

Cerebral monitoring in newborn infants by magnetic resonance and near infrared spectroscopy

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Phosphorus magnetic resonance spectroscopy (MRS) and near infrared spectroscopy (NIRS) have been used to study the brains of normal newborn infants and infants with cerebral disorders admitted to a neonatal intensive care unit. MRS, which involves transporting the infant to the spectrometer, allows measurement of mobile phosphorus compounds such as adenosine triphosphate and phosphocreatine in brain tissue, and has been performed on over 160 babies. NIRS gives cotside information about cerebral oxygenation and haemodynamics and has recently been introduced. These techniques, especially when used together, show promise of providing important information about the mechanisms and prognostic significance of hypoxic-ischaemic damage to the brain – the most important cause of permanent neurodevelopmental disabilities in infants who require intensive care.

Key words: Blood oxygenation, blood volume, cytochrome aa3, phosphorus, tissue oxygenation, haemoglobin.

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Hypoxic-ischaemic brain injury accounts for at least one quarter of all perinatal deaths and in surviving infants is the commonest cause of permanent neurodevelopmental disabilities arising in the perinatal period. Modern non-invasive methods for investigating the brain, notably ultrasound-imaging, have recently provided much important information about the pathogenesis of hypoxic-ischaemic injury and also about cerebral haemorrhage, which particularly affects preterm infants. However, neither ultrasound nor any other non-invasive technique allows observations to be made at a very early stage

of hypoxic-ischaemic injury, when treatment might have some hope of success.

We have therefore explored two new methods for obtaining direct information about cerebral metabolism and haemodynamics in newborn infants, magnetic resonance spectroscopy and near infrared spectroscopy. Magnetic resonance spectroscopy (MRS) is used to measure, in brain tissue, the concentrations of 'high-energy' phosphorus compounds that are important in energy metabolism, and also to estimate intracellular pH. Near infrared spectroscopy (NIRS) provides cotside information

about blood and tissue oxygenation and cerebral haemodynamics.

This review summarises the methods used, and gives some results from normal infants and infants with cerebral disorders studied at University College London.

MAGNETIC RESONANCE SPECTROSCOPY (MRS)

BACKGROUND AND METHODS

The fundamental principles of MRS and its application to living systems have been reviewed by Gadian [1]. For studying human subjects large bore superconducting magnets had first to be developed. We have used an Oxford Research Systems TMR 32-200 spectrometer with a clear bore of 26.5 cm and operating at a field strength of 1.89 tesla. To perform MRS studies the infants are transported to the magnet and positioned within the bore. Because many of the infants are seriously ill, facilities for mechanical ventilation and close monitoring of vital functions are mandatory. This has necessitated the development of a transport incubator system capable of operating in a high magnetic field and which does not interfere with the operation of the spectrometer [2]. The infants are studied with a single-turn radiofrequency coil [3] mounted in a pillow. The diameter of the coil is 5.0 cm or 7.4 cm, depending on the size of the head, which is positioned so that the magnetic resonance signals received by the coil come from the temporo-parietal cortex [4]. For studies of phosphorus (^{31}P) compounds, spectra are usually obtained by Fourier transformation of 256 summed free induction decays (FIDs) 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 caused by the rapid pulse repetition rate have been measured for each metabolite, and a set of saturation factors derived [5] so that the values for gated spectral peak areas can be corrected and made proportional to concentration.

RESULTS

Normal infants. Over 160 babies have, since 1982, been studied by MRS at University College London (UCL), mostly because of a suspected or proven cerebral disorder but also including normal preterm and term infants. Similar studies have been done in

Philadelphia [6]. Fig. 1 shows a ^{31}P spectrum from a normal term infant. The peaks are attributable, from right to left, to magnesium-complexed nucleotide triphosphates, mainly adenosine triphosphate (ATP), phosphocreatine (PCr), phosphodiester, inorganic orthophosphate (Pi) and phosphomonoesters: the broad signal underlying the left-hand peaks is probably due largely to mobile phospholipids [7]. The phosphomonoester peak has been attributed largely to phosphoethanolamine, a major precursor of membrane phospholipid and myelin [8]: it becomes less prominent with increasing maturation of the brain and is small in adult brain tissue in man and experimental animals [9, 10]. The areas of the PCr and Pi peaks change in opposite directions with increasing maturation, causing PCr/Pi, which is an index of the 'energy status' or phosphorylation potential of brain tissue, to increase (Fig. 2). Intracellular pH (pH_i) in brain tissue, measured from the difference in resonance frequency (or 'chemical shift') between the PCr and Pi peaks is 7.0-7.1 and probably does not alter with maturation of the brain [5, 11].

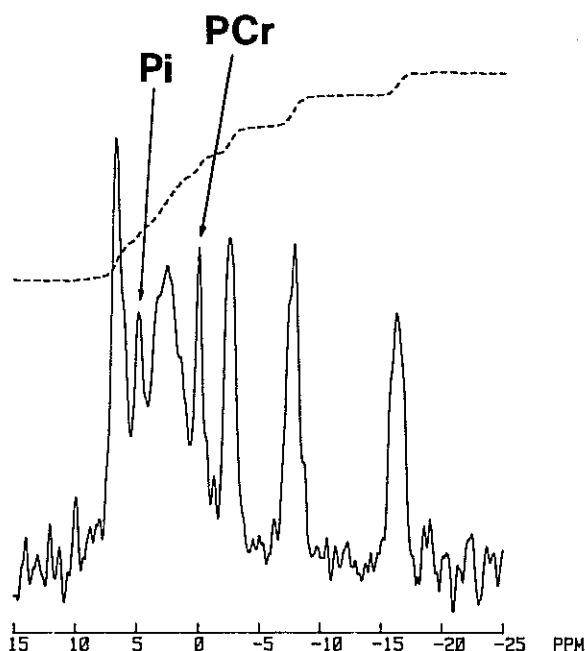


FIG. 1. ^{31}P MRS spectrum from a normal infant born at 40 weeks of gestation and studied aged 1 day. The x-axis is frequency expressed in ppm relative to the phosphocreatine (PCr) peak. The y-axis is signal intensity: the interrupted line is the integral of the spectrum. Peak assignments are, from right to left, the β , α , γ , phosphorus nuclei of nucleotide triphosphates, mainly adenosine triphosphate, PCr, phosphodiester, inorganic orthophosphate (Pi) and phosphomonoesters, mainly phosphoethanolamine. (Data of Hamilton et al [14]).

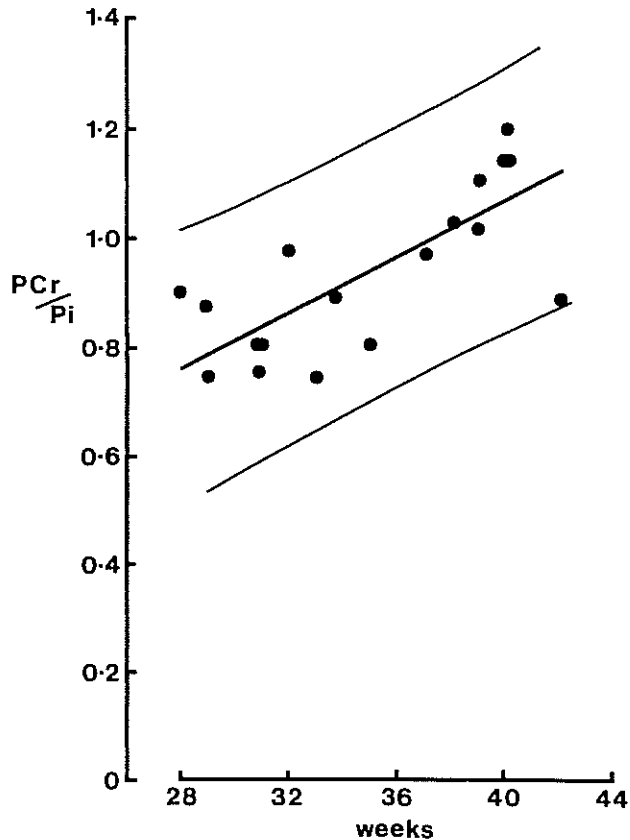


FIG. 2. Relation between PCr/Pi (calculated from the integrals of the spectra and corrected for saturation) and gestational plus postnatal age in 18 normal infants. The regression line and 95% confidence limits are shown. (Data of Hamilton et al [14]).

Abnormal infants. Infants with a variety of cerebral disorders have been studied at UCL [5, 11-14]. Abnormalities in the ^{31}P spectra have been found almost exclusively in infants who have sustained a suspected or proven hypoxic-ischaemic episode.

Birth asphyxia. Early studies [5, 13] and subsequent experience have shown that following severe birth asphyxia the ^{31}P spectra are commonly normal in the first hours of life. A progressive fall in PCr/Pi then takes place, to a minimum value at about 3-5 days of age (Fig. 3). In badly damaged babies, when PCr/Pi is very low, the ATP concentration (expressed as a fraction of total phosphorus signal, Ptot) may also fall, and death ensues. In surviving infants PCr/Pi recovers to normal by about two weeks. Perhaps unexpectedly, pH_i is often abnormally high in infants with low values for PCr/Pi. The mechanisms which account for this progression of events are by no means clear. In experimental animals, a severe acute hypoxic-ischaemic episode [15, 16] – analogous to an episode of birth asphyxia – causes acute changes in the ^{31}P spectra, first a fall in PCr/Pi and then in ATP/Ptot – as expected from consideration of the creatine kinase reaction [5]: these changes are accompanied by a fall in pH_i to very low levels, due to the production of lactic acid.

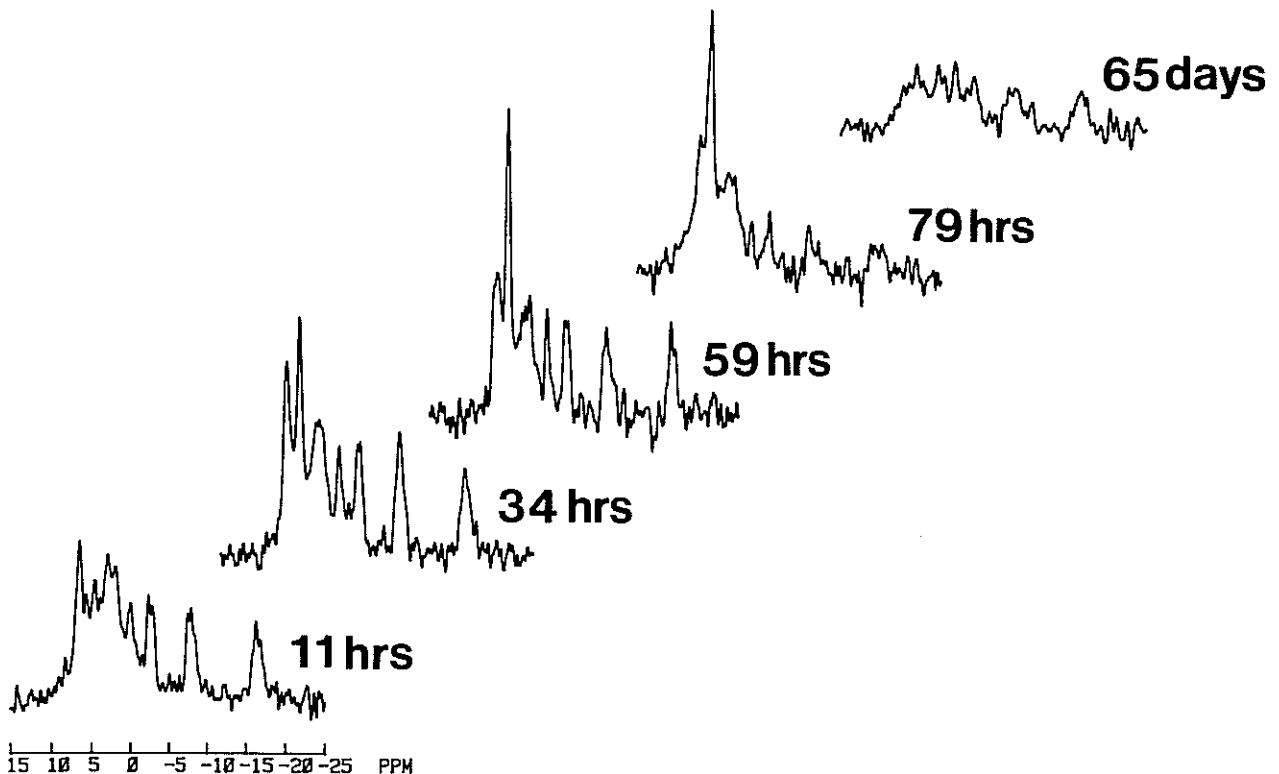


FIG. 3. Sequence of ^{31}P spectra from the brain of a baby who was born at 40 weeks of gestation following severe birth asphyxia. The ages at study are indicated. The spectrum on the first day of life was normal. PCr/Pi then progressively fell. By 65 days extensive cerebral atrophy had developed: he died aged one year.

On restoration of cerebral oxygenation all the abnormalities can revert to normal over a period of about one hour [16]. We assume that severely birth-asphyxiated babies, when studied soon after delivery, are often in this state of apparent normality. The subsequent development of 'secondary' energy failure is a phenomenon that is very familiar in cerebral pathophysiology and is attributable to various influences associated with reperfusion of the tissue [17]. Precisely which influences are the most important are unknown. Damage to the mitochondrial respiratory electron transport chain seems very likely, due for example to free radicals, calcium entry to cells, and the toxic effects of excitatory neurotransmitters [18]: inadequate oxygen supply may also play a part. Further information about these mechanisms is urgently required, so that the effects of rationally applied early treatment can be tested. The use of NIRS to observe changes in brain oxygenation and haemodynamics during the development

of secondary energy failure may provide useful clues about which interventions are most appropriate.

Other forms of hypoxic-ischaemic injury. Changes in ^{31}P spectra similar to those encountered following severe birth asphyxia have been found in infants with various other forms of hypoxic-ischaemic injury, for example, infants with the ultrasound appearance of periventricular leucomalacia and of middle cerebral artery infarction [13, 14].

Prognosis. Low levels for PCr/Pi are highly prognostic of death or subsequent loss of brain tissue, which carries a bad prognosis for neurodevelopment. For example, in infants selected for study because of ultrasound findings suggestive of hypoxic-ischaemic damage, no infant with a value below 95% confidence limits for normal infants survived without cerebral atrophy (Fig. 4). Long term follow-up studies are in progress which suggest that the neurodevelopmental status of infants who have sustained hypoxic-ischaemic injury is quite closely related to the lowest PCr/Pi value recorded [19].

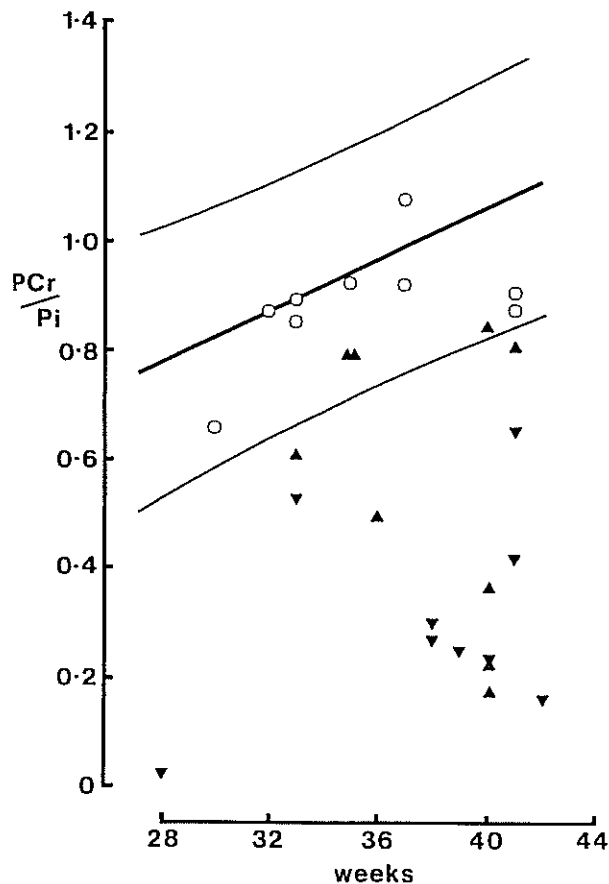


FIG. 4. Relation between PCr/Pi and outcome in 27 infants with ultrasound brain scans suggestive of hypoxic-ischaemic damage. The regression line and 95% confidence limits for normal infants (Fig. 2) are shown. O = survived without cerebral atrophy; ▲ = survived with cerebral atrophy ▼ = died. (Data of Hamilton et al [14]).

NEAR INFRARED SPECTROSCOPY (NIRS)

BACKGROUNDS AND METHODS

Measurements of blood and tissue oxygenation by spectral analysis of transmitted or reflected light is a well established method [20, 21]. Until the mid-1970s, light in the visible part of the spectrum was usually employed, where strong absorbance peaks are detectable [22]. In 1977 [23] Jobsis showed that if near infrared light with a wavelength of 700-1000 nm was used instead of visible light, absorption by tissue was low enough for spectral measurements to be made across animal heads with diameters of 5-6 cm. In the near infrared region, absorption due to oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (HbR) can be observed (Fig. 5) and also absorption (around 830 nm) due to the oxidised form of cytochrome aa3 (cyt aa3-O_2), the terminal enzyme in the respiratory mitochondrial electron transport chain, which passes electrons to molecular oxygen. The oxidative (or redox) state of this enzyme gives an indication of intracellular oxygen availability. Several groups of investigators have developed apparatus for continuous monitoring of these variables [24-27].

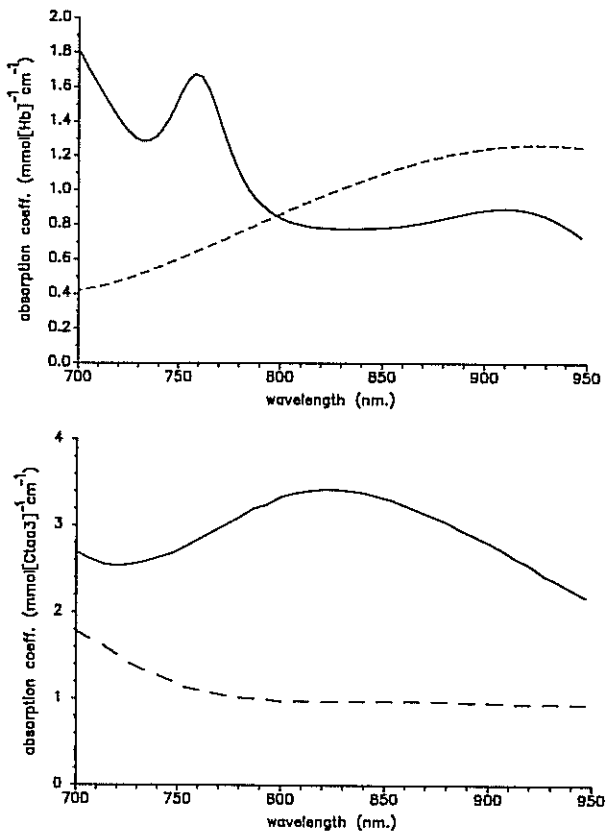
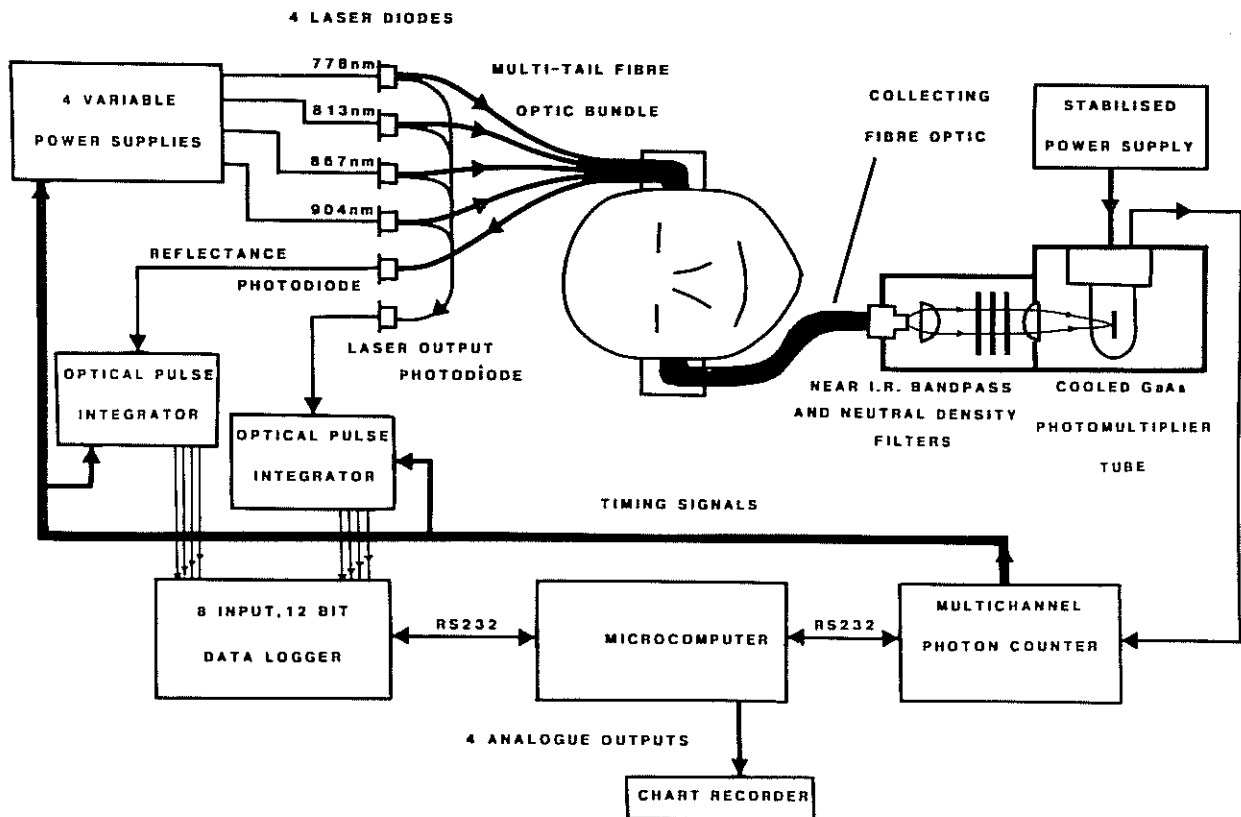


FIG. 5. (Upper) NIR absorption spectrum of HbO₂ (---) and HbR (—). (Lower) NIR absorption spectrum of oxidised (—) and reduced (---) cytochrome aa3.

Instrumentation. Although penetration of tissue by light is much greater in the NIR than in the visible part of the spectrum, attenuation is still high, approximately one optical density (OD) per cm. Many workers have therefore developed equipment to monitor the light reflected from the tissue rather than to transilluminate it. This approach has drawbacks. It is extremely difficult to define the region in the tissue from which the signal is being reflected, and also the optical path length, which has to be known if the data are to be made quantitative.

We have therefore designed and built new apparatus for NIRS which can make measurements across 10 OD [28]. The transillumination of the heads of preterm and most term infants then becomes possible. With this geometry it is much easier to model mathematically the light transport through the tissues and hence estimate the optical path length [29]. The instrument is shown schematically in Fig. 6. Four semiconductor laser diodes are used as light sources, which are pulsed sequentially at 4 kHz with a pulse length of 100 nsec. The diode output power is approximately 10 watts peak, but because of the low duty cycle, the total average power is approximately 16 mW. This is an order of magnitude below the exposure limits allowed (BS4803).



INFRA-RED TRANSMILLUMINATION SYSTEM

FIG. 6. Diagram of the NIR measurement system.

Light from the diodes is transmitted to the infant's head via a flexible fibre optic cable. The ends of the cable (optodes) are attached to the side of the head by a double-sided adhesive ring. Light emerging from the other side is collected by a similar fibre and conveyed to a photomultiplier detector which is operated in photon counting mode, to ensure maximum sensitivity. The detected photon counts are stored in a multichannel counter (one channel per laser diode). Interference by stray light entering the fibre optic cable is reduced to a low level by wrapping a light-proof cloth around the infant's head. The sensitivity of the detector to stray light is minimised by gating the photon counter so that photons are only counted when the laser diodes are firing, and also by sampling the background photon count when no diode is operating: this background count is then subtracted by the controlling microcomputer.

To resolve the absorption changes due to cyt aa3-O₂ from those of haemoglobin, the resolution of the instrument must be better than 0.01 OD. Also, for long term monitoring, drift in the system must be much less than 0.01 OD per hour. To ensure this level of performance, the temperature of the photomultiplier is held constant by a Peltier cooler and drift in the output intensity of the laser diodes is continuously monitored and compensated for by the computer.

After obtaining measurements representing absorption changes at several wavelengths, conversion to quantities of HbO₂, HbR and cyt aa3-O₂ in the light-path is performed by a relatively simple matrix inversion, which assumes that absorption changes due to each compound can be linearly summed. This assumption is only valid in a scattering medium if the range of variation of absorption coefficient is small [30]. There is considerable evidence that this is true in the NIR range, where absorption is low and the absorption spectra show no sharp peaks [31]. The absorption factors for HbO₂ and HbR used in the calculation were derived from measurements made on haemoglobin solutions; and for oxidised cyt aa3-O₂ they were measured *in vivo* in rats whose blood had been replaced by a fluorocarbon blood-substitute [32, 33]. The factors used with our prototype machine are as follows:

Compound	Multiplying factors		
	778 nm	813 nm	867 nm
HbO ₂	-0.499	-1.756	+2.577
HbR	+1.768	-0.877	-0.421
Cyt aa3-O ₂	-0.559	+1.659	-0.949

Using these factors, concentration changes are expressed in mmol · l⁻¹ · cm⁻¹ of path length. If a value for brain specific gravity of 1.05 is assumed, the data can be converted to mmol · 100 g⁻¹ of tissue. In our preliminary studies we have assumed that the path length of light through the head is the same as the distance between the optodes: this assumption is considered further below.

RESULTS

Preliminary studies, some of which have been published [26], have been carried out on 25 preterm and term babies. Seven had no evidence of a cerebral abnormality, but the other 18 had sustained various forms of cerebral injury - particularly hypoxic-ischaemic injury.

Figs. 7 and 8 show typical alterations in HbO₂, HbR and cyt aa3-O₂ in an infant with a normal brain,

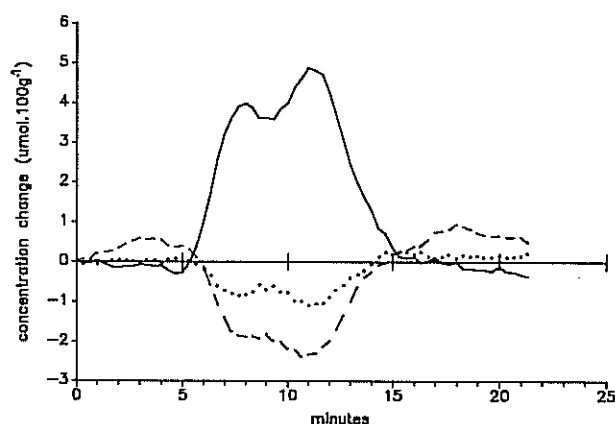


FIG. 7. Changes in the concentrations of HbO₂ (---), HbR (—) and cyt aa3 (.....) in the brain of a 3-day old infant during a transient decrease in SaO₂, from 95% at 5 minutes to 70% at 10 minutes. He had been born at 39 weeks of gestation with *Listeria septicaemia* but had no evidence of cerebral abnormality.

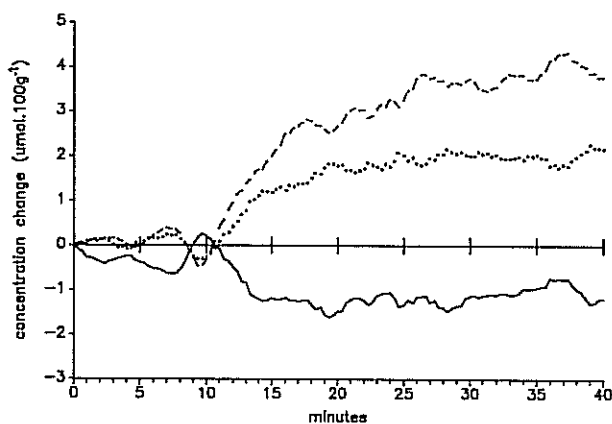


FIG. 8. Changes in the concentrations of HbO₂ (---), HbR (—) and cyt aa3 (.....) in the brain during an increase in PaCO₂ from 3.5 kPa at 10 minutes to 5.8 kPa at 30 minutes. Same infant as in Fig. 7.

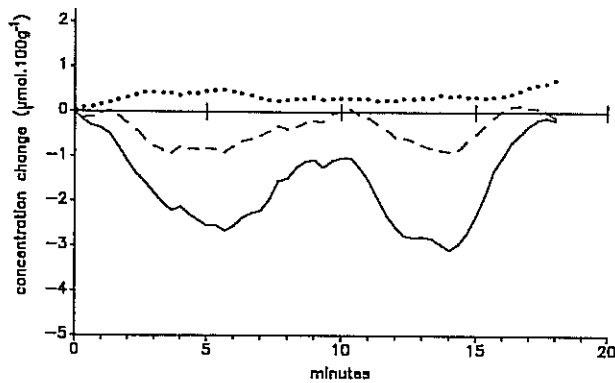


FIG. 9. Changes in the concentrations of HbO₂ (— — —), HbR (————) and cyt aa3 (·····) in the brain following 10° head-up tilts at 0 and 10 minutes. The infant was 5 weeks old and had been born at 28 weeks of gestation. (Data of Wyatt et al [26]).

in response to changes in arterial oxygen saturation (SaO₂) and carbon dioxide tension (PaCO₂).

Increases in SaO₂ were regularly associated with an increase in cerebral HbO₂ and a corresponding fall in HbR, indicating a rise in mean cerebral haemoglobin saturation. Cyt aa3-O₂ also showed a small but consistent increase, indicating increased intracellular oxygen availability. In infants with normal brains increases in PaCO₂ caused similar changes and also a striking increase in cerebral blood volume, up to 2 ml · 100 g⁻¹ · kPa⁻¹, as estimated from the sum of the HbO₂ and HbR signals: however, in 3 babies who had been very severely birth-asphyxiated, cerebral blood-volume did not respond to changes in PaCO₂ [34]. Although the sum of HbO₂ and HbR provides information about changes in cerebral blood volume, it does not provide absolute values for volume: these can, however, be obtained by observing the effect of changes in SaO₂ at constant PaCO₂ on the 'haemoglobin oxygenation index' (HbO₂-HbR) [26]. If cerebral blood volume (CBV) and oxygen consumption remain constant during transient changes in SaO₂, then HbO₂-HbR should vary linearly with SaO₂ and volume can be derived from the expression

$$CBV = d(\text{HbO}_2 - \text{HbR})/d(\text{SaO}_2) \times 50$$

Such a linear relation has been found, yielding values for CBV between 2.2 and 10.5 ml · 100 g⁻¹ of tissue in the population studied.

Estimates of cerebral venous saturation have been made by observing the effects of a small head up or head down tilt (Fig. 9). If no change in SaO₂ and PaCO₂ occur, acute alterations in cerebral HbO₂ and HbR are likely to be due to changes in the size of the intracranial venous compartment (the

head is very compliant in newborn infants, particularly preterm ones). Cerebral venous saturation (SvO₂) may then be calculated from the formula:

$$SvO_2 = \frac{d \text{HbO}_2}{d(\text{HbO}_2 + \text{HbR})}$$

This calculation does not require any assumptions about optical path length. Values obtained for SvO₂ have been between 18% and 66%. A rough estimate of changes in cerebral blood flow can be obtained by observing changes in the HbR signal, provided the assumption can be made that cerebral oxygen consumption remains unchanged.

DISCUSSION

The value of MRS as a method for exploring events in the brains of newborn infants has become established. Normal values for ³¹P spectra have been defined and maturational changes detected which require further explanation. In particular in the PDE and PME regions of the spectrum there is a need to determine firstly which metabolites contribute to these peaks *in vivo*, and then to determine their functional roles. It is clear from normal brain spectra that these regions do not contain just one resonance peak (Fig. 1). Studies have been made of brain extracts since these yield narrower lines and hence make identification easier [35]. However, the methods necessary to extract the metabolites and their unphysiological state (e.g. examined at pH 10), means that much caution is needed in applying these results to *in vivo* brain spectra [36]. A more satisfactory approach may be to perform *in vivo* animal studies using very high field magnets - which give increased spectral resolution.

One of the major contributors to the PME peak is phosphoethanolamine which is a precursor of membrane components. It is not clear why other related PME precursors, for example phosphocholine, do not appear to be present in such high concentrations. The decrease in phosphoethanolamine with postnatal age is thought to reflect the decrease in cell synthesis and myelination with increased development. PDE arise from the turnover of larger membrane components. They can also be produced in response to osmotic stress [37]. Changes in the brain PDE peak during degenerative diseases, such as Alzheimer's, have been reported [38]. However, there does not appear to have been any investigation of how this peak, or individual components of it, change with postnatal development.

The mechanisms involved in the 'secondary' energy failure which, after a latent period, follows a

severe asphyxial episode are of great interest, and their elucidation provides a major stimulus to the development of NIRS. The ability to observe indices of cerebral oxygenation and haemodynamics before and during the observed deterioration of PCr/Pi, and eventually of ATP/Ptot, may give crucial clues about which methods of treatment are likely to be the most effective as well as enabling the effects of treatment to be tested objectively.

Further development of the methodology of NIRS is, however, required before it can become a routine investigative or clinical tool. One apparently simple practical problem that has to be solved is improved attachment of the optodes to the scalp. The optical fibres must be kept as small and flexible as possible, but the cladding material on commercial fibres is stiff: thinner or more flexible cladding tends to let light in, hence increasing the background photon count. We are currently investigating various flexible plastic materials for this purpose. Another difficulty is the response time of the system. By operating in photon counting mode we are working at maximum possible sensitivity: nevertheless, 10,000 photons must be detected for the standard deviation to be less than 1%. The photon counter can operate at 10 MHz, but so as to maintain a high degree of system linearity, a count rate of 100 KHz is used. Given the repetition frequently of the laser diodes, 15 seconds are required for 10,000 photons to accumulate. The response time could be reduced by using a faster photon counter, or counting at a faster rate and then correcting for the non linearity [39]. Reduction of the response time to 1 second would permit the measurement of cerebral blood flow by following the passage of a bolus of cardiogreen dye – which has a strong NIR absorption band – through the brain [40].

The final technical problem that awaits solution is the most difficult one. We have shown that with the aid of measurements of SaO₂ and PaCO₂, it is possible to use NIRS to quantitate a variety of cerebral physiological indices [26]. In some of the calculations an assumption is needed about average photon path length. In theory, the NIR signal can be analysed using a multicomponent curve fitting technique [41] – but additional data from extra laser diodes operating at as many wavelengths as feasible would then be required. The standard error of estimation achieved by sophisticated curve fitting can also be minimised by careful choice of the wavelengths at which the measurements are made [42]. Work presently under way on the modelling of light transport in tissue is likely to provide data on aver-

age photon path length which can be incorporated into curve-fitting routines [29].

CONCLUSIONS

Magnetic resonance spectroscopy (MRS) has proved to be a very useful method for exploring the developmental changes in mobile phosphorus compounds and intracellular pH which take place in the brains of newborn infants and experimental animals, and also for observing events during and after a hypoxic-ischaemic episode. Furthermore, the spectra obtained provide important prognostic information. Near infrared spectroscopy (NIRS) gives instantaneous information about cerebral oxygenation and haemodynamics. Particularly when used in conjunction with one another these two techniques should enable much to be learnt about the mechanisms of hypoxic-ischaemic brain injury in newborn infants, as well as allow prevention and treatment to be tested in a rational way.

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