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Proton NMR Studies of Brain Metabolism

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As a result of several technical developments that have taken place over the past few years, it is now possible to obtain ^{1}H spectra of very high quality from localized regions of the human brain. ^{1}H spectroscopy provides scope for detecting a wide range of metabolites, and offers spatial resolution that is superior to that available with other nuclei.

The animal and clinical studies that have so far been reported indicate that abnormal ^{1}H spectra are associated with a variety of disorders of the brain. Among the metabolites of interest are lactate and *N*-acetylaspartate. The signal from lactate can provide information about abnormal glycolytic metabolism, for example in brain tumours and cerebrovascular disease. *N*-Acetylaspartate is believed to be located primarily in neurons, and its signal could prove to be particularly useful as a non-invasive marker for neurons.

1. Introduction

In the 1970s, many of the developments in NMR imaging and spectroscopy of intact biological systems proceeded independently of each other, with an emphasis in imaging on the use of the ^{1}H nucleus, and in spectroscopy on ^{31}P studies. However, the techniques of imaging and spectroscopy share many common features, and they are really variants or extensions of the more traditional uses of NMR as a branch of spectroscopy. Furthermore, imaging and spectroscopy are necessarily linked by the new generation of experiments that provide both spatial and chemical information. They become particularly closely linked when they both use the same nucleus. This is happening increasingly for investigations of the brain, with the growing use of ^{1}H NMR for metabolic studies. Therefore, although the emphasis in this article is on metabolism, it should be appreciated that, particularly for clinical studies, ^{1}H spectroscopy of the brain should be regarded as just one part of an integrated imaging/spectroscopy examination.

2. Why use ^{1}H NMR?

The development of ^{1}H NMR for metabolic studies lagged behind ^{31}P NMR for both technical and biochemical reasons. Technically, ^{1}H NMR is more complex than ^{31}P NMR because of the need to suppress the large signals from water and, in some cases, from fats, and because of the large number of metabolites that produce signals in a relatively narrow chemical shift range. However, techniques for solvent suppression and spectral 'editing' are now sufficiently well developed to permit the non-invasive

monitoring of several metabolites of interest, including lactate, alanine, *N*-acetylaspartate, glutamine, glutamate and total creatine (i.e. creatine + phosphocreatine). For the brain, excellent field homogeneity can often be obtained, so that even at the relatively low (by spectroscopists' standards) field strengths of 1.5–2.0 T that are commonly used for clinical spectroscopy, adequate spectral resolution can be achieved for many of the signals. In addition, the normal human brain (as opposed to the scalp and bone marrow) does not generate any signal from fats, and this facilitates the detection of the co-resonant signal from lactate.

From a biochemical viewpoint, ^{1}H NMR lagged behind ^{31}P studies because of the perceived strength of ^{31}P NMR in monitoring energy metabolism by means of the signals of ATP, phosphocreatine and inorganic phosphate and by the measurement of intracellular pH. However, although such studies of energy metabolism have undoubtedly been of great value, it has become apparent that there are many disease states where ^{1}H spectroscopy might reveal abnormalities despite a relatively normal energy status as measured by ^{31}P spectroscopy. Therefore at the very least ^{1}H spectroscopy can complement the information that is available from ^{31}P studies.

A key feature of ^{1}H spectroscopy is the high sensitivity of the ^{1}H nucleus in comparison with other nuclei. In principle, this means that metabolites could be detected at relatively low concentrations. However, it is not necessarily straightforward to observe metabolites at low concentrations, as their signals may be masked by larger signals from other compounds that are present at higher concentrations. In practice, therefore, the higher sensitivity is generally exploited by trading signal-to-noise ratio with spatial resolution. The higher sensitivity of ^{1}H spectroscopy means that adequate signal-to-noise ratios can be obtained from smaller volume elements, and the consequent improvement in spatial resolution is proving of considerable value.

3. Animal studies

Following the first *in vivo* ^{1}H NMR studies reported by Behar *et al.* (1983), numerous research groups have used ^{1}H NMR, often together with ^{31}P NMR, to investigate metabolic abnormalities in animal models of disease. Below, we describe illustrative examples from our own studies of normal and abnormal cerebral metabolism. All of the *in vivo* studies were carried out at a field strength of 8.5 T, the animals being under halothane/oxygen anaesthesia.

(*a*) *An inborn error of metabolism*

The first studies that we carried out were of mutant mice with histidinaemia, an inborn error of metabolism which results in elevated concentrations of histidine in the brain (Gadian *et al.* 1986). Figure 1 shows ^{1}H and ^{31}P spectra from a mutant mouse that had been fed a histidine-enriched diet. The ^{1}H spectra were obtained using a spin-echo sequence incorporating an initial $1\bar{3}3\bar{1}$ pulse for solvent suppression, with total echo times of 40 ms (figure 1*a*) and 120 ms (figure 1*b*). The large (albeit strongly suppressed) signal at 4.7 p.p.m. is from water, and the broad signal centred at 1.5 p.p.m. is from the fats of bone marrow and scalp. Narrow signals can be observed from *N*-acetylaspartate (NAA), creatine + phosphocreatine (Cr) and choline-containing compounds (Cho). These are the most prominent signals that are routinely observed in the normal brain. The additional signals at 7.83 and 7.08 p.p.m., which are not seen in control studies, can be assigned to the C_2 and C_4 protons of histidine (His). With the aid of various control studies, we were able to

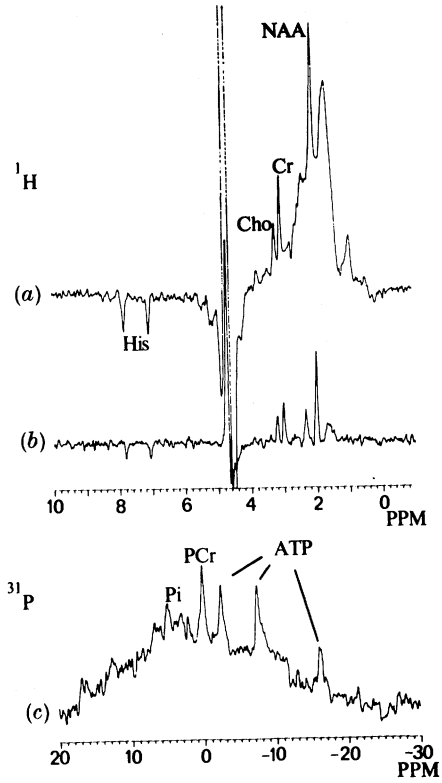

Figure 1. ^{1}H and ^{31}P spectra obtained at 8.5 T from the brain of an intact histidinaemic mouse anaesthetized with halothane/oxygen. The mouse had been fed on a histidine-enriched diet. Further details are given in the text and in Gadian *et al.* (1986).

quantify the brain histidine concentration, which in a series of 12 animals varied from about 2 to 10 mmol (kg wet wt)$^{-1}$. These values correlated well with values determined from the same animals by amino acid analysis of brain extracts, confirming that *in vivo* ^{1}H NMR can, if all the appropriate controls are performed, give reliable concentration measurements. Further studies at 1.9 T showed that histidine is visible at the lower field strengths that are available for whole body spectroscopy. The ^{31}P spectra of these mutant mice were normal, consistent with the fact that there are no major derangements of brain energy metabolism in these mice.

These studies suggested a clinical role for ^{1}H spectroscopy in the investigation of patients with inborn errors of metabolism. The mechanisms whereby such errors can result in brain disease remain obscure, and the non-invasive monitoring of metabolites that accumulate in the brains of such patients (one example being the detection of phenylalanine in patients with phenylketonuria) could assist in the elucidation of these mechanisms and in the evaluation of therapy.

(*b*) *A stroke model*

The metabolic changes that are associated with reduced cerebral blood flow have been extensively studied. When the flow falls sufficiently, there is a decline in high energy phosphates, an increase in inorganic phosphate, an increase in lactate and a decline in pH. However, the precise relationship between flow and metabolism has

been difficult to establish, because of problems in making concurrent measurements of flow and metabolic state *in vivo* in a single animal as a function of time.

In our studies of a gerbil stroke model, we have been able to correlate changes in ^{31}P and ^{1}H spectra during and following ischaemia with changes in regional cerebral blood flow measured simultaneously with the hydrogen clearance technique (Gadian *et al.* 1987; Crockard *et al.* 1987; Allen *et al.* 1988). We have demonstrated that there is a flow threshold of about 20 ml (100 g)$^{-1}$ min^{-1} at which energy failure ensues. This threshold 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 gerbil brain. These results therefore provide evidence suggesting that the thresholds for electrical function and oedema are a direct consequence of energy failure. The model could prove to be of value in studying modifications to flow and metabolism produced by pharmaceuticals and other forms of therapy.

During recirculation following ischaemia, we have found that there is a period when the lactate is still elevated, while the ^{31}P spectrum and intracellular pH are close to normal. This reflects extrusion of protons independently of lactate, possibly by Na^+–H^+ exchange. In contrast to this, following severe hypoxia in a guinea pig cortical brain slice preparation, the lactate concentration recovers to normal slightly faster than the normalization of intracellular pH (Kauppinen & Williams 1990). On the basis of this observation, together with additional data utilizing 1-[^{13}C]glucose, it was suggested that the slow return of lactate to normal levels *in vivo* is not a consequence of continued lactate production, but presumably reflects a delay in the removal of lactate to the circulation.

The delayed removal of lactate suggests that ^{1}H NMR spectroscopy of lactate may be a more sensitive monitor than ^{31}P NMR of a previous ischaemic episode, or of repeated transient ischaemia. This is consistent with early ^{1}H studies of cerebrovascular disease in man (Bruhn *et al.* 1989).

(*c*) *A model of acute liver failure*

We have used ^{31}P and ^{1}H NMR to investigate brain metabolism in rats with acute liver failure, induced by a single intragastric dose of carbon tetrachloride (Bates *et al.* 1989*b*). The ^{31}P spectra showed that the brain can maintain normal energy metabolite ratios despite the metabolic challenge produced by an increase in blood ammonia to about 500 μmol l^{-1}. ^{1}H spectra that were edited for the detection of specific metabolites showed a significant increase in brain glutamine and lactate, and a decrease in glutamate (figure 2). These results suggest a role for ^{1}H NMR in clinical studies of metabolic encephalopathies.

(*d*) *Metabolic changes during development*

Many of the metabolites that are observable by ^{1}H NMR undergo significant alterations in their concentrations as part of normal cerebral development. As an aid to interpreting the changes with development that occur both in animals and in man, we decided to study perchloric acid extracts of brain tissue from rats, as the improved spectral resolution that extracts provide facilitates assignment and quantification of the metabolite signals.

Among our results, we found that development of the brain is associated with a particularly large increase in the ratio of *N*-acetylaspartate to choline-containing compounds; in fact the absolute concentration of *N*-acetylaspartate rose by a factor of 9 during the period from 1 to 21 days of birth (Bates *et al.* 1989*a*). These metabolic

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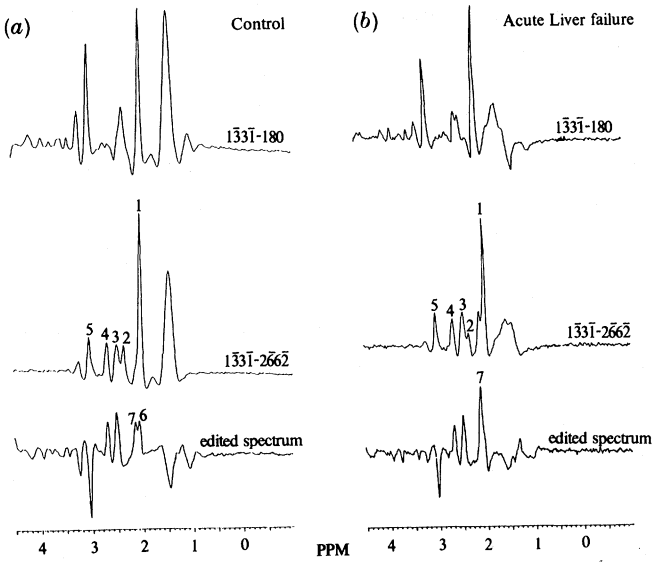

Figure 2. ^{1}H spectra obtained at 8.5 T from the brain of (*a*) a control rat, and (*b*) a rat with acute liver failure. The top and middle spectra were obtained using 1$\bar{3}$3$\bar{1}$–180 and 1$\bar{3}$3$\bar{1}$–2$\bar{6}$6$\bar{2}$ pulse sequences, with the solvent suppression pulses adjusted to give maximal excitation at the *N*-acetylaspartate signal at 2.0 p.p.m. The edited spectra were obtained by subtracting the top from the middle spectra. Dominant contributions to the resonances can be assigned as follows: peak 1, *N*-acetylaspartate (NAA); peak 2, glutamate; peak 3, glutamine and NAA; peak 4, NAA and aspartate; peak 5, creatine + phosphocreatine; peak 6, glutamate; and peak 7, glutamine and glutamate. Further details are given in Bates *et al.* (1989*b*).

changes may be a reflection of the maturation of nerve cells or of myelination which is particularly active during the period between 9 and 21 days when the spectra change most extensively. Studies of hypomyelinating mutant mice are in progress (Small *et al.* 1990), and these should clarify the relationship of these metabolic changes to neuronal maturation and/or myelination.

4. Acute and chronic changes

Animal studies such as those described above show that there could be many situations in which the ^{1}H spectra are more responsive than ^{31}P signals to brain disease. However, if animal studies are to be related to clinical investigations, it must be appreciated that many animal models are studied in the acute phase, whereas the majority of clinical cases will be investigated in the sub-acute or chronic phase. This is just one reason why caution must be exercised in making comparisons between studies of animal models and of man.

The gerbil model discussed above provides an example of this. Following 30 min of complete ischaemia in the gerbil brain, the ^{31}P spectrum returns to normal within 30 min of recirculation, the lactate clearance taking somewhat longer. However, functional recovery would not be complete following such an insult; secondary damage would occur. Therefore although the acute metabolic processes are obviously of importance, they do not tell the whole story, and it would clearly be of interest to follow the sub-acute and chronic metabolic responses to the ischaemic period. In particular, an ischaemic insult may result in neuronal death and gliosis, and it is

apparent that ^{1}H spectroscopy is very much more sensitive than ^{31}P NMR to pathological changes of this type. The reason for this is that one of the prominent signals in the ^{1}H spectra is from *N*-acetylaspartate. Although the function of this compound is unclear, it is believed to be located primarily in neurons, and can therefore be used in ^{1}H spectroscopy as a 'neuronal marker'. Many clinical studies have now been reported showing a reduction in *N*-acetylaspartate in brain disorders, totally consistent with neuronal loss. Thus ^{1}H spectroscopy should be useful in the investigation of many disorders involving neuronal degeneration and glial proliferation. ^{31}P spectroscopy is unlikely to be as useful in this respect, as there are no equivalent phosphorus-containing markers that can be exploited. Indeed, cell culture studies suggest that neurons and glia may have similar PCr/ATP ratios (Gill *et al.* 1989).

5. Clinical studies

The development of clinical ^{1}H spectroscopy has relied on a number of technical developments, in particular on improved methods for obtaining spectra from selected volumes of interest. For investigations of the brain, several techniques have been used both for solvent suppression and for spatial localization. A common localization approach involves the use of three frequency-selective radiofrequency pulses to generate a spin echo or stimulated echo from the volume of interest (Ordidge *et al.* 1985; Frahm *et al.* 1987). Solvent suppression can be achieved by presaturation or by the use of binomial pulses, and additional pulsed gradients can be incorporated into the sequence to generate 'spectroscopic images' or 'metabolite maps' of the selected volume.

Several groups have reported excellent ^{1}H spectra from localized regions of the human brain, showing clear-cut signals from lactate, *N*-acetylaspartate, creatine + phosphocreatine and choline-containing compounds. Spectral abnormalities have been observed in some patients with brain tumours (Bruhn *et al.* 1988; Luyten *et al.* 1988), cerebrovascular disease (Bruhn *et al.* 1989), and severe multiple sclerosis (Arnold *et al.* 1990). Here, we describe some of our recent clinical studies, beginning with studies of intracranial tumours (Gill *et al.* 1990).

(*a*) *In vitro and in vivo studies of intracranial tumours*

Figure 3*a* shows a spectrum obtained *in vivo* from a Grade III astrocytoma. The dominant signals are assigned as shown to *N*-acetylaspartate (NAA), creatine + phosphocreatine (Cr) and choline-containing compounds (Cho), while the signal at 1.3 p.p.m. could be from lactate and/or fat. In comparison with the spectra of normal subjects, the main abnormality is a marked reduction in the ratio of NAA to Cr and to Cho. In order to aid interpretation of spectra obtained *in vivo*, where feasible we have been correlating *in vivo* results with NMR data obtained *in vitro* from perchloric acid extracts of biopsy specimens. For example, figure 3*b* shows the *in vitro* spectrum from one of five serial stereotactic biopsies that were taken from the same patient in the course of neurosurgical treatment. On the basis of the relative signal intensities observed *in vitro*, the residual NAA observed *in vivo* from this patient may reflect a contribution from normal tissue, or it could possibly be from other *N*-acetyl-containing compounds not extracted by perchloric acid.

The suggestion that other compounds contribute to the 'NAA' signal was first made when it was found that the NAA/Cr ratio measured from ^{1}H spectra of the human brain *in vivo* was higher than anticipated on the basis of the known

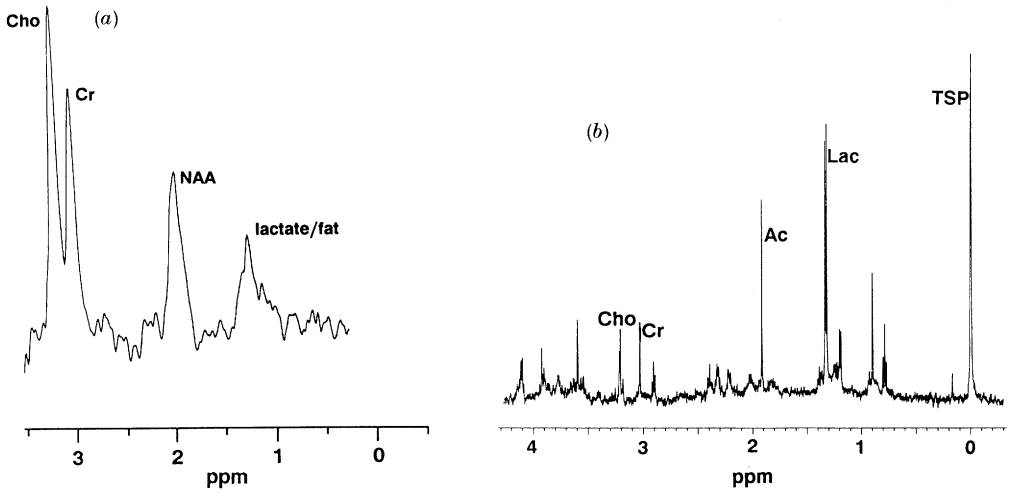

Figure 3. (*a*) ^{1}H spectrum of an astrocytoma obtained *in vivo* at 1.6 T, at the Hammersmith NMR unit. The spectrum was obtained from a $4 \times 4 \times 4$ cm volume using a multiple spin-echo sequence incorporating a $1\bar{3}3\bar{1}$ solvent suppression pulse optimized for NAA. The echo time was 270 ms, and the repetition time was 2 s. (*b*) ^{1}H spectrum obtained at 11.7 T (using the University of London NMR facilities at Birkbeck College) from a perchloric acid extract of one of five serial stereotactic biopsies from the same tumour. Acetate (Ac) enters as an impurity in the course of extraction and storage of the specimen. TSP is added as a chemical shift and concentration standard. For further details see Gill *et al.* (1990).

metabolite levels within the brain (Hanstock *et al.* 1988; Frahm *et al.* 1989). This NAA/Cr ratio is also higher than values that we observed in biopsy specimens of normal white matter. Out data therefore add weight to this suggestion, but indicate that any such additional compounds are not extracted by perchloric acid.

In general, the improved spectral resolution of the biopsy spectra facilitates assignment and quantification of the signals, and since the volume of tissue that is removed is so small in comparison with the selected volume *in vivo*, comparison of the *in vivo* and *in vitro* data provides additional information about tissue heterogeneity and 'partial volume' effects.

We examined biopsy specimens from 47 patients, 11 of whom were also examined *in vivo*. Biopsy data from 26 astrocytomas showed that the NAA/Cr ratio differs significantly in all grades of tumour from its value in normal white matter, and that the Cho/Cr ratio differs significantly in Grade IV tumours from its value in the other grades. Meningiomas have an unusually high ratio of alanine to total creatine and to other metabolites. Spectra obtained *in vivo* were consistent with *in vitro* results from the same patients. The lactate signal that is sometimes observed *in vivo* provides additional information about abnormal metabolism; however, for this particular metabolite, the extracts have limited value as lactate accumulates during the unavoidable period between surgical removal of the biopsies and freezing of the tissue.

We concluded from this combined study that *in vivo* ^{1}H spectroscopy could play a useful role in the diagnosis and grading of tumours, and in the assessment of treatment.

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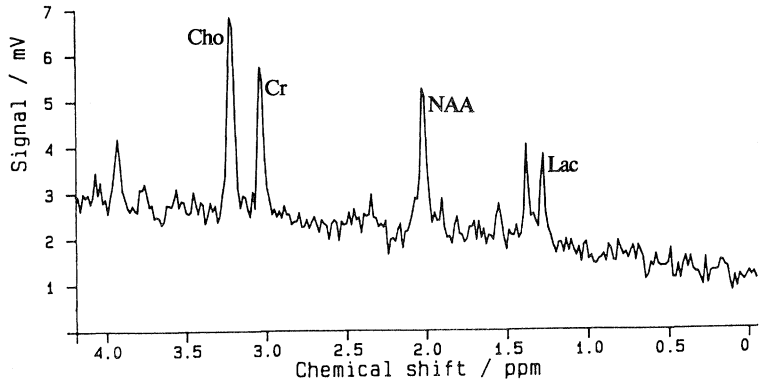

Figure 4. ^{1}H spectrum obtained *in vivo* from the brain of an eight-month-old child with suspected pyruvate dehydrogenase deficiency. The spectrum was obtained from a $2 \times 2 \times 2$ cm volume using a spin-echo sequence incorporating a frequency-selective 90° pulse followed by two frequency-selective 180° pulses, with presaturation for solvent suppression. 512 data acquisitions were accumulated, with an echo time of 270 ms, and repetition time of 1600 ms. (Courtesy of the Hospital for Sick Children, Great Ormond Street.)

(*b*) *Neuronal loss*

As discussed in §4, *N*-acetylaspartate is believed to be located primarily in neurons and so, at least in adults, an abnormally low NAA signal can be attributed to loss of neurons (although the cautionary note about other contributions to the 'NAA' signal should be borne in mind). A reduced NAA/Cr ratio has been seen in many intracranial tumours (Bruhn *et al.* 1988; Luyten *et al.* 1988), cerebrovascular disease (Bruhn *et al.* 1989), and severe multiple sclerosis (Arnold *et al.* 1990). Early studies from the Hammersmith NMR Unit have shown similar effects in a child with herpes simplex encephalitis (Menon *et al.* 1990*b*), in some patients with AIDS (Menon *et al.* 1990*a*), and in infants who had suffered birth asphyxiation (Peden *et al.* 1990).

For paediatric studies, interpretation of a reduced NAA signal is less straightforward, as the NAA/Cr and NAA/Cho ratios increase during normal development, so a reduced NAA in comparison with controls could presumably reflect abnormal development as well as neuronal loss. In such cases, follow-up studies will be of value. In fact, from the studies of birth asphyxia referred to above, it seems likely that ^{31}P spectroscopy will be of must value in the acute and sub-acute phases as a monitor of energy failure, while ^{1}H spectroscopy will be more informative in the chronic phase as an indicator of neuronal loss (Peden *et al.* 1990).

(*c*) *Inborn errors of metabolism*

As illustrated by the animal studies of histidinaemia discussed above, ^{1}H NMR could prove to be of considerable value in the investigation of inborn errors of metabolism. In our studies, extensive spectral abnormalities have been observed in a number of disorders, for example in Canavan's disease, which only recently has been shown to involve a deficiency of the enzyme aspartoacylase. As another example, figure 4 shows a ^{1}H spectrum from an eight-month-old child with suspected pyruvate dehydrogenase deficiency, demonstrating the characteristic doublet of lactate centred at 1.32 p.p.m. In addition, the NAA/Cr and NAA/Cho ratios are reduced relative to values in age-matched controls, presumably reflecting neuronal loss. The lactate level in the cerebrospinal fluid of this child was 8.4 mM. In fact, it

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will be interesting to compare intracellular metabolites as measured by ^{1}H spectroscopy *in vivo* with NMR measurements of body fluids. In particular, ^{1}H NMR analysis of urine samples from patients with inborn errors of metabolism can provide a relatively simple method of diagnosis and of extending our understanding of the biochemical changes associated with many disease processes (Iles & Chalmers 1988).

6. Concluding remarks

^{1}H spectra of the human brain display abnormalities in a wide variety of disorders. Interpretation of the spectra, which can be obtained with linear resolution of about 1 cm, is facilitated by parallel studies of biopsy specimens, body fluids, cell cultures, isolated tissue, and animal models of disease. There appears to be a bright future for the combined use of ^{1}H imaging and spectroscopy in the diagnosis and biochemical assessment of brain disease, and in the evaluation and monitoring of therapy.

This article draws on the research of many colleagues from a number of research groups. In particular, I acknowledge the following colleagues and collaborations: R. M. Gardiner and E. B. Cady (University College London) for their work on the histidinaemic mice; K. L. Allen and H. A. Crockard (Institute of Neurology) and R. S. J. Frackowiak (MRC Cyclotron Unit) for the gerbil studies; R. K. Small and her group (Institute of Neurology) for their work on the developing rat brain, hypomyelinating mice (together with B. Watkins (NINDS, NIH, Bethesda)), and neural cell preparations; R. A. Iles (London Hospital Medical College) and J. D. Bell (Hamersmith Hospital NMR Unit) for their work on cerebral development and intracranial tumours; D. G. T. Thomas and S. S. Gill (Institute of Neurology) and additional members of the Hammersmith NMR Unit (especially C. J. Peden, I. J. Cox, D. K. Menon, R. Porteous, D. J. Bryant and G. A. Coutts) for the studies of intracranial tumours; A. Connelly, S. J. Austin and J. V. Leonard (Institute of Child Health) for the studies of inborn errors of metabolism in children, and finally all my colleagues at the Hunterian Institute, (in particular T. E. Bates, A. L. Busza, R. A. Kauppinen, E. Proctor, J. Urenjak, N. Van Bruggen and S. R. Williams) who have contributed so much to the research described here.

This article is written in memory of my father Theodore Gadian, 1913–1990.

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