

# New non-invasive methods for assessing brain oxygenation and haemodynamics

**E O R Reynolds, J S Wyatt, D Azzopardi,  
D T Delpy, E B Cady, M Cope, S Wray**

*Departments of Paediatrics, Medical Physics & Bioengineering, and Physiology,  
University College and Middlesex School of Medicine, The Rayne Institute,  
London*

Hypoxic-ischaemic injury to the brain is the commonest cause of permanent neurodevelopmental disability in very preterm and other infants who survive after neonatal intensive care.

Two new methods allow normal and abnormal oxidative metabolism to be investigated non-invasively in brain tissue. **Magnetic resonance spectroscopy** is used to measure the concentrations of important phosphorus compounds that are involved in energy metabolism—adenosine triphosphate, phosphocreatine and inorganic orthophosphate—and also to measure intracellular pH. Normal developmental changes with gestation have been defined and abnormalities indicating impaired oxidative phosphorylation detected in a range of conditions of suspected or proven hypoxic-ischaemic injury. **Near infrared spectroscopy** provides continuous cot-side information about cerebral oxygenation and haemodynamics. Quantitative measurements can be made of oxyhaemoglobin, deoxyhaemoglobin, oxidized cytochrome aa3 and various haemodynamic indices.

These two complementary techniques are likely to prove increasingly valuable for: monitoring brain-oxygenation and haemodynamics in babies; investigating the mechanisms of damage to the brain; assessing the results of treatment; and assigning long-term prognosis.

## NON-INVASIVE INVESTIGATION OF THE BRAIN

Cerebral periventricular haemorrhage and hypoxic-ischaemic injury are very common in infants born well before term.<sup>1</sup> The introduction of portable ultrasound apparatus for scanning the brain repeatedly in the intensive care nursery has been followed by the publication of a large number of studies of the prevalence, pathogenesis and prognostic significance of these cerebral lesions.<sup>2</sup> The initial studies concentrated on periventricular haemorrhage, which was easy to visualize, even with early linear-array scanners, and it soon became clear that the prevalence of haemorrhage increased sharply with decreasing maturity, so that in infants born before 28 weeks of gestation it was about 80%. More recently attention has shifted towards the role of hypoxic-ischaemic brain injury as a cause of death and long-term disability in very preterm infants. This change of direction arose for two reasons. Firstly follow-up studies of cohorts of infants whose brains had been prospectively scanned with ultrasound showed that periventricular haemorrhage, though extremely common, was responsible for disability only in a minority of disabled very preterm survivors: the haemorrhages appeared innocuous, or almost so, unless they led to post-haemorrhagic hydrocephalus or intraparenchymal haemorrhage (often due to venous infarction associated with disrupted venous drainage.<sup>3</sup>) Secondly the improving quality of ultrasound imaging, especially following the introduction of modern mechanical sector scanners has allowed hypoxic-ischaemic injury to be diagnosed from the development of cystic periventricular leucomalacia, or inferred from the presence of ventricular dilatation in the absence of evidence of obstruction to the drainage of cerebrospinal fluid.<sup>2,4-9</sup> It is now clear that hypoxic-ischaemic injury such as periventricular leucomalacia is a more frequent cause than haemorrhage of permanent damage to the brain in surviving very preterm infants.<sup>4-6,10</sup> The presence of hypoxic-ischaemic injury may be suspected before frank loss of brain tissue occurs from the appearance of increased echodensities on ultrasound scan.<sup>2,7,8</sup> However, studies in which the ultrasound-appearance of the brain has been compared with autopsy findings show that increased echodensities are poor indicators of hypoxic-ischaemic injury<sup>9</sup>—unless they are extremely obvious and persistent.<sup>7,8</sup> It must be concluded that, unlike the situation for cerebral haemorrhage, ultrasound scanning does not provide useful information about early events in this type of injury.

If periventricular leucomalacia and other forms of hypoxic-ischaemic injury to the brain (such as birth asphyxia in term infants) are to be rigorously investigated, new methods are required for identifying them as they occur, and for exploring the mechanisms involved, particularly so that therapeutic interventions can be tested objectively and the prognosis for the infants determined. It was the realization that hypoxic-ischaemic brain injury was the major cause of long-term neurodevelopmental disability in very immature, and other, infants who survive after intensive care that provided the impetus for the introduction of new techniques for investigating this type of injury. The aim of this review is to describe the use of two such techniques—magnetic resonance spectroscopy and near infrared spectroscopy—which show great promise, particularly when used in combination.

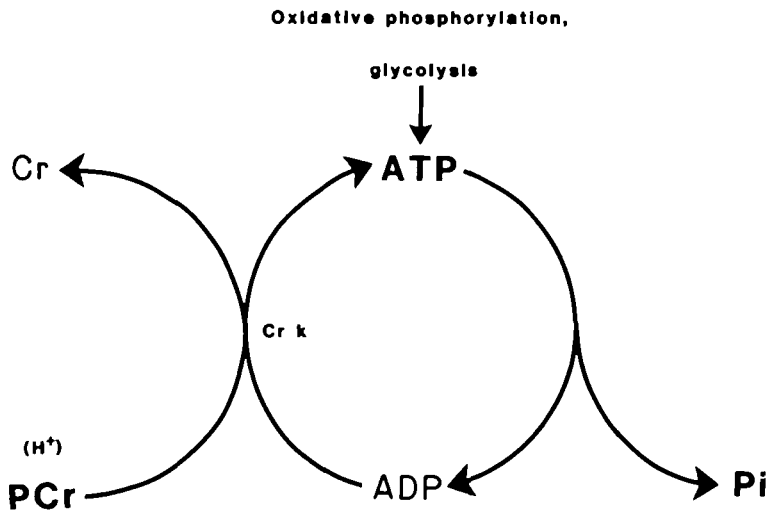
## MAGNETIC RESONANCE SPECTROSCOPY

### **Background and theory**

Magnetic resonance spectroscopy has been used for many years in analytical chemistry, but has only recently become applicable to studies of animals and human subjects.<sup>11</sup> This development depended on the production of the large, usually superconducting, magnets that are necessary for the provision of a sufficiently strong uniform magnetic field. The principle of the technique depends on the tendency of certain atomic nuclei, notably phosphorus ( $^{31}\text{P}$ ), hydrogen ( $^1\text{H}$ ), an isotope of carbon with 1.1% abundance ( $^{13}\text{C}$ ), sodium ( $^{23}\text{Na}$ ) and fluorine ( $^{19}\text{F}$ ), to line up along a magnetic field. This alignment can be disturbed by applying a suitable radio-frequency pulse at right angles to the field. When the pulse ceases, the nuclei return to their previous alignment and in so doing generate a magnetic resonance signal which can be detected. The exact frequency of the signal depends on the chemical compound in which the nuclei reside, and the signal-intensity is proportional to concentration. In practice, the part of the body to be examined is placed within the horizontal bore of the magnet, and a succession of radiofrequency pulses is transmitted by a surface coil, which also acts as an aerial to detect the magnetic resonance signals returning from the tissue. These signals are summed and processed by Fourier transformation to generate a spectrum.

The technique is particularly suited to the investigation of hypoxic-ischaemic injury because it can be used to measure, non-

invasively in brain tissue, the relative intracellular concentrations of phosphorus metabolites that are important in energy metabolism, notably adenosine triphosphate (ATP), phosphocreatine (PCr) and inorganic orthophosphate (Pi). Intracellular pH ( $pH_i$ ) can also be estimated. ATP is the major energy source for the metabolism of the body and is dependent for its synthesis on the processes of oxidative phosphorylation and glycolysis. In conditions of inadequate oxygen supply or when the mechanisms involved in the utilization of oxygen are disrupted, the concentration of ATP will tend to fall. This tendency is however, counteracted by the creatine kinase reaction (Fig. 1). Because of the very high equilibrium constant of this reaction, phosphocreatine acts as a buffer for ATP, so that any impairment of oxidative phosphorylation will result initially in a fall in the concentration of PCr and an approximately equal rise in that of Pi, in other words a fall in the ratio of PCr to Pi. This ratio is a sensitive indicator of the efficacy of oxidative phosphorylation, because it is related directly to the phosphorylation potential  $[ATP]/([ADP][Pi])$  and to the free energy change of hydrolysis of ATP. Only when PCr/Pi has fallen to a low level will the concentration of ATP begin to fall.



**Fig. 1** Creatine kinase reaction. Any tendency for the concentration of adenosine triphosphate (ATP) to fall due to inadequate oxidative phosphorylation is buffered by the creatine kinase reaction. The net effect is a fall in the concentration of phosphocreatine (PCr) and a rise in that of inorganic phosphate (Pi).

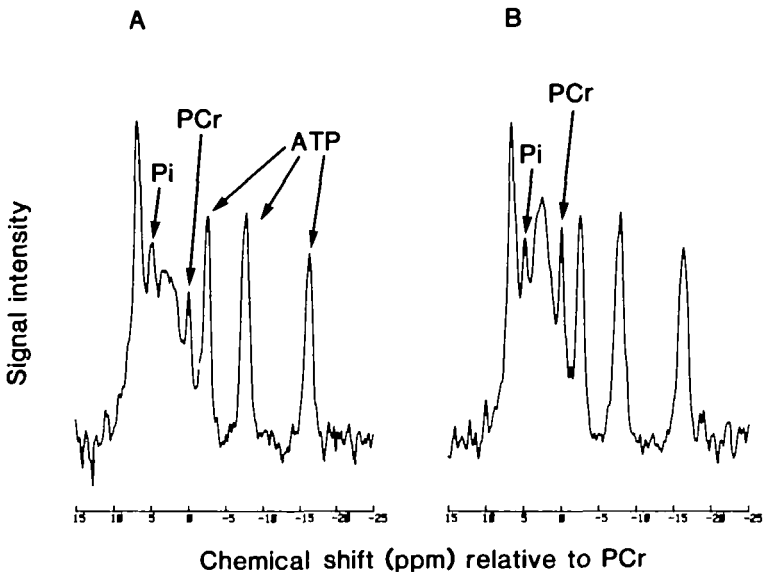
## Equipment and methods for studying the brain in newborn infants

We have used an Oxford Research Systems TMR 32-200 spectrometer operating at a field strength of 1.89 T. The diameter of the bore of the magnet was initially 20 cm and more recently 27 cm. For transporting and studying infants safely in a strong magnetic field, a special transport system is necessary, especially since ferromagnetic metal cannot, for reasons of safety, be allowed within 3 m of the magnet.<sup>12</sup> The system is based on a transport incubator with the baby carried in a perspex cylinder mounted on the top. This cylinder can be detached and inserted within the bore of the magnet while monitoring and mechanical ventilation, if necessary, are continued. Inside the cylinder, the baby's head lies on a surface coil 5 cm or 7.4 cm in diameter, which transmits the exciting radiofrequency pulses into the brain and also receives the returning magnetic resonance signals. The baby's head is positioned so that signals are obtained from the adjacent cerebral hemisphere, mainly from the temporoparietal cortex.<sup>13</sup> The surface coil can be tuned to the resonance frequencies of both <sup>31</sup>P (32.5 MHz) and <sup>1</sup>H (80.3 MHz). The <sup>31</sup>P spectra are usually obtained by Fourier transformation of 256 summed free induction decays following radiofrequency pulses repeated at intervals of 2.256 s. The pulse length is chosen to produce a flip angle of 90° at the centre of the coil. The effects of saturation (atomic nuclei not returning fully to their resting state between pulses) caused by the rapid pulse repetition rate have been measured for each metabolite and correction factors derived, so that the values for the gated spectral peak areas can be corrected and made proportional to concentration.<sup>14,15</sup> The resonance frequency of the Pi peak changes with pH<sub>i</sub>, which can therefore be calculated from the difference in frequency between the PCr and Pi peaks, using a version of the Henderson-Hasselbach equation.<sup>16</sup>

Studies have been performed at University College Hospital on over 180 infants, often on several occasions, usually because of suspicion of hypoxic-ischaemic brain injury, but also including normal infants.<sup>14,17-21</sup> Similar studies have been done in Philadelphia.<sup>22,23</sup> Few data are available so far from infants born at less than 28 weeks of gestation, but it is nevertheless possible to draw inferences about maturational events in the brain, and about the general effects of hypoxic-ischaemic injury on the <sup>31</sup>P metabolites and pH<sub>i</sub>, which are not likely to differ greatly with maturity.

### Normal changes with brain maturation

Figure 2 shows  $^{31}\text{P}$  spectra from two healthy infants without evidence of cerebral abnormalities, one born at 28 weeks of gestation (A) and the other born at term (B). Seven spectral peaks can be seen which are attributable, from left to right, to phospho-monoesters, Pi, phosphodiester, PCr, and the  $\gamma$ ,  $\alpha$  and  $\beta$   $^{31}\text{P}$  nuclei of nucleotide triphosphates, mainly ATP.<sup>11</sup> The  $\gamma$  and  $\alpha$  peaks include undetectably small contributions from adenosine diphosphate (ADP) and the  $\alpha$  peak, a contribution from nicotinamide adenine dinucleotide (NAD). The PCr and Pi peaks are of particular importance for the consideration of oxidative metabolism, for reasons that have been given above. In the immature infant, the area of the PCr peak appears relatively smaller, and that of the Pi peak larger, than in the term one. Data from 30 normal preterm and term infants, born between 24 and 42 weeks of gestation and studied at a median age of 5 days after birth show that PCr/Pi increases in a systematic way with gestation (Fig. 3).<sup>15</sup> It appears, therefore, that the phosphorylation potential of brain



**Fig. 2**  $^{31}\text{P}$  spectra from two infants with normal brains.  
**A:** Born at 28 weeks' gestation and studied 7 days after birth; PCr/Pi=0.80.  
**B:** Born at 40 weeks' gestation and studied aged one day; PCr/Pi=1.27.  
 For peak-assignments, see text.

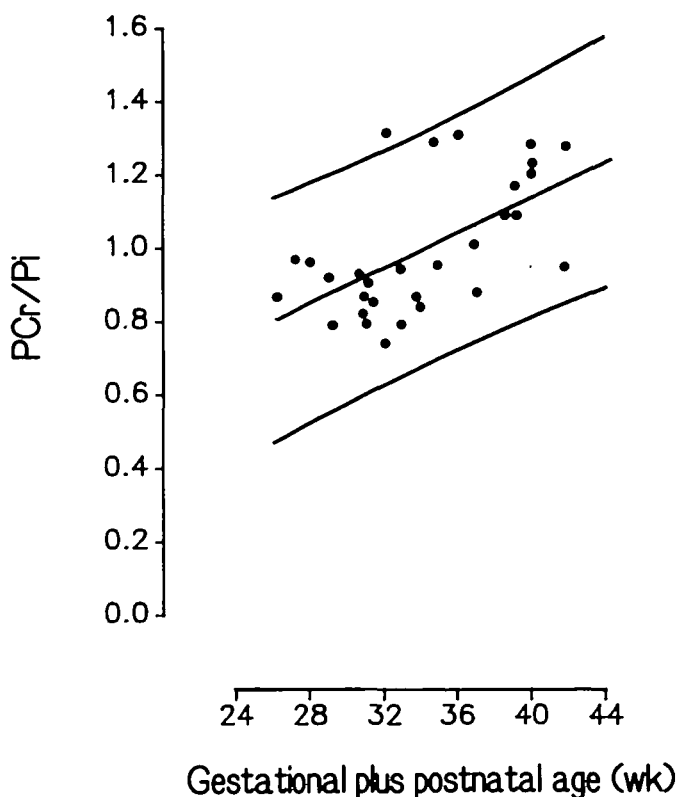


Fig. 3 Relation between PCr/Pi and gestational plus postnatal age in 30 infants with normal brains. The regression line and 95% confidence limits are shown.

tissue increases with maturation of the brain. Data for this and other metabolite concentration ratios are given in Table 1; the demonstration that PCr/total P (total phosphorus signal) and PCr/ATP increase, and Pi/total P and Pi/ATP fall with maturation are consistent with this view. Data from newborn animals show similar changes.<sup>24</sup> By comparison, the newborn human infant seems, in terms of phosphorus energetics, to be at about the same stage of maturation as other altricial animals such as the newborn rat but less so than the guinea pig or lamb<sup>24,25</sup> which are precocious. Table 1 also gives changes in other metabolite ratios. The prominent phosphomonoester (PME) peak has been shown by chemical analysis of the brain in the newborn of several animal species to be almost entirely attributable to phosphoethanolamine,<sup>26</sup> so it must be assumed that the same is true of the newborn

**Table 1** Changes in phosphorus metabolite concentration ratios and intracellular pH with gestational plus postnatal age in 30 infants with normal brains<sup>15</sup>

	28 wk	42 wk	r	P
PCr/Pi	0.85 ± 0.33	1.18 ± 0.33	0.58	< 0.001
ATP/total P	0.09 ± 0.03	0.10 ± 0.03	0.19	ns
PCr/total P	0.09 ± 0.02	0.11 ± 0.02	0.56	< 0.01
Pi/total P	0.10 ± 0.02	0.09 ± 0.02	-0.40	< 0.05
PME/total P	0.32 ± 0.04	0.26 ± 0.04	-0.68	< 0.001
PDE/total P	0.19 ± 0.04	0.22 ± 0.04	0.47	< 0.01
PCr/ATP	0.97 ± 0.42	1.09 ± 0.42	0.20	ns
Pi/ATP	1.13 ± 0.50	0.95 ± 0.50	-0.25	ns
PME/ATP	3.49 ± 1.23	2.79 ± 1.25	-0.37	< 0.05
PDE/ATP	2.11 ± 1.03	2.34 ± 1.05	0.15	ns
pH <sub>i</sub>	7.13 ± 0.39	7.13 ± 0.40	0.00	ns

Mean values ± 95% confidence limits from the regression lines are given.

PCr = phosphocreatine, Pi = inorganic orthophosphate, ATP = adenosine triphosphate, total P = total phosphorus, PME = phosphomonoester, PDE = phosphodiester, pH<sub>i</sub> = intracellular pH

human infant. Phosphoethanolamine is a major precursor of phospholipid in membranes and myelin. PME/total P and PME/ATP fall with maturation of the brain (see table) and it is known that the PME peak is small in spectra from the brains of adult animals and human subjects.<sup>24,27-29</sup> At the same time as the reduction in PME, phosphodiester (PDE)/total P and PDE/ATP increase. The PDE peak is due mainly to phosphatidylethanolamine and phosphatidylcholine, which are important constituents of membranes and myelin.<sup>30</sup> The fall in PME and rise in PDE with maturity have also been found in newborn animals and appear to reflect myelination and the proliferation of membranes.<sup>24</sup> With current developments in MRS, it will shortly be possible to provide data for absolute rather than relative concentrations of metabolites in brain and other tissue.<sup>31,32</sup>

### Small-for-gestational-age (SGA) infants

SGA infants are at increased risk of neurodevelopmental disabilities. In one study, several such infants were found to have evidence of impaired oxidative phosphorylation (low PCr/Pi ratios) in brain tissue in association with increased cerebral echodensities.<sup>19</sup> To find out whether SGA babies had intrinsic abnormalities of <sup>31</sup>P spectra indicating abnormal energy metabolism, or defective cell synthesis or myelination (as judged from metabolite ratios) a group of 13 babies with birthweights below the 3rd centile for gestation was studied.<sup>15</sup> Their gestational ages

ranged from 28 to 40 weeks (median 34 weeks) and birthweights from 780–2532 g (median 1405 g). The infants were selected because, apart from being SGA, they appeared completely normal as indicated by their clinical features and cranial ultrasound scans. No evidence was found of any difference in metabolite ratios or  $\text{pH}_i$  between the SGA infants and appropriately grown infants. Derangements of  $^{31}\text{P}$  spectra previously found in SGA babies were thought likely, therefore, to have been due to superimposed perinatal events, rather than any intrinsic cerebral abnormality.

### **Hypoxic-ischaemic brain injury**

The  $^{31}\text{P}$  spectra have been found to show similar abnormalities in a variety of situations associated with hypoxic-ischaemic injury, for example following birth asphyxia, during the progression of periventricular leucomalacia and other forms of cerebral infarction, and in the presence of intraparenchymal periventricular haemorrhage (Figs 4, 5). The evolution of the abnormalities has been investigated more thoroughly following birth asphyxia than in the other conditions, because birth-asphyxiated infants are often born at term and can be studied repeatedly, whereas this is not possible in small preterm infants.

#### *Birth asphyxia*

The evolution of the changes in the  $^{31}\text{P}$  spectra following birth asphyxia has been illustrated elsewhere.<sup>14,20</sup> It has been found that the spectra are often normal in the first hours of life. A progressive fall in  $\text{PCr}/\text{Pi}$  then takes place, to a minimum value at about 3 days of age. In the worst-affected babies, when  $\text{PCr}/\text{Pi}$  is very low,  $\text{ATP}/\text{total P}$  then also falls and death is likely to ensue. In surviving infants,  $\text{PCr}/\text{Pi}$  recovers to normal by about two weeks, though the total phosphorus signal may by then be reduced, due to loss of brain tissue. Intracellular  $\text{pH}$  is often elevated when  $\text{PCr}/\text{Pi}$  is low. The mechanisms responsible for this progression of events are by no means clear. In the newborn lamb<sup>25</sup> (as in adults of various species)<sup>28,29</sup> a severe acute hypoxic-ischaemic episode, analogous to an episode of birth asphyxia, causes immediate changes in the  $^{31}\text{P}$  spectra—first a fall in  $\text{PCr}/\text{Pi}$  and then in  $\text{ATP}/\text{total P}$ , as expected from consideration of the creatine kinase reaction (Fig. 1). At the same time,  $\text{pH}_i$  falls to a very low level, due to the production of lactic acid. When cerebral

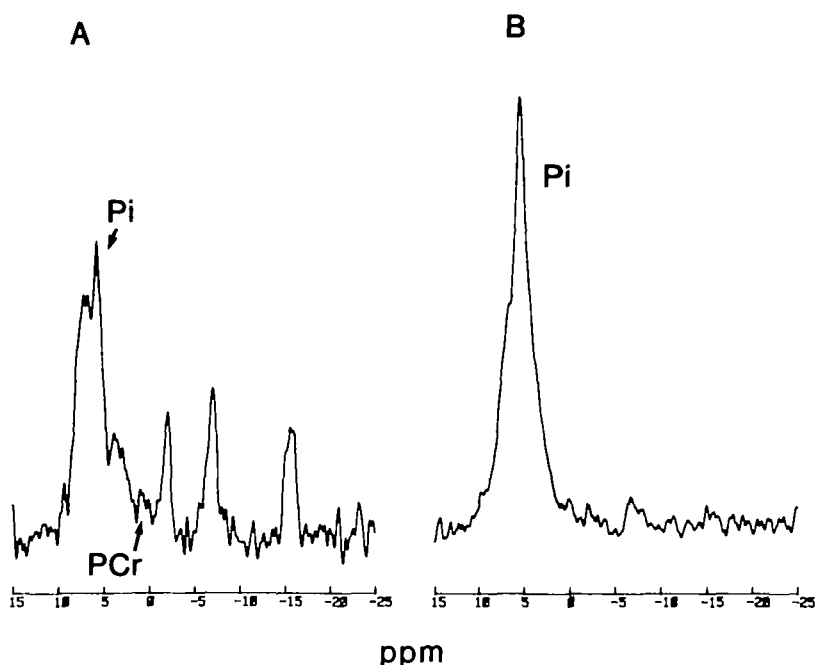


Fig. 4  $^{31}\text{P}$  spectra from two infants with severe brain injury.

**A:** Born at 25 weeks' gestation and studied aged 3 days (right hemisphere). Bilateral intraventricular haemorrhages and haemorrhagic parenchymal echodensities were present.  $\text{PCr}/\text{Pi}=0.22$ . Died aged 7 days.

**B:** Born at 27 weeks' gestation and studied aged 8 days (left hemisphere). He had suffered an extremely severe asphyxial episode aged 5 days. Bilateral intraventricular haemorrhages and diffusely increased parenchymal echodensities were present. PCr and ATP were virtually absent. Died aged 11 days.

oxygenation is restored all the abnormalities can revert to normal within about one hour.<sup>25</sup> It must be assumed that severely birth-asphyxiated babies, when studied during the first hours of life have been through this phase of acute (or primary) energy failure and are often in a state of apparently normal oxidative phosphorylation—possibly due partly to reduced demands for energy associated with cerebral depression. The subsequent development of 'secondary' energy failure is a well-recognized phenomenon of cerebral pathophysiology which is attributable to various imprecisely defined influences associated with reperfusion and reoxygenation of the tissue.<sup>33,34</sup> Which influences are the most important are unknown. Damage to the mitochondrial respiratory electron chain is very likely, following the abnormal entry of calcium ions into the cells, the generation of free radicals and

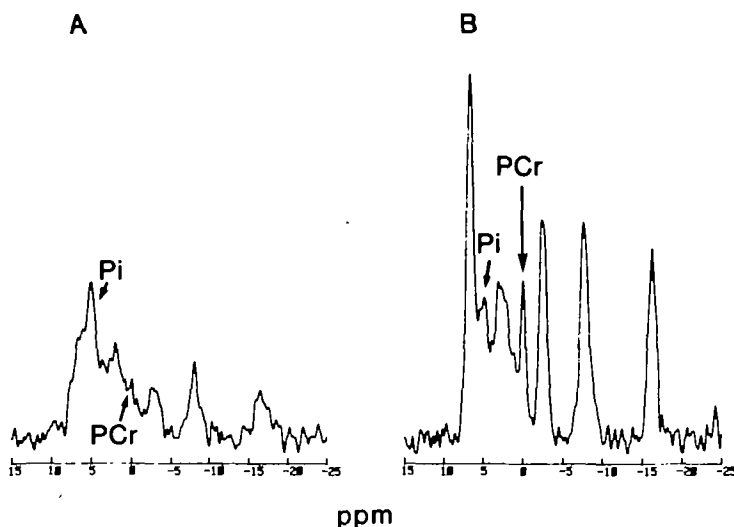


Fig. 5  $^{31}\text{P}$  spectra from the left (A) and right (B) hemispheres of a baby with infarction of the left hemisphere. He was born at 26 weeks' gestation and studied aged 6 days. Diffusely increased echodensities were present on the left.

The spectrum from the right hemisphere is normal;  $\text{PCr}/\text{Pi}$  0.93. On the left,  $\text{PCr}/\text{Pi}$  = 0.40, and the total  $^{31}\text{P}$  signal is reduced indicating death of cells.

He survived with a large left-sided porencephalic cyst.

the toxic effects of excitatory neurotransmitters.<sup>33-35</sup> Inadequate oxygen supply to the tissue because of capillary stasis and progressive cerebral oedema also probably plays a part. The increase in  $\text{pH}_i$  above normal may be due to abnormalities of sodium-hydrogen exchange. Further information about these mechanisms is urgently required, so that the effects of rationally based early treatment can be tested. Several investigations by MRS of the effects of an acute reduction in cerebral oxygenation,<sup>25</sup> and of manipulations of arterial carbon dioxide tension in newborn experimental animals, have been reported,<sup>36</sup> and the results of systemic glucose and bicarbonate administration during or immediately after a hypoxic-ischaemic episode have been documented.<sup>37</sup> These studies provide valuable information about brain buffering capacity, glucose consumption and lactate production in response to an acute hypoxic-ischaemic insult. They also show that lactate (measured by  $^1\text{H}$  MRS) may persist in brain tissue after the phosphorus spectra and  $\text{pH}_i$  have returned to normal.<sup>25</sup>

### **Periventricular leucomalacia and other forms of infarction**

Periventricular leucomalacia is infarction of the periventricular region of the brain in preterm infants following a hypoxic-ischaemic episode. The localization of the lesion to the periventricular tissue is probably due to the poorly developed blood supply to this region of the immature brain, which therefore sustains damage before other regions.<sup>1</sup> Periventricular leucomalacia may progress to cystic lesions or passive ventricular dilatation and is an extremely important cause of permanent neurodevelopmental disability, notably spastic diplegia. The timing of the initial injury is difficult to define precisely but appears usually to be in the peripartum period, sometimes initiated before delivery and sometimes afterwards. It is quite possible that damage to the periventricular region in very immature ill infants is cumulative, due to repeated episodes of hypotension and hypoxaemia.

Periventricular leucomalacia and other forms of infarction may be suspected before cysts or ventricular dilatation develop, because of the appearance on ultrasound scans of increased periventricular echodensities. However, as indicated earlier, the presence of increased echodensities of this type is not accurately diagnostic. A study with MRS has therefore been done of 27 infants with increased echodensities suggestive of periventricular leucomalacia, as well as of other forms of hypoxic-ischaemic injury—such as middle cerebral artery infarction and birth asphyxia.<sup>19</sup> The aim was to see if evidence of impaired energy metabolism could be found, and if so whether the severity of the impairment was related to subsequent death or loss of brain tissue (cysts or generalized loss) as assessed by ultrasound scans. Fifteen of the 27 infants had PCr/Pi ratios below normal 95% confidence limits, indicating impaired oxidative metabolism. Nine of these 15 died and in all 6 survivors loss of brain tissue developed. By contrast, all 12 infants whose ratios remained within the normal range survived, although loss of brain tissue developed in 3 infants with ratios towards the lower limit of normal. It was concluded that when impaired metabolism was detected in infants with increased echodensities the immediate prognosis was very bad.

Abnormal <sup>31</sup>P spectra associated with periventricular leucomalacia have been illustrated previously.<sup>9,19</sup> Figures 4 and 5 show abnormalities in other forms of hypoxic-ischaemic injury. The progression of the abnormalities in different types of injury

appears similar. In situations where the eventual outcome is loss of brain tissue, the total  $^{31}\text{P}$  signal becomes reduced<sup>17</sup> (Fig. 5).

### Neurodevelopmental prognosis

The MRS data have been found to provide prognostic information for neurodevelopmental outcome at one year of age.<sup>21</sup> More recently the brains of 43 infants born at 31–42 weeks of gestation were studied who were known or suspected on various grounds to have suffered hypoxic-ischaemic brain injury. (Ref. 38 and unpublished observations). The surviving infants were examined by independent observers. All 8 infants whose ATP/total P values fell below 95% confidence limits died. Of the 21 infants whose PCr/Pi values fell below 95% confidence limits, 14 died and 6 of the 7 survivors had disabling neurodevelopmental impairments. Only one of the 22 infants with a normal value for PCr/Pi died, and although 5 of the 21 survivors had impairments, these were less serious.

The data from this study and from the study of infants with increased echodensities cited above demonstrate that when severe failure of oxidative phosphorylation following a hypoxic-ischaemic insult is found, death ensues. Less severe, or localized, failure often leads to loss of brain tissue in survivors and is associated with a very high risk of neurodevelopmental disability.

### NEAR INFRARED SPECTROSCOPY (NIRS)

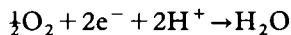
NIRS provides continuous bedside information about a wide range of indices of cerebral oxygenation and haemodynamics. The technique therefore shows great promise for the monitoring of ill babies, and it is complementary to MRS in exploring the mechanisms of impaired oxidative phosphorylation.

### Background and theory

Spectral analysis of transmitted or reflected visible light is a well-established method for measuring various indices of oxygenation in blood or tissue. However, visible light penetrates tissue very poorly, and information about oxygenation deep inside organs such as the brain cannot be obtained. In 1977 Jobsis showed that if near infrared light with a wavelength of 700–1000 nm was used instead of visible light, penetration of tissue was much greater and spectral measurements could be made across the heads of small

animals.<sup>39</sup> At near infrared wavelengths, absorption can be detected due to oxyhaemoglobin (HbO<sub>2</sub>), deoxyhaemoglobin (Hb) and the oxidized form of cytochrome aa<sub>3</sub>, the terminal enzyme of the mitochondrial respiratory electron transport chain, which passes electrons to molecular oxygen. The oxidative (redox) state of this enzyme is an index of intracellular oxygen availability.

Apart from its potential value in enabling brain oxygenation in ill babies to be monitored and regulated, data obtained by the combined use of MRS and NIRS may help elucidate some of the mechanisms of brain damage following a hypoxic-ischaemic episode. Measurements of HbO<sub>2</sub> and Hb in brain tissue give data about the circulating supply of oxygen, and measurement of oxidized cytochrome aa<sub>3</sub>, about the intracellular availability of oxygen to fuel oxidative phosphorylation. Figure 6 is a very simplified diagram summarizing oxidative phosphorylation. It shows that as a result of the activity of the citric acid cycle, substrate is consumed, and carbon dioxide and electrons are generated. The electrons pass down the respiratory chain, causing protons to be pumped out of the mitochondrial matrix, thereby generating a membrane potential. ATP is synthesized from ADP and Pi when the protons flow back to the matrix through a channel in an ATP-synthesizing complex (mitochondrial ATP-ase). At the end of the chain the electrons reduce cytochrome aa<sub>3</sub>, which becomes reoxidized when they combine with molecular oxygen:



This reaction accounts for nearly all of the oxygen uptake of the body.

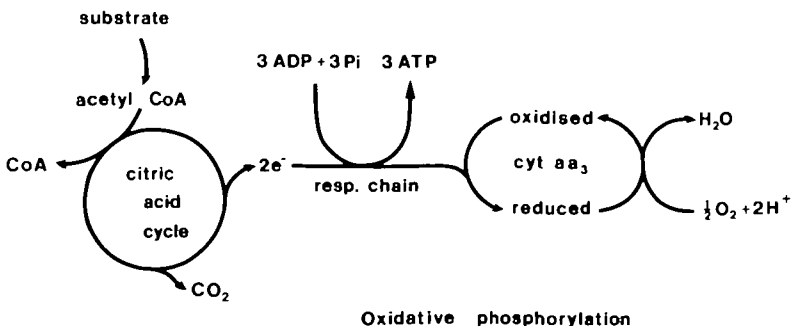


Fig. 6 Simplified diagram to illustrate oxidative phosphorylation. For explanation, see text.

Inadequate oxidative phosphorylation leads first to a fall in the PCr/Pi ratio and then in ATP concentration, as described above. The inadequacy could be due to several different mechanisms which can be investigated by observing the coexisting changes in haemoglobin and cytochrome aa3 oxygenation. For example, if insufficient oxygen supply to tissue was to blame, deoxygenation of haemoglobin and reduction of cytochrome aa3 would be expected, whereas failure of electron transport due to lack of substrate or to mitochondrial disruption would cause cytochrome aa3 to become highly oxidized.

### **Equipment and methods for studying the brain in newborn infants**

Fluctuations in signals from haemoglobin and oxidized cytochrome aa3 have previously been detected from the brains of newborn infants during hypoxaemic episodes<sup>40</sup> and transient elevations of blood pressure,<sup>41</sup> but until very recently quantitation of the data has not been possible.<sup>42</sup>

For quantitation certain variables must be defined. In particular, the spectral absorption properties of the compounds being investigated, and the optical path length (represented by the average distance travelled by photons through the tissue), must be known.

The Beer-Lambert law describing optical absorption in a scattering medium can be expressed as:

$$\text{absorption (in optical densities)} = \alpha cLB$$

where  $\alpha$  is the absorption coefficient ( $\text{mM}^{-1} \text{cm}^{-1}$ ),  $c$  is concentration (mM),  $L$  is the distance between the points where light enters and leaves the tissue (cm), and  $B$  is a 'path length factor' which takes account the scattering of light in the tissue (which causes the optical path length to be greater than  $L$ ). If  $\alpha$ ,  $L$  and  $B$  are known, then changes in absorption are directly proportional to changes in concentration.

Because of uncertainty about the absorption spectrum of oxidized cytochrome aa3 *in vivo*, observations have been made across the heads of rats exchange-transfused with a fluorocarbon blood-substitute, thus removing the signals from haemoglobin.<sup>43</sup> These observations allowed the absorption difference between oxidized and reduced cytochrome aa3 to be defined and, together with *in vitro* data for the absorption spectra of HbO<sub>2</sub> and Hb provide

information for obtaining the necessary values of  $\alpha$ . Measurements of optical path length across the head have only recently been made. In initial studies of babies, a path length factor (B) of  $1^{42,44}$  or  $2^{45}$  was assumed, but recent studies, including those in which the time taken for near infrared light to traverse the head of the rat was measured in picoseconds, suggest a value for B of between 4 and 5.<sup>43,46,47</sup>

Since more than one light-absorbing compound is present in the tissue, several NIR wavelengths must be transmitted and an algorithm is required to convert changes in optical absorption to changes in concentration. The observations described above have enabled a suitable algorithm to be devised.

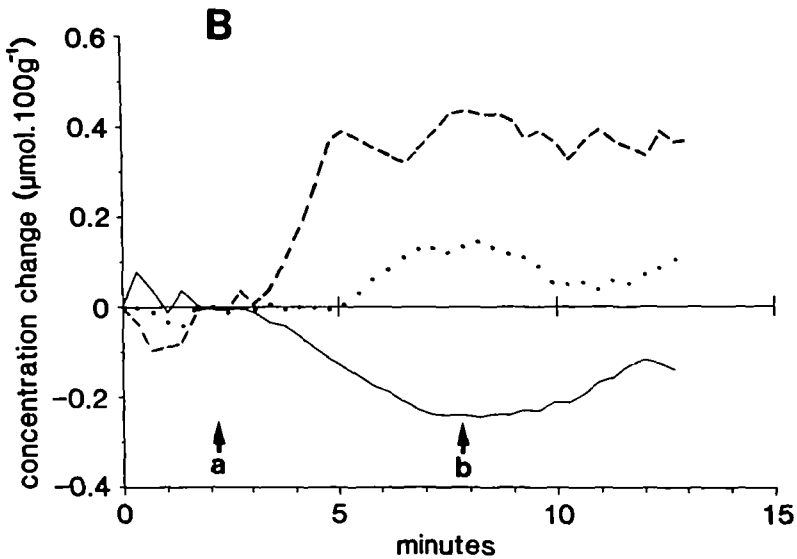
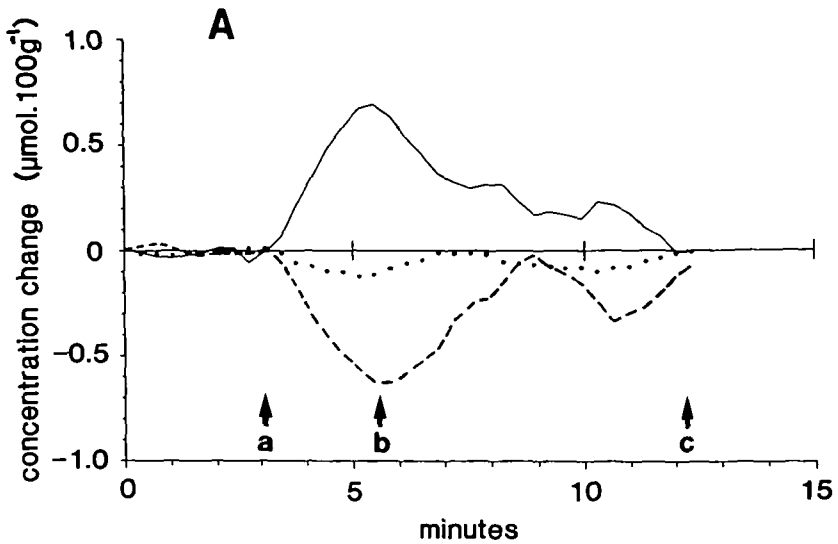
For current studies, three wavelengths 778, 813 and 867 nm, are employed and the factors for converting changes in optical density at these wavelengths to changes in concentration are given in Table 2. Concentration changes in HbO<sub>2</sub>, Hb and oxidized cytochrome aa3 are calculated by summation of absorption changes (measured in optical densities) multiplied by the appropriate factors. The values obtained are expressed in mM l<sup>-1</sup> cm<sup>-1</sup> of path length. If a value for tissue density in the light path of 1.05 is assumed, the data can be converted to mM · 100 g<sup>-1</sup>.

To study the brains of newborn infants, portable apparatus has been designed and built.<sup>42,48</sup> Near infrared light at the three wavelengths is emitted from laser diodes and directed into the head through a fibre-optic bundle positioned at a site equidistant from the anterior fontanelle and the external auditory meatus. Light emerging from the opposite side of the head is conveyed through another fibre-optic bundle to a photomultiplier tube operating in photon-counting mode. The ends of the fibres (optodes) are fixed to the scalp with adhesive rings, and optical contact is ensured by means of standard ultrasound contact gel. Background light is excluded by an opaque bonnet.

**Table 2** The factors for converting changes in optical density at 778, 813 and 867 nm to changes in concentration

Compound	Multiplying factor		
	778 nm	813 nm	867 nm
HbO <sub>2</sub>	0.32	-2.31	2.89
Hb	2.40	-2.67	0.66
Oxidized cytochrome aa3	-0.77	2.28	-1.30

The total light energy emitted by the laser diodes is about half that of a standard cold-light source, and the amount of energy absorbed by brain tissue is several orders of magnitude below British Standards Institute safety limits (BS 4803).



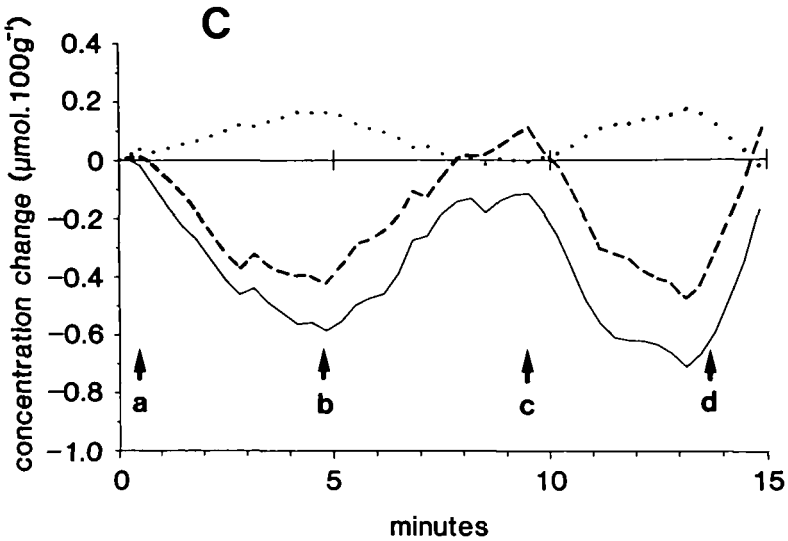


Fig. 7 Changes in the cerebral concentrations of HbO<sub>2</sub> (-----), Hb (—) and oxidized cytochrome aa3 (.....) in two infants with normal brains, born at 27 weeks' gestation and studied aged one day (A and B), and at 25 weeks and studied aged 70 days (C).

Changes are shown during:

A: Alterations in  $S_aO_2$ , which was 96% at a and c, and 84% at b.

B: Alterations in  $P_aCO_2$  which was 4.6 kPa at a and 5.7 kPa at b.

C: Head up tilting by 10° at a and c; and back to level at b and d.

Figure 7 shows changes in the concentrations of HbO<sub>2</sub>, Hb and oxidized cytochrome aa3 in response to small alterations in arterial oxygen saturation ( $S_aO_2$ ) and carbon dioxide tension ( $P_aCO_2$ ), and to head-up tilting.

### Quantitation of haemodynamic variables

NIRS primarily provides information about **changes** in the concentration of light-absorbing compounds in the brain as shown in Figure 7. In addition, **absolute** values for a number of important haemodynamic variables can be estimated, if the results of various manoeuvres are observed.<sup>42,44,45,49</sup> For example, total cerebral haemoglobin concentration (tHb) in  $mM \cdot 100 g^{-1}$  of tissue can be derived from the alterations in the concentration difference  $[HbO_2] - [Hb]$  which occur during a small transient change in  $S_aO_2$ . Provided cerebral blood flow and oxygen consumption remain constant during this change,

$[\text{HbO}_2] - [\text{Hb}]$  will vary linearly with  $S_a\text{O}_2$ <sup>42,49</sup> and tHb can be estimated from the expression:

$$\text{tHb} = \Delta([\text{HbO}_2] - [\text{Hb}]) \times 0.5 / \Delta(S_a\text{O}_2)$$

Cerebral blood volume in  $\text{ml} \cdot 100 \text{ g}^{-1}$  of tissue can then be obtained if a value for mean cerebral haematocrit is estimated.

It has also been suggested that an estimate of mixed cerebral venous saturation ( $S_v\text{O}_2$ ) can be made from observing the results of a small head-up or head-down tilt (Fig. 7c).<sup>42</sup> The skull of a newborn, particularly preterm, infant is much more compliant than later in life; hence the acute alterations in the cerebral concentrations of  $\text{HbO}_2$  and Hb which result from a tilt may reasonably be assumed to be due initially to changes in the size of the intracranial venous compartment.  $S_v\text{O}_2$  can then be calculated from the formula:

$$S_v\text{O}_2 = \Delta[\text{HbO}_2] / \Delta([\text{HbO}_2] + [\text{Hb}])$$

In the near future it is likely that methods will be described for quantitating a number of other important variables, notably cerebral blood flow and cerebral arterial saturation.

### Results in newborn infants

Quantitative results from NIRS of the brain in infants are so far scanty.<sup>42,44,45,49</sup> A number of tentative inferences may however be drawn from the available information. For example, the cerebral blood volume was  $2.3 (\text{SD} \pm 0.3) \text{ ml} \cdot 100 \text{ g}^{-1}$  in 8 babies born at 27–43 weeks of gestation who were thought to have normal brains. By contrast, it was significantly elevated,  $4.4 (\text{SD} \pm 1.0) \text{ ml} \cdot 100 \text{ g}^{-1}$ , when studied in 9 infants 24–42 hours after they had sustained brain injury—hypoxic-ischaemic in 7, and associated with cerebral haemorrhage in 2.<sup>45</sup> (It should be noted that the technique used does not measure blood-clot as part of the cerebral blood volume.) The response of the cerebral blood volume to small alterations in  $P_a\text{CO}_2$  was investigated in these same infants. The change in blood volume ranged from 0.2 to  $0.8 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{kPa}^{-1}$  in infants with normal brains, with evidence of reduced sensitivity to changes in  $P_a\text{CO}_2$  in the least mature ones. Infants with brain injury showed a significant decrease in response to changes in  $P_a\text{CO}_2$ . These preliminary findings suggest that fixed vasodilatation of the cerebral circulation and reduction of the responses to changes in  $P_a\text{CO}_2$  are common following brain injury.

This situation has been found to precede and be associated with the development of secondary energy failure, as detected by MRS and discussed above. The combined use of MRS and NIRS in experimental animals is beginning to provide further information about this sequence of events.<sup>50</sup>

Alterations in cytochrome aa3 oxidation have been quantified during transient alterations in  $S_aO_2$  and  $P_aCO_2$ .<sup>42,43</sup> Changes in the oxidation-state of the enzyme appear to follow those of haemoglobin (Fig. 7) demonstrating the relation between haemoglobin saturation and intracellular oxygen availability.

Repeated observations in five infants with convulsions have shown a fall in cerebral  $HbO_2$  concentration during the fit, together with a rise in that of Hb and a significant increase in cerebral blood volume.<sup>44</sup> At the same time cytochrome aa3 became less oxidized—except in one infant who was muscle-relaxed and showed increased haemoglobin oxygenation and cytochrome oxidation during paroxysmal electrical activity.

These findings all require confirmation in larger studies.

## SUMMARY AND CONCLUSIONS

Very preterm infants and other infants who require intensive care are at considerable risk of death or long-term neurodevelopmental disability. Non-invasive methods have therefore been sought for assessing the structure and function of the brain in the immediate newborn period. The availability of these methods allows the prevalence and mechanisms of potentially brain-damaging influences to be explored, preventive strategies and treatment to be tested and the prognosis for the infants to be assigned. Several methods have proved their worth: for example, ultrasound imaging, electroencephalography, including the testing of evoked potentials, and Doppler ultrasonography for measuring flow-velocity in intracerebral vessels.

Ultrasound-imaging is particularly valuable for investigating cerebral haemorrhage in very preterm infants, but is of little use for elucidating the early events of hypoxic-ischaemic brain injury, which is a more important cause than haemorrhage of long-term disability in very preterm and other infants who survive after intensive care. Furthermore, recent evidence suggests that lesser degrees of injury may go unrecognized by current techniques and could account for some of the subtle neurodevelopmental abnormalities that are found at school age in surviving infants. Non-

invasive methods have therefore been introduced for studying hypoxic-ischaemic injury. The purpose of this review has been to discuss two new methods, magnetic resonance spectroscopy and near infrared spectroscopy.

Magnetic resonance spectroscopy is used to investigate phosphorus energy metabolism and intracellular pH. Clear evidence of deranged energy metabolism has been found in a range of conditions of hypoxic-ischaemic injury. A latent period of many hours often follows acute injury before energy failure develops, suggesting the possibility of effective early treatment. Abnormalities of oxidative phosphorylation are predictive of a very poor prognosis for normal survival. Many new developments in magnetic resonance spectroscopy are imminent, particularly employing proton ( $^1\text{H}$ ) spectroscopy which will enable a wide range of metabolites, including neurotransmitters, to be measured. The combination of magnetic resonance spectroscopy with imaging will allow quantifiable data to be obtained from small selected volumes within the brain, and measurements of blood and cerebrospinal fluid flow are becoming possible.

Near infrared spectroscopy provides continuous information about cerebral oxygenation and haemodynamics at the cotside. Quantitative measurements of oxyhaemoglobin, deoxyhaemoglobin and oxidized cytochrome aa3 can be made, and a range of haemodynamic indices can be derived. The technique is already beginning to provide information about the haemodynamic events occurring in the brain after a hypoxic-ischaemic insult and before overt energy failure develops, both in newborn infants and experimental animals. It is likely eventually to go into routine use for monitoring brain oxygenation in ill babies and guiding therapy. Imaging of brain-oxygenation at the cotside will in the future probably be feasible.

The deployment of non-invasive techniques for examining the brains of very preterm and other ill infants looked after in neonatal intensive care units provides the best hope for achieving the major aim of intensive care, namely to ensure the maximum chances of survival for potentially normal children, but at minimum risk of salvaging hopelessly disabled ones.

## ACKNOWLEDGEMENTS

We thank for their help, R Aldridge, Dr AD Edwards, E Eldon, Dr PA Hamilton, Dr PL Hope, Miss C Richardson, and many members of the staff of the Neonatal

Unit and the Departments of Paediatrics, Medical Physics and Bioengineering, and Physiology; and we are grateful to Mrs G Harris for preparing the manuscript. This work was supported by Action Research for the Crippled Child, the DHSS, Hamamatsu Photonics, the MRC and the Wellcome Trust.

## REFERENCES

- 1 Pape KE, Wigglesworth JS. Haemorrhage, ischaemia and the perinatal brain. For: Spastics International Medical Publications, London: Heinemann, 1979
- 2 Levene MI, Williams JL, Fawer C-L. Ultrasound of the infant brain. Clinics in developmental medicine, No. 92. For: Spastics International Publications, Oxford: Blackwell, 1985
- 3 Gould S, Howard S, Hope PL, Reynolds EOR. Periventricular intraparenchymal cerebral haemorrhage in preterm infants: the role of venous infarction. *J Pathol* 1987; 151: 197-202
- 4 Stewart AL, Thorburn RJ, Hope PL, Goldsmith M, Lipscomb AP, Reynolds EOR. Ultrasound appearance of the brain in very preterm infants and neurodevelopmental outcome at 18 months of age. *Arch Dis Child* 1983; 58: 598-604
- 5 De Vries LS, Dubowitz LMS, Dubowitz V et al. Predictive value of cranial ultrasound in the newborn baby: a reappraisal. *Lancet* 1985; ii: 137-140
- 6 Sinha SK, Davies JM, Sims DG, Chiswick ML. Relation between periventricular haemorrhage and ischaemic brain lesions diagnosed by ultrasound in very preterm infants. *Lancet* 1985; ii: 1154-1156
- 7 Fawer C-L, Calame A, Perentes E, Anderegg A. Periventricular leukomalacia: a correlation study between real-time ultrasound and autopsy findings. *Neuroradiology* 1985; 27: 292-300
- 8 Trounce JQ, Fagan D, Levene MI. Intraventricular haemorrhage and periventricular leucomalacia: ultrasound and autopsy correlation. *Arch Dis Child* 1986; 61: 1203-1207
- 9 Hope PL, Gould SJ, Howard S et al. Precision of ultrasound diagnosis of pathologically verified lesions in the brains of very preterm infants. *Dev Med Child Neurol* 1988 (In press)
- 10 Stewart AL, Reynolds EOR, Hope PL et al. Probability of neuro-developmental disorders estimated from ultrasound appearance of brain in very preterm infants. *Dev Med Child Neurol* 1987; 29: 3-11
- 11 Gadian DG. Nuclear magnetic resonance and its application to living systems. Oxford, Clarendon Press, 1982
- 12 Chu A, Delpy DT, Thalayasingam S. A transport and life support system for newborn infants during NMR spectroscopy. In: Rolfe P, ed. Fetal and neonatal physiological measurements. London: Butterworths, 1986: pp. 409-415
- 13 Tofts PS, Cady EB, Delpy DT et al. Surface coil NMR spectroscopy of brain. *Lancet* 1984; i: 459
- 14 Hope PL, Costello AMdeL, Cady EB et al. Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants. *Lancet* 1984; ii: 366-370
- 15 Azzopardi D, Hamilton PA, Wyatt JS et al. Phosphorus metabolites and intracellular pH in the brains of normal and small-for-gestational age infants investigated by magnetic resonance spectroscopy (Submitted)
- 16 Petroff OAC, Prichard JW, Behar KL, Alger JR, den Hollander JA, Shulman RG. Cerebral intracellular pH by  $^{31}\text{P}$  magnetic resonance spectroscopy. *Neurology* 1985; 35: 781-788
- 17 Cady EB, Costello AMdeL, Dawson MJ et al. Non-invasive investigation of cerebral metabolism in newborn infants by phosphorus nuclear magnetic resonance spectroscopy. *Lancet* 1983; i: 1059-1062
- 18 Hope PL, Reynolds EOR. Investigation of cerebral energy metabolism in

- newborn infants by phosphorus magnetic resonance spectroscopy. *Clin Perinatol* 1985; 12: 261-275
- 19 Hamilton PA, Hope PL, Cady EB, Delpy DT, Wyatt JS, Reynolds EOR. Impaired energy metabolism in brains of newborn infants with increased cerebral echodensities. *Lancet*. 1986; i: 1242-1246
  - 20 Delpy DT, Cope MC, Cady EB et al. Cerebral monitoring in newborn infants by magnetic resonance and near infrared spectroscopy. *Scand J Clin Lab Invest* 1987; 47 (Suppl 188): 9-17
  - 21 Reynolds EOR, Hamilton PA. Magnetic resonance spectroscopy of the brain and early neurodevelopmental outcome. In: Kubli F, Patel N, Schmidt W, Linderkamp O, eds. *Perinatal events and brain damage in surviving children*. Berlin: Springer-Verlag, 1987: pp. 247-253
  - 22 Younkin DP, Delivoria-Papadopolous M, Leonard J et al. Unique aspects of human newborn cerebral metabolism evaluated with phosphorus nuclear magnetic resonance spectroscopy. *Ann Neurol* 1984; 16: 581-586
  - 23 Lawson B, Anday E, Guillet R, Wagerle LC, Chance B, Delivoria-Papadopoulos M. Brain oxidative phosphorylation following alteration in head position in preterm and term infants. *Pediatr Res* 1987; 22: 302-305
  - 24 Tofts PS, Wray S. Changes in brain phosphorus metabolites during the postnatal development of the rat. *J Physiol* 1985; 359: 417-429
  - 25 Hope PL, Cady EB, Chu A, Delpy DT, Gardiner RM, Reynolds EOR. Brain metabolism and intracellular pH during ischaemia and hypoxia. An in vivo  $^{31}\text{P}$  and  $^1\text{H}$  nuclear magnetic resonance study in the lamb. *J Neurochem* 1987; 49: 75-82
  - 26 Gyulai L, Bolinger L, Leigh JS, Barlow C, Chance B. Phosphorylethanolamine—the major constituent of phosphomonoester peak observed by  $^{31}\text{P}$ -NMR in developing dog brain. *FEBS Lett* 1984; 178: 137-142
  - 27 Bottomley PA, Hart HR, Edelstein WA et al. Anatomy and metabolism of the normal human brain studied by magnetic resonance at 1.5 tesla. *Radiology* 1984; 150: 441-446
  - 28 Delpy DT, Gordon RE, Hope PL et al. Non-invasive detection of cerebral ischemia by phosphorus nuclear magnetic resonance. *Pediatrics* 1982; 70: 310-313
  - 29 Behar KL, den Hollander JA, Stromski ME et al. High resolution  $^1\text{H}$  nuclear magnetic resonance study of cerebral hypoxia in vivo. *Proc Natl Acad Sci USA* 1983; 80: 4945-4948
  - 30 Cerdan S, Subramanian H, Hilberman M et al.  $^{31}\text{P}$  NMR detection of mobile dog brain phospholipids. *J Mag Res Med* 1986; 3: 432-439
  - 31 Wray S, Tofts PS. Direct in vivo measurement of absolute metabolite concentrations using  $^{31}\text{P}$  nuclear magnetic resonance spectroscopy. *Biochim Biophys Acta* 1986; 886: 399-405
  - 32 Tofts PS, Wray S. Non-invasive measurement of molar concentrations of  $^{31}\text{P}$  metabolites in vivo using surface coil NMR spectroscopy. *J Mag Res Med* 1988; 6: 84-86
  - 33 Siesjo BK. *Brain energy metabolism*. New York: Wiley, 1978
  - 34 Siesjo BK. Cerebral circulation and metabolism. *J Neurochem* 1984; 60: 883-908
  - 35 Schwarcz R, Meldrum B. Excitatory amino acid antagonists provide a therapeutic approach to neurological disorders. *Lancet* 1985; ii: 140-143
  - 36 Cady EB, Chu A, Costello AmdeL et al. Brain intracellular pH and metabolism during hypercapnia and hypocapnia in the new-born lamb. *J Physiol* 1987; 382: 1-14
  - 37 Hope PL, Cady EB, Delpy DT, Ives NK, Gardiner RM, Reynolds EOR. Brain metabolism and intracellular pH during ischaemia: The effects of systemic glucose and bicarbonate administration studied by  $^{31}\text{P}$  and  $^1\text{H}$  nuclear magnetic resonance spectroscopy in vivo in the lamb. *J Neurochem* 1988; 50: 1394-1402

- 38 Azzopardi D, Wyatt JS, Cady EB et al. Prognosis of infants with hypoxic-ischaemic brain injury assessed by phosphorus magnetic resonance spectroscopy *Pediatr Res* 1987; 22: 220
- 39 Jobsis FF. Non-invasive infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* 1977; 198: 1264-1267
- 40 Brazy JE, Lewis DV, Mitnick MH, Jobsis van der Vliet. Non-invasive monitoring of cerebral oxygenation in preterm infants: preliminary observations. *Pediatrics* 1985; 75: 217-225
- 41 Brazy JE, Lewis DV. Changes in cerebral blood volume and cytochrome aa3 during hypertensive peaks in preterm infants. *J Pediatr* 1986; 108: 983-987
- 42 Wyatt JS, Cope M, Delpy DT, Wray S, Reynolds EOR. Quantitation of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet* 1986; ii: 1063-1066
- 43 Wray S, Cope M, Delpy DT, Wyatt JS, Reynolds EOR. Characterisation of near infrared absorption spectra of cytochrome aa3 and haemoglobin for the non-invasive monitoring of cerebral oxygenation. *Biochim Biophys Acta* 1988; 933: 184-192
- 44 Wyatt JS, Cope M, Delpy DT, Wray S, Reynolds EOR. Cerebral oxygen and haemodynamics during neonatal seizures assessed by continuous near infrared spectrophotometry. *Early Hum Dev* 1987; 15: 307
- 45 Wyatt JS, Cope M, Delpy DT, Wray S, Richardson C, Reynolds EOR. Responses of cerebral vasculature to changes in arterial carbon dioxide measured by near infrared spectroscopy in newborn infants. *Pediatr Res* 1987; 22: 230
- 46 van der Zee P, Delpy DT. Simulation of the point spread function for light in tissue by a Monte Carlo technique. *Adv Exp Med Biol* 1988; 215: 179-192
- 47 Cope M, van der Zee P, Arridge SR, Wray S, Wyatt JS, Delpy DT. Direct measurement of optical pathlength during NIR transillumination of the brain. *Phys Med Biol* (In press)
- 48 Cope M, Delpy DT. A system for long term measurement of cerebral blood and tissue oxygenation in newborn infants. *Med Biol Eng Comput* 1988 (In press)
- 49 Wyatt JS, Cope M, Delpy DT, Richardson C, Edwards AD, Reynolds EOR. Measurement of cerebral blood volume in newborn infants by near infrared spectroscopy (Submitted)
- 50 Aldridge R, Cady EB, Cope MC et al. Simultaneous measurements of cerebral oxygenation and metabolites by near infrared (nir) and <sup>31</sup>P nuclear magnetic resonance (nmr) spectroscopy during hypoxia in rats. *J Physiol* 1988; 396: 96P