imaging technology, both for defining the region of interest (by obtaining a ^{1}H image before accumulating spectra) and for achieving the required spectral localization. For this reason, the modern generation of spectrometers for whole-animal and human studies incorporates imaging facilities.

Regardless of the quality of the localization procedures that may be developed, it has to be appreciated that NMR is an intrinsically insensitive technique, and this imposes two main limitations. First, metabolites need to be present at fairly high concentration in order to produce detectable signals, and second the spatial resolution that can be achieved in localized spectroscopy is limited by the signal:noise ratio that a single volume element can generate. Bearing in mind that the metabolites of interest have typical concentrations of a few millimolar, it can be anticipated that for ^{31}P spectroscopy one will have to accept typical spatial resolution for human studies of 20–50 mm, i.e. resolution of the type that has been achieved with the phase-encoding technique (Bailes *et al.* 1988) mentioned previously. For ^{1}H NMR studies, particularly of lactate, the achievable resolution should be better than this because of the higher sensitivity of ^{1}H NMR, coupled with the high concentration to which lactate can accumulate in ischaemia.

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Printed in Great Britain