THE USE OF INTENSITY MODULATED OPTICAL SPECTROSCOPY TO MEASURE CEREBRAL SATURATION AND HAEMOGLOBIN CONCENTRATION IN THE HUMAN FETUS DURING LABOUR

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ABSTRACT

Background

Although intrapartum hypoxia-ischaemia is an important cause of death and permanent brain injury, current available methods for the detection of damaging fetal hypoxia are unsatisfactory and unreliable. Electronic fetal heart rate monitoring (EFM) is considered to be the "gold standard" for intrapartum fetal surveillance. However, monitoring of the fetal heart rate provides an indirect measure of fetal hypoxia and provides little indication of the adequacy of cerebral perfusion. Consequently, EFM has a false positive rate of 99.8% in the detection of fetuses that subsequently develop cerebral palsy. A direct consequence of the poor specificity of fetal heart rate monitoring is a high rate of unnecessary Caesarean sections with associated fetal and maternal morbidity.

Most fetuses subjected to intrapartum hypoxia will be protected from brain injury by appropriate changes in cardiovascular distribution. It is the aim of fetal surveillance to detect the minority of fetuses in whom this response is absent and who are at risk of permanent brain injury.

Intrapartum fetal pulse oximetry is a promising new method of fetal surveillance, enabling measurement of fetal arteriolar saturation but the technique provides no direct information on cerebral oxygen delivery.

Intrapartum measurement of fetal cerebral saturation *and* changes in blood volume has been described using conventional near infrared spectroscopy (NIRS). However, the clinical relevance of these values may be limited. This is because these values are derived from **changes** in the concentrations of oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (Hb) from an arbitrary baseline. Furthermore, the contribution of artefact to NIRS measurements of Hb and HbO_2 changes, arising from possible changes in the geometry of the NIRS fetal probes during uterine contractions, has not been clearly defined.

Using novel methods of collection and analysis of NIRS data, the technique of Intensity Modulated Optical Spectroscopy (IMOS) has the unique potential to provide direct information on fetal cerebral oxygenation and perfusion during labour from measured **absolute** values of fetal cerebral HbO_2 and Hb.

Furthermore, the technique of IMOS has the potential to provide more information on the contribution of probe movement during uterine contractions to conventional NIRS data.

Aims

The aims of this project were therefore to use this new technique of intensity modulated optical spectroscopy to (a) provide the first measurements of absolute cerebral blood volume and cerebral saturation in healthy normoxic human fetuses during labour and (b) to compare these values with those calculated from fetuses that develop hypoxia-ischaemia during labour and (c) to assess the contribution of probe movement during uterine contractions.

Methods

After assessing and optimising the technical performance of a specially designed and constructed intensity modulated optical spectrometer, a specially designed optical probe was placed against the scalp of 29 fetuses after rupture of amniotic membranes during labour and connected to the spectrometer.

Results

Of these 29 fetuses, data were collected from 18 fetuses during the first and second stage of labour through to delivery. Of these 18, data was suitable for analysis in 10 of these fetuses. In these 10 fetuses, a mean (+/-1 S.D.) value of cerebral saturation of **59 +/-12 %** and a mean absolute cerebral blood volume of **2.8 +/-1.0mls/100g** over 3 uterine contractions were derived from the mean concentrations of Hb and HbO_2 of **30 +/-18** and **46 +/-21 μmol/l**, respectively.

Concentration changes rather than artefact appeared to dominate the NIR signal in the calculation of these values.

Conclusion

This work has provided the first measurements of absolute values of fetal cerebral oxygenation and of cerebral perfusion, whilst the contribution of artefact to the data, certainly in the healthy fetus, appears to be negligible. However, despite these advances in near infrared technology and knowledge of intrapartum fetal cerebral haemodynamics, the number of fetuses studied with near infrared spectroscopy, in particular IMOS, remains small. In order for IMOS to be subjected to larger studies to assess it's usefulness as a realistic adjunct to fetal heart rate monitoring, advances in the technology are still required.

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GLOSSARY

AROM	artificial rupture of amniotic membranes
CTG	cardiotocograph
DK	David Kirkby, Medical Physicist
ECG	electrocardiograph
EFM	electronic fetal heart rate monitoring
FBS	fetal blood sampling
FHR	fetal heart rate
FSE	fetal scalp electrode
[HbO2]	oxyhaemoglobin concentration
[Hb]	haemoglobin concentration
[HbT]	total haemoglobin concentration
IMOS	Intensity modulated optical spectroscopy
IOS	interoptode spacing
JC	Jeremy Chipchase
LSCS	lower segment caesarean section
NIRS	near infrared spectroscopy
NVD	normal vaginal delivery
OD	optical density
PO_2	pulse oximetry
MC	Mark Cope
μ_a	Absorption coefficient

CHAPTER 1

INTRODUCTION

Despite striking advances in obstetric and neonatal care, encephalopathy induced by hypoxia-ischaemia of the fetus and newborn infant continues to be an important problem in society. Cerebral palsy develops in approximately 2-3 out of 1000 live births during the first years of life (Bakketeig 1999), and its association with the intrapartum period has contributed to the speciality of obstetrics and gynaecology having the highest proportion of medical litigation and damages paid for hospital claims. Known liabilities for brain damaged babies have been valued at between £660 million and £1 billion (Symonds 1993) and over a third of obstetric claims notified to the Medical Defence Union arise from allegations that intrapartum hypoxia resulted in permanent brain damage or perinatal death (James 1991).

Thus one of the tasks of the obstetrician is the prevention of any condition which could jeopardise the proper functioning and viability of the fetus and its brain. Because of this, considerable attention over the last few decades has been devoted to the understanding of the neuropathological sequelae of hypoxia-ischaemia during the perinatal period in the hope that this will lead to the development of improved diagnostic and predictive modalities. Both clinical studies and experiments in laboratory animals have attempted to describe the effect of hypoxia-ischaemia on the developing fetal brain and to explain the physiological as well as the abnormal fetal and newborn cardiovascular and respiratory responses, which may be relevant to human fetuses during labour.

This chapter explains the background upon which this thesis is based. Firstly, the role of hypoxic intrapartum events in the aetiology of brain injury and long term neurological handicap is discussed. Current knowledge of the underlying pathophysiology of hypoxic

fetal brain injury and the fetal response to hypoxia are then reviewed. This provides the background to assess a) the clinical outcome measures used to identify those neonates thought to be at risk of subsequent long term neurological handicap and b) the usefulness of the current available methods to monitor fetuses to detect those at risk of cerebral damage during labour.

1.1 FETAL BRAIN INJURY

1.1.1 Intrapartum events and cerebral palsy

Historically, the association of cerebral palsy and intrapartum events has led to much controversy, which has continued up to the present day. In 1843 William John Little, a London Orthopaedic surgeon published his lectures on Deformities of the Human Frame, and described a 'spasmodic tetanus-like rigidity and distortion of the limbs of new-born infants' following asphyxia neonatorum, and mechanical injury to the fetus immediately before or during parturition. His subsequent thesis concluded that cerebral palsy was primarily due to perinatal cerebral injury. Little's work was supported by William Osler in 1888 who first used the term 'cerebral palsy' and stressed its association with birth asphyxia in many cases. This view that cerebral palsy necessarily followed birth asphyxia or trauma, was subsequently challenged by Sigmund Freud who stressed that there was a lack of correlation of clinical findings with either purported aetiology or neuropathological findings in the neonate. Within the last decade evidence has emerged to challenge the old concept that most cases of cerebral palsy begin in labour. Clinical epidemiological studies have indicated that in most cases the events leading to cerebral palsy occur in the fetus before the onset of labour, or in the newborn after delivery (Blair 1988,1993). A large proportion of cases of cerebral palsy is now thought to arise antenatally (Badawi et al 1998). In addition, neonatal causes of cerebral palsy which include metabolic abnormalities (Volpe 1995) autoimmune and coagulation disorders (Nelson1998a), infections (Grether 1997) and developmental abnormalities (Stanley 1984, Nelson 1986) require exclusion before a case of cerebral palsy could be considered to arise from intrapartum events.

Intrauterine infection

What does seem clear is that in many instances the cause of perinatal brain injury is multi-factorial. A source of current speculation is the possible impact of coexisting infection and intrapartum hypoxia-ischaemia on the fetal brain. A recent meta-analysis found that among full term infants there was a positive correlation between clinical chorioamnionitis and cerebral palsy (Wu 2000), while a similar relation exists between histologically diagnosed fetal chorioamnionitis and brain injury (Redline 2000). Thus combined exposure to infection and intrapartum hypoxia-ischaemia dramatically increases the risk of spastic cerebral palsy compared with hypoxia alone (Nelson 1998b). However, the correlation between severity of maternal disease and neonatal outcome is not clear, as many infants born to mothers with obvious signs of chorioamniotis have a normal outcome. Thus the precise relationship between antepartum factors, such as inflammatory activation as a result of infection, intrapartum hypoxia –ischaemia stress and perinatal brain injury is still unclear (Peebles 2002).

Defining a causal relationship between intrapartum events and cerebral palsy, therefore, remains difficult (MacLennan et al 1999). It is now estimated that only 7 to 15% of perinatal deaths and cases of neurological impairment in children are the result of hypoxic damage occurring during the course of labour and delivery (Blair1988, Badawi 1998, Nelson 1988, Grant 1989).

Possible antenatal causes of neurological impairment are listed in Table 1.1(MacLennan et al 1999). The presence of any one of these factors greatly reduces the likelihood that acute intrapartum hypoxia was the cause, or the sole cause, of any subsequent neurological impairment. The absence of any of these factors does not exclude an antenatal cause, as much of the evidence can be very difficult to ascertain retrospectively.

Table 1.1 Factors that suggest a cause of cerebral palsy other than acute intrapartum hypoxia.

Umbilical arterial base deficit less than 12 mmol/l or pH greater than 7.00
Infants with major or multiple congenital or metabolic abnormalities
Central nervous system or systemic infection
Early imaging evidence of longstanding neurological abnormalities for example, ventriculomegaly, porencephaly, multicystic encephalomalacia
Infants with signs of intrauterine growth restriction
Reduced fetal heart rate variability from the onset of labour
Microcephaly at birth (head circumference less than a third of the centile)
Major antenatal placental abruption
Extensive chorioamnionitis
Congenital coagulation disorders in the child
Presence of other major antenatal risk factors for cerebral palsy for example, preterm birth at less than 34 weeks' gestation, multiple pregnancy, or autoimmune disease
Presence of major postnatal risk factors for cerebral palsy for example, postnatal encephalitis, prolonged hypotension, or hypoxia due to severe respiratory disease
A sibling with cerebral palsy, especially of the same type

Neonatal brain imaging

Different imaging modalities have been used to define the origin and timing of brain lesions in term infants with neonatal encephalopathy. Ultrasound, because of its portability and ease of performing serial evaluations, has become the most common imaging method used for this purpose. However, resolution is limited to lesions greater than 5-8mm, and the peripheral field of view is limited. Computerised tomography likewise has limited ability to detect small lesions and to differentiate the grey-white matter interface (Barkowitz 1997). Magnetic resonance imaging has far superior resolution and currently it is being used more frequently to assess brain structure (Robertson and Wyatt 2004). In the most recent study using MRI to assess the timing of brain injury, Cowan (2003) suggested that events in the immediate perinatal period are

most important in neonatal brain injury. Thus, brain images showed evidence of an acute insult without established injury or atrophy in 197 (80%) of infants with neonatal encephalopathy and evidence of perinatal asphyxia (see table 1.1a). Only 2 infants (<1%) had MRI evidence of established injury. Of those infants without other evidence of encephalopathy, but who presented with seizures within 3 days of birth, acute focal damage was noted in 62(69%) of infants whereas only two (3%) also had MRI evidence of antenatal injury.

Table 1.1a MRI abnormalities to define the timing of brain injury (Cowan 2003).

Signs of an acute perinatal insult
Brain swelling
Cortical highlighting
Focal or global loss of grey-white matter differentiation
Abnormal signal intensity in the basal ganglia and thalami
Loss of normal signal intensity in the posterior limb of the internal capsule
Acute and subacute parenchymal, intraventricular or extracerebral haemorrhage
Acutely evolving focal infarction in an arterial territory or in a parasaggital or watershed distribution
Signs of an antenatal insult
Irregular ventricular dilatation, widening of the interhemispheric fissure, enlarged extracerebral space
Established cystic lesions e.g. cystic periventricular leucomalacia
Focal infarction with atrophy
Longstanding haemorrhage
Marked asymmetries e.g. in ventricular shape or size
Developmental abnormalities e.g. abnormal cerebellar development

1.1.2 PATHOPHYSIOLOGY OF BRAIN INJURY

1.1.2.1 Normal cerebral physiology

The survival of the brain is dependent on a continuous and adequate supply of oxygen and nutrients. Maintenance of cerebral perfusion seems to be essential for long-term survival (Gunn 1992). Failure of the cerebral circulation results in almost instantaneous (less than 2 minutes) neuronal dysfunction with rapid progression to cell death. The factors controlling cerebral blood flow are, therefore, of considerable importance. In the fetus and newborn, the most common cause of cerebral hypoxia (relative lack of oxygen in the tissues) is inadequate oxygen delivery because of lower than normal oxygen levels coupled with inadequate cerebral blood flow (ischaemia).

Under normal conditions transport form the maternal circulation is the primary source of oxygen for the fetus. Values of the arterial partial pressure of oxygen in the mother are higher (aorta: 95mmHg) than that of the fetal umbilical vein (35 mmHg), thus allowing a steep gradient to facilitate diffusion of oxygen from mother to fetus. In spite of the normally low arterial partial pressure of oxygen, the transport of oxygen to fetal tissues appears more than adequate. This is in part due to the increased oxygen carrying capacity of the fetal blood, in turn due to the higher haemoglobin concentration, and to the higher oxygen affinity, which permits the saturation of fetal blood with oxygen at a lower oxygen partial pressure. Thus although the rate of oxygen uptake by the placenta is about 50% greater per gram of tissue than that of the fetus, oxygen affinity is higher in fetal than in human blood thus ensuring that an adequate supply of oxygen reaches the fetal tissues. The fetal carcass (skin, bone, muscle) has the highest (50% of total) consumption of absolute oxygen while the brain with the liver and heart make up a further 35-40% of total oxygen consumption (Fowden 1995).

Autoregulation

Oxygen delivery to the brain is a product of oxygen content of the blood (derived from haemoglobin concentration and saturation) and tissue blood flow (or perfusion,

measured in ml/min/100g). It is commonly stated that the blood flow to the fetal brain in the near term infant is approximately 100ml/min/100g-brain tissue (Longo 1997). Knowledge of the factors controlling cerebral circulation is based on estimates of *total* blood flow to the brain. The perfusion pressure of the blood supplying the brain is the difference between the arterial blood pressure and the cerebral venous pressure. The mean arterial blood pressure can be altered over a fairly wide range without affecting cerebral blood flow.

This phenomenon is called 'autoregulation' and is defined as the intrinsic ability of cerebral resistance vessels to alter their calibre and maintain a constant blood volume and flow in the face of alterations in perfusion pressure (Harper 1990). Cerebral arteriolar vasodilatation, resulting in an increase in cerebral blood volume, occurs in response to hypotension, hypercapnia (increase in arterial carbon dioxide) and hypoxia. A reduction in blood pressure causes at transient reduction in flow and a build up of metabolites, such as adenosine and hydrogen ions, which in turn causes vasodilatation and a restoration of flow and maintenance of blood volume.

1.1.2.2 Cerebral metabolism

The other nutrients required for fetal growth have important roles in oxidative metabolism. Glucose is the main substrate for oxidative metabolism in utero and facilitated diffusion of glucose across the placenta occurs. The fetus can also produce glucose endogenously by gluconeogenesis in the liver and kidneys, though this usually occurs in adverse conditions such as intrauterine growth restriction. Lactate is the second most important carbohydrate fuel in the fetus and is mostly derived from glucose. The majority of the lactate is consumed by the fetus and utilised in the heart and liver. Amino acids are essential for oxidation and their supply is dependent on placental metabolism as well as transplacental transport. Lipids are essential for fetal growth and play a minor role in oxidative metabolism (Nordstrom 1998).

Under normal conditions, with sufficient oxygenation and perfusion to the fetus, aerobic metabolism occurs in which glucose is broken down to pyruvate along the glycolytic

pathway, within the cytosol. Oxygen is directly consumed at the inner membrane of the mitochondrion in the catabolic reaction of NADH to form NAD+ and water. It is this reaction that releases large amounts of energy to produce the principle energy source within the cell, namely adenosine triphosphate (ATP), via oxidative phosphorylation. ATP helps to maintain cellular integrity, as cellular functions require ion gradients, which in turn require ATP to function. With sufficient oxygen supply, the cell maintains oxidative metabolism and there is a steady state between lactate and pyruvate concentrations. If the oxygen supply is less than adequate then the fetus has to engage in anaerobic metabolism, from which the end products are hydrogen ions and lactate. With anaerobic metabolism the lactate/pyruvate ratio increases. Regeneration of NAD+ is provided by the oxidation of pyruvate to lactate, concomitantly producing H+ ions (lactic acidosis) (see figure 1.1). With depletion in cellular energy, cellular functions can not be maintained, as ATP regeneration is stopped (Windle 1944, Dawes 1960).

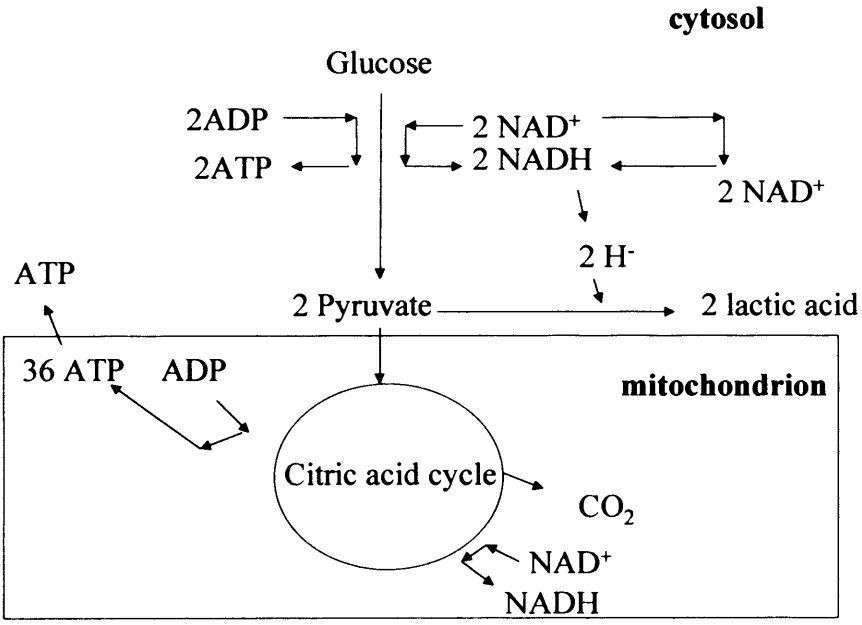


Figure 1.1. Fetal cellular glucose metabolism schematically represented. Glucose is broken down along the glycolytic pathway to form pyruvate. Under aerobic conditions large amounts of energy (ATP) are produced in the citric acid cycle, whereas anaerobic glycolysis renders less energy and lactacidosis.

Thus three different levels of cellular energy status may occur. The first is aerobic metabolism in which there is a sufficient amount of oxygen to produce a large supply of energy (ATP). The second level is anaerobic metabolism, in which a lack of oxygen forces the cell to produce lactate while generating a limited amount of energy and concomitantly producing lactacidosis. Early in this stage energy supply is still satisfactory in relation to demand and the cellular energy status is compensated. In the third level, regeneration of ATP can no longer be maintained and energy supply to the cell can no longer keep up with demands and is thus decompensated. Because the fetal brain has a high rate of oxygen consumption and a relative lack of oxygen stores, at flow rates below 50ml/min/100g tissue protein synthesis is impaired, while at rates below 15 to 20ml/min/100g tissue, adenosine triphosphate (ATP) synthesis is impaired and electrical activity fails (Longo 1997). If this low rate persists, cellular transmembrane ionic gradients will eventually be lost. These ionic gradients can also be lost almost instantaneously if cerebral blood flow is reduced to ~10 ml/min/100g brain tissue (Hossman 1994). It is at this level that neuronal cell damage is thought to occur during a hypoxic-ischaemic event (Heuser 1975, Giffard 1990, Levine 1993).

Therefore it is now well established that there is a primary phase of neuronal loss during a hypoxic/ischaemic event likely to be due to deterioration of the cellular steady state including acidosis, disturbed ion distributions and altered tissue perfusion. Additionally, neuronal cell damage has been shown to occur during a secondary phase of re-oxygenation and re-perfusion after a hypoxia-ischaemic event (Pulsinelli 1982), which can take place hours to days after the event (Bennet 1999). Delayed neuronal and glial death occurring in the hours and days after a hypoxic insult by apoptotic and related processes are observed following severe injury (Gluckman 2001). The severity of cortical neuronal loss may be related to the degree of hypoperfusion in the immediate reperfusion period and inversely related to the magnitude of the delayed hyperperfusion (Raad 1999). The damaging mechanisms that are thought to be involved in secondary neuronal loss are metabolic changes, neurotoxicity and circulatory changes. Oxygen-derived free radicals and excitatory amino acids have been implicated (Kjellmar 1989). Oxygen–derived free radicals are highly reactive atoms or molecules with an uneven number of electrons in their outer-shell. These are generated during normal aerobic

metabolism but are effectively inactivated by naturally occurring scavenger systems. A state of severe metabolic acidosis will cause phospholipid release, providing a substrate store for the production of prostaglandins when the brain is subsequently re-perfused as the hypoxic insult resolves. The production of prostaglandins has an inevitable by-product, oxygen radicals. Oxygen free radical production also results from the breakdown of xanthine and reperfusion of the areas in which xanthine has accumulated leads to the formation of uric acid with oxygen radical production again being a by-product (Nordstrom 1998). The role of oxygen free radicals however remains unclear, although it is thought that they may disrupt the integrity of blood capillaries, which may lead to breakdown of the blood-brain barrier leading to vasogenic oedema.

Similarly, glutamate and other excitatory amino acids have been proposed to have a role in the secondary phase of hypoxia-ischaemia neuronal injury (Hagberg 1992). These act as neurotransmitters in the brain, with the N-methyl-D-aspartate (NMDA) being the most important receptor, and can be released in response to a metabolic acidosis caused by profound hypoxia. Stimulation of the NMDA receptor opens up the passage of Na+ and Ca2+ ions through the cell membrane, with concomitant intracellular hypercalcaemia. In animal models, a high density of NMDA receptors has been found in areas of the brain that are vulnerable to hypoxia-ischaemia (Greenmayre 1987). During moderately severe isocapnic hypoxia fetal plasma amino acids increase significantly (Walker 2000). This may reflect reflex peripheral vasoconstriction in skeletal muscle beds and decreased hepatic blood flow, demonstrating the importance of skeletal muscle in branched-chain amino acid metabolism. In a fetus that is able to successfully buffer any production of lactate and maintain cerebral perfusion, the level of amino acids is insufficient to produce neuronal damage. However, as the level of lactate rises in response to the hypoxia, the amino acid accumulation threatens neuronal integrity. The underlying mechanisms responsible are thought to be acute osmotic uptake of sodium and chloride ions and a secondary calcium ion accumulation in the neurone (Nordstrom 1998).

It is important to note that there is a constant readjustment of local blood flow to various areas to satisfy the metabolic needs of the brain cells. Thus some areas of the brain such

as the hippocampus, areas of the neocortex, and portions of the caudate nucleus and cerebellum have neurones that are most vulnerable to hypoxia-ischaemia (Paschen 1989). Damage from ischaemia may occur in a graded fashion, depending on the collateral circulation surrounding the ischaemic 'core'. This surrounding relatively ischaemic 'penumbra' may receive blood flow that is inadequate to preserve normal cellular function, but adequate to allow some level of recovery relative to the ischaemic 'core' (Longo 1997). The concept of the ischaemic penumbra is important, as the compromised status of such a region may be improved if effective early intervention, such as delivery of a compromised fetus, is achieved (Obrenovitch 1995).
In conclusion, a fetus suffering from hypoxia initially compensates by producing energy through anaerobic metabolism and physiological neuro-protective mechanisms. At some stage, the fetus becomes decompensated and basic cellular functions fail. The neuronal loss occurs in two phases: during the primary hypoxic event and later during reperfusion/reoxygenation phase, with risks of permanent neurological morbidity and mortality.

1.1.3 Fetal adaptive response to hypoxia-ischaemia

Deficiency of oxygen (and the other nutrients) supplies antenatally have been shown to have an effect on body and organ growth in utero (Steyn 2000). Reduced oxygen supply to the fetus for most of the pregnancy will cause intrauterine growth restriction and potential subsequent long-term neurological handicap (Blair1993, 2000). At a cellular level, animal studies have shown that induced, prolonged placental insufficiency in the last third of gestation resulting in persistent moderate fetal hypoxaemia disrupt myelinasation and the growth of the cerebellum (Mallard 1998). They also show that, in mid –gestation, an episode of 12 hours hypoxaemia is enough to cause damage to white matter and neuronal death in the hippocampus, cerebral cortex and cerebellum (Rees 1998).

In contrast, a normally grown term fetus is well adapted to the intermittent periods of hypoxia, which are frequently, if not universally, experienced during labour. The fetal circulation normally works at or near its functional limit with a high cardiac output. A limited improvement in cardiac output can occur, which is chemoreceptor-mediated, which results in an increase in fetal heart rate (Thornburg 1994).

The cardiovascular response to acute moderate hypoxia in the ovine fetus is redistribution of the cardiac output, with an increase in blood flow to vital organs (brain, heart and adrenals) and a fall in blood flow to the carcass muscle, kidney and intestine (Peeters 1979, Jensen 1991 Ashwal 1984, Kiserud 2001). Decreasing breathing and gross body movements to reduce fetal metabolic activity will lower the oxygen demand (Bekedam 1985) and, as fetal haemoglobin has a higher oxygen affinity than in the adult, more oxygen can be attached to the haemoglobin molecule to be available for extraction by fetal tissue (Bocking 1992).

Other physiological mechanisms that may protect the brain are the release of catecholamines from the adrenal glands with activation of the sympathetic nervous system (Nylund 1979), utilisation of liver glycogen reserves (Irestedt 1984) and stressed-associated release of endorphins (Ionides 1995) and a rise in adenosine which may be neuroprotective (Newman 2001). Another described aspect of neuroprotection that is thought to occur is hypoxic or ischaemic 'pre-conditioning' in which tissues are rendered resistant to the deleterious effects of hypoxia-ischaemia by prior exposure to brief periods of hypoxia or ischaemia. However, the mechanisms of this phenomenon are poorly understood (Gidday 1994, Chopp 1989).

In contrast to the response to acute moderate hypoxia, severe hypoxia results in a fall in cerebral perfusion, as a result of a fall in perfusion pressure and cerebral vasoconstriction (Jensen 1987). In the sheep fetus the severity of histological brain injury correlates better with the degree of hypotension than of hypoxaemia, indicating that cerebral hypoperfusion is the central factor in the aetiology of hypoxic-ischaemic brain injury (Gunn 1992).

Similar findings have been described in the hypoxic human fetus using Doppler ultrasonography in the antenatal period (Vyas 1990). Fetal hypoxaemia, demonstrated by sampling umbilical venous blood, is associated with increased blood flow velocity in the middle cerebral artery, presumably as a protective mechanism enabling cerebral oxygen delivery to be maintained. As the fetal condition deteriorates further, the adaptive responses are overwhelmed and Doppler studies indicate the development of cerebral hypoperfusion. A partial failure of this redistribution and near terminal

peripheral hypoperfusion. Table 1.1b provides a summary of the fetal pathophysiology events in response to acute and chronic insults.

Table 1.1b Summary of pathophysiological sequence of events

Fetal Exposure	Anoxia (Minutes)	Hypoxia (hours)
	Hypercapnia	Respiratory acidosis
	Hypoxaemia	Metabolic acidosis
Fetal response	Compensation	Decompensation
Blood Pressure	↑	↓
Cerebral blood flow	↓	↓
Cerebral O2 Consumption	↑↓	↓
Outcome		
Cerebral dysfunction	None / minor	Moderate/severe
Brain damage	0	+
Mortality	0	+

1.1.4 Clinical endpoints of brain injury

It is therefore hypothesised that the human fetus will show a similar cerebrovascular response to hypoxaemia during labour. Moderate hypoxaemia, which is relatively common phenomenon during labour due to impaired placental perfusion during uterine contractions, will lead to cerebral vasodilatation and a rise in cerebral blood volume. In contrast, severe hypoxaemia, such as may be found when uterine contractions are superimposed on an already inadequately functioning placenta, will lead to cerebral vasoconstriction and a fall in cerebral perfusion and blood volume. This results in intracellular failure and eventual brain cell death. If this sequence of events is to be detected during labour and the consequences in the neonate it is necessary to obtain continuous measurements of fetal cerebral oxygenation and perfusion.
Attempts have been made to define the association of the pathophysiological processes with clinical endpoints in the neonate and measurable variables in the fetus during labour in order to predict subsequent neurological outcome.

Apgar scores

The Apgar score since its presentation in 1953 has been the most common end point defining intrapartum hypoxia (Apgar 1953). Apgar scoring is a quick and somewhat subjective method of assessing the condition of newborn infants. However, by themselves, Apgar scores have been shown to be poor predictors of neurological outcome and in particular, in preterm infants it is highly limited in this respect (Topp 1997). Sykes et al (1982) reported the poor correlation between Apgar scores and umbilical artery acidaemia and Ruth (1988) found the sensitivity and the positive predictive value of a low 5 minute Apgar score (<7) were as little as 12 and 19%. However, it is known that the risk of poor outcome increases with decreasing Apgar score and increasing duration of low scores. Thus a 5 minute Apgar score below 4 but improving thereafter was associated with an increase in the risk of cerebral palsy from 0.35 to1% for births in the 1950s and 1960s (Nelson1981). It is thought now that the duration of low Apgar scores is more likely to indicate the effectiveness of resuscitation rather than predicting long-term neurological outcome (American Academy of Pediatrics1996). More recently, it is thought that an Apgar score of 0-6 for longer than 5 minutes is only 'suggestive' of intrapartum hypoxia (MacLennan 1999).

Metabolic acidaemia

Although its presence does not define the timing of its onset, metabolic acidaemia at birth is considered an essential criterion in defining a causal relationship between an intrapartum event and cerebral palsy (MacLennan et al 1999). Metabolic acidaemia may be detected from fetal, umbilical and neonatal blood samples. Attempts have been made to correlate pathological fetal acidaemia with an increasing risk of neurological deficit. Thus a pH of less than 7.00 with a base deficit of more than 16 mmol/l have been suggested (Sehdev 1997) however cases of cerebral palsy with base deficits of between 12-15 mmol/l have been reported. Thus a base deficit of less than 12 is now considered to be an exclusion criterion (Low 1997) in defining a case of cerebral palsy to intrapartum hypoxia. Others have suggested that if both arterial and venous cord blood gases are obtained, then a difference in the partial pressure of carbon dioxide of more than 25mmHg suggest an acute rather than chronic acidosis (Belai 1998). However the

technique of measuring both samples is crucial and is not universally practised (Westgate1994). Individual partial pressures of oxygen are not helpful as they correlate poorly with outcome (Belai 1998). However, acidaemia from neonatal samples may reflect difficult resuscitation and thus carry less weight in determining an intrapartum cause. Furthermore cord blood gas analysis is not universally practised, often only venous blood is obtained, and sodium bicarbonate, which is used as part of resuscitation, may influence the results of neonatal samples.

Encephalopathy

Sarnat and Sarnet (1976) originally described the term 'hypoxic-ischaemic encephalopathy' (HIE), the presence of which was shown to predict future neuro-developmental handicap. They described a specific condition with a typical course in the neonatal period, which included hypotonia, seizures and coma occurring within 24 hours of delivery. However other causes of encephalopathy such as infection, hypoglycaemia, intra-cranial haemorrhage and metabolic disorders in the neonate need to be excluded before an intrapartum insult can be considered to be the cause of neurodevelopmental handicap (Nelson 1991). Furthermore, spastic quadriplegia and less commonly dyskinetic cerebral palsy are the only subtypes of cerebral palsy associated with acute hypoxic events (Rosenbloom 1994). However, even spastic quadriplegia is not specific to intrapartum hypoxia as in one population-based series 24% of cases were not related to intrapartum events (Stanley 1993). MacLennan et al (1999) suggest that only early onset of severe or moderate neonatal encephalopathy in infants of more than 34 weeks gestation and cerebral palsy of the spastic quadriplegic or dyskinetic type should be considered in the causal relationship of intrapartum events to cerebral palsy. Thus, although HIE seems to be the best clinical endpoint for intrapartum hypoxia the condition is rare, with an incidence of 0.9 to 6.0 per thousand deliveries (Levene 1985).

More recently, MacLennan proposed a template of evidence required to suggest the occurrence of damaging intrapartum hypoxia was sufficient to cause permanent neurological impairment. Taken together, criteria 4 to 8 may help to time the hypoxic event to the intrapartum period.

Table 1.2 Criteria suggesting that acute intrapartum hypoxia was the cause of cerebral palsy.

Essential criteria

1 Evidence of a metabolic acidosis in intrapartum fetal, umbilical arterial cord, or very early neonatal blood samples (pH <7.00 and base deficit >= 12 mmol/l)

2 Early onset of severe or moderate neonatal encephalopathy in infants of >=34 weeks' gestation

3 Cerebral palsy of the spastic quadriplegic or dyskinetic type

Criteria that together suggest an intrapartum timing but by themselves are non-specific

4 A sentinel (signal) hypoxic event occurring immediately before or during labour

5 A sudden, rapid, and sustained deterioration of the fetal heart rate pattern usually after the hypoxic sentinel event where the pattern was previously normal

6 Apgar scores of 0-6 for longer than 5 minutes

7 Early evidence of multisystem involvement

8 Early imaging evidence of acute cerebral abnormality

1.2 CURRENT METHODS OF INTRAPARTUM SURVEILLANCE

Intrapartum surveillance methods should therefore aim to detect hypoxia when the fetus is still compensated with the aim of prophylactic intervention before the fetus becomes decompensated. However, due to the rarity of cases of intrapartum-related cerebral palsy, a large number of fetuses are required to detect an effect of an intrapartum monitoring tool on neurological outcome. Consequently, the majority of intrapartum studies have used surrogate clinical endpoints such as Apgar scores, SCBU admissions and obstetric intervention rates. The current methods available for intrapartum monitoring will now be reviewed.

1.2.1 Fetal Heart Rate (FHR) monitoring

The association between the FHR and fetal well being was considered as early as the 19th century. Laennec's invention of the stethoscope in 1806 was followed by auscultation of the fetal heart by Kergaradac. On hearing "beats occurring 143 to 148 times per minute, and the patient's pulse was only 72 beats per minute" he wondered "whether it would be possible to judge the state of health or disease of the fetus from the variations that occur in the beat of the fetal heart" (Pinkerton 1969). Normal fetal heart rates of 100 to 180 beats per minute were subsequently proposed and Schwartz recommended frequent counting of the fetal heart rate during labour, both during and between contractions, on the grounds that "asphyxic intoxication" could alter the rate (Goodlin 1979).

The presence of meconium-stained amniotic fluid and an abnormal FHR on auscultation were the criteria used for fetal distress until the introduction of continuous electronic monitoring of the fetal heart (EFM) through an electrode applied to the fetal scalp during labour (Hon 1960).
For the first time continuous traces were obtained, showing patterns that appeared to correlate with the condition of the baby at birth. Various fetal heart rate changes in relation to uterine contractions were described with terms such as "baseline bradycardia", "early and late decelerations" and "variable decelerations".
By the early 1970's, reports on a decrease in perinatal mortality (Simmons 1974, Edington 1975) in units using electronic FHR monitoring led to the rapid uptake of EFM. The value of EFM, however was being questioned (Hellman 1965) and it was recognised that there was a need for 'a controlled trial to determine whether FHR monitoring significantly alters perinatal morbidity and mortality' (Renou 1974). Subsequently, the first controlled trial on the effects of EFM in high-risk labours did not detect any benefit, but showed an increased rate of caesarean section in women monitored continuously (Haverkamp 1976).

Several randomised-controlled trials of EFM versus intermittent auscultation were to follow. Thacker et al (1995) made a comprehensive review of 12 of these, and

concluded that the only clinically significant benefit from the routine use of EFM was in the reduction of neonatal seizures. The rates of intrapartum and neonatal death, short-term morbidity (with the exception of seizures) and long-term morbidity including cerebral palsy were found to be similar whether the fetal heart rate had been monitored continuously or intermittently. Follow–up of the children born in the largest of these trials (Macdonald 1985), involving 13000 women, revealed that 78% of the cases of cerebral palsy had not shown clinical signs suggestive of intrapartum asphyxia (Grant 1989).

Thus, it was thought that compared with intermittent intrapartum monitoring, intensive monitoring had little, if any, protective effect against cerebral palsy. More recently, Nelson (1996) reported that even the most severe FHR abnormalities of multiple late decelerations and decreased variability might have a false positive rate of 99.8% in the prediction of cerebral palsy. Thus the high frequency (up to 79%) of non-reassuring FHR patterns make both the decision on optimal management of the labour and the prediction of current or future neurological status of the fetus very difficult (Umstad 1994).

1.2.2 Fetal Blood Sampling (FBS)

In the early 1960s, Saling introduced the technique of FBS in response to the 1958 Perinatal Mortality Survey which had shown that intermittent auscultation of the FHR had failed to prevent deaths caused by trauma and/or hypoxia in labour. This method of assessing the fetus during labour became established as a complementary assessment in cases of FHR abnormalities. A mean normal pH was established as 7.33 with a range (+/- 2sd) of 7.2-7.5 (Saling 1962). Corresponding values for umbilical cord pH at birth were 7.27(7.12-7.42) for the artery and 7.33(7.2-7.47) for the vein. Thereafter a pH value of 7.2 rapidly became established as the lower limit of normal and a value below this suggested a clinically significant acidaemia, with delivery of the fetus recommended (Beard 1971a).

However, it was soon noted that in a "certain number" of neonates there was a discrepancy between the condition of the neonate predicted from the measured pH and its actual condition (Beard 1971b). The clinical value of FBS remains controversial as metabolic acidaemia is comparatively common (2 % of all births), and the vast majority do not develop cerebral palsy (Ruth 1988).

However, the more that EFM is supplemented with FBS, the lower the percentage of babies with pH at birth of less than 7.20 (Sykes 1983). This reduction in acidosis at birth however may have little value in the prediction of long term neurological outcome. Although a reduction in Caesarean Section rates may be achieved with CTG and FBS, there is little evidence of benefit in terms of short- term neonatal morbidity. Apart from a reduction in neonatal seizures, there is no evidence of benefit in the long –term with regards to a reduction in cerebral palsy (Grant 1993).

Furthermore, the use of FBS during labour is not without practical problems. Repeat samples are often necessary to establish a trend and caput succadaneum and amniotic fluid contamination can interfere with the results. Physiologically, the fact that the peripheral circulation is being sampled may lead to erroneous results as redistribution of the circulation occurs in hypoxia with peripheral vasoconstriction and increased blood flow to the brain and heart (Peeters et al. 1979).
Often difficulties are encountered in obtaining accurate and timely samples. Furthermore, the economic consequences of maintaining analytic equipment, and the poor correlation between pH and neurological consequences, has led to the technique of FBS in routine use in only 50% of maternity units (Wheble 1989). Others question the feasibility of retaining this practice and propose that FBS should be removed from any possible association with purported 'standards of care' (Perkins1997).

1.2.3 Pulse Oximetry

The use of pulse oximetry is routine in intensive care situations both on adults and neonates. The ratio of non-absorbed red light to near infrared light is measured to provide continuous, non-invasive data of peripheral arterial oxygen saturation of

haemoglobin. The light is transmitted by two light-emitting diodes (LEDs) and detected by a photodiode that is on the opposite side on a finger or ear.

Over the last two decades, pulse oximetry has been adapted for fetal use with the use of sensors that rely on the reflectance of the emitted light and so incorporate the LEDs and the photodiode together. These sensors can therefore be applied to the fetal cheek or temple to avoid hair or caput, with the use of the Nellcor N-400 fetal pulse oximeter and the FS-14 sensor being the most frequently studied.

Several studies have evaluated the feasibility of fetal pulse oximetry and it's use appears to be acceptable to mothers (Arikan 1998), with values of fetal oxygen saturation being obtained in up to 95% of studies, and sensor placement is considered to be easier than FBS (Gofinet 1997).

A wide range of normal values of fetal oxygen saturation has been obtained during labour. However, there is now general agreement that a fetal oxygen saturation value of 30% represents the lower limit of normal (Chua 1997,East 1997, Kuhnert M 1998, Seelbach-Gobel 1999). This level has been shown to correlate with increased blood flow velocity in the middle cerebral artery in the human fetus during labour (Sutterlin1999). Using this level as a threshold seems to have the same predictive value for low arterial umbilical pH (7.15 or less) as a fetal scalp pH level of 7.20 (Carbonne 1997).

However, transient fetal arterial oxyhaemoglobin saturation values below 30% may occur during normal labour. Thus it is thought that the duration of values less than 30% better correlates with fetal compromise. Arterial oxygen saturation below 30% for at least 10 minutes has been correlated with scalp pHs of less than 7.20 (Kuhnert 1998). Bloom (1999) reported that saturations of less than 30% for at least 10 minutes correlated with either CS for non-reassuring fetal heart rate pattern, umbilical artery pH less than 7.20, admission to the special care nursery, and low 5-minute Apgar score. The addition of pulse oximetry in cases of non-reassuring FHR patterns may improve the predictive value of CTG in terms of metabolic acidosis and neonatal resuscitation

but has not been shown that its use is associated with a reduction in the overall Caesarean Section rate (Garite et al 2000).

There is therefore little evidence that the use of pulse oximetry reduces operative deliveries or long-term neurological handicap. This maybe because the number of fetuses detected by pulse oximetry suffering from hypoxia seems to be low (Luttkus 1998). Scalp perfusion may decrease in hypoxic fetuses who are peripherally vasoconstricted, thus reducing the size of the arterial signal in those fetuses that need to be monitored more closely. Furthermore, sensor placement still seems to be important as in one study, significantly lower values of oxygen saturation were found at the forehead compared with the fontanelle, the parietal and occipital position, and the temporal area. Thus, a difference of up to 13.4% in oxygen saturation may occur between the forehead and the occipital area (Dassel1997).

1.2.4 Fetal Electrocardiograph (FECG)

Over the past decade, attention has focused on using the fetal ECG waveform as a discriminator of FHR changes. Many parts of the fetal ECG complex have been studied, most relate to the ST waveform and the PR interval.

Preliminary studies reported that intrapartum hypoxia may be associated with ST segment changes (Lewinsky 1992, Murphy 1992, Morgan 1991,Widmark 1991,Westgate 1990,Arulkumaran 1990). Subsequently, a randomised trial incorporating ST segment analysis to assess its effect on obstetric intervention rates and neonatal outcome in over 2400 high-risk obstetric labours was undertaken (Westgate 1992). It was reported that the addition of ST waveform analysis during labour significantly reduced the proportion of operative deliveries for presumed fetal distress with no detrimental effect on neonatal outcome. A large multi-centred study suggested that ST analysis, in addition to CTG, has a high sensitivity to predict fetal acidosis and is associated with a marked increase in positive predictive values compared with conventional CTG (Amer-Wahlin et al 2002).

Other studies, which analysed the PR interval, reported a relationship between the PR interval and the FHR, which varied according to the acid-base status of the fetus. Thus

in the normal fetus there is a negative correlation between these two parameters and as acidosis develops, the relationship becomes positive (van-Wijngaarden 1996a).

A retrospective study (Reed 1996) reported that the use of this relationship may reduce unsuspected acidosis at birth by 50% and unnecessary FBS by 60% without an increase in the assisted delivery rate may be achieved. Subsequently, a randomised trial showed a significant reduction in the number of cases having an FBS performed without an increase in adverse outcome, when analysis of the PR-FHR relationship with conventional CTG was used (van Wijngaarden1996b). Unfortunately, however, the most recent and largest randomised study using FECG reported no difference in perinatal outcome (Strachan 2000).

1.2.5 Lactate measurement

As previously discussed in section 1.1.2.2, tissue hypoxia may lead to accumulation of lactate and hydrogen ions and therefore lactacidosis. Measurements can safely and easily be determined in fetal scalp and umbilical artery blood with a microvolume (5 microliters) lactate meter (Nordstrom 1994). Mean values of fetal scalp lactate and umbilical artery lactate have been defined and intrapartum lactate measurements have been correlated with scalp pH and cord artery lactate (Nordstrom 1994, 1995. 2001). Significantly higher concentrations of lactate are found in scalp blood from babies with low Apgar scores compared with those with higher Apgar scores (Smith 1983). Westgren (1998) performed a randomised trial where scalp lactate was compared to scalp pH. The lactate group underwent significantly more successful blood sampling procedures and required fewer scalp incisions per blood sampling attempt. Umbilical artery lactate concentration blood may have the same predictive properties as pH or base deficit in relation to poor neonatal outcome (Westgren 1998).
Kruger et al (1999) found that that determination of the lactate concentration in fetal scalp blood is a more sensitive diagnostic tool than is determination of the pH value for predicting either an Apgar score <4 at 5 minutes or moderate to severe hypoxic-ischaemic encephalopathy. Similarly, lactate levels in the first hour of life and serial measurements of lactate appear to be important predictors of moderate-to-severe HIE

(Shah 2004). The measurement of lactate in fetal scalp blood therefore appears to be an attractive alternative to pH analysis, and determination of the lactate concentration in fetal scalp blood seems to be a useful tool for monitoring the condition of the fetus.

1.3 Conclusion

Having considered the mechanisms of hypoxic-ischaemic brain injury and the available monitoring techniques it is possible to set down the requirements for the ideal method of fetal surveillance.

1) As the primary organ of concern is the fetal *brain* it is this that should be investigated. Studies on the peripheral circulation or fetal heart rate provide only indirect evidence of cerebral oxygenation and inferences concerning brain function may be misleading.
2) It has become clear that the aetiology of hypoxic-ischaemic brain injury is complex. Studying a single physiological parameter is unlikely to provide a complete picture of cerebral function. Information on brain *oxygenation* needs to be combined with knowledge of cerebral *perfusion* and possibly of cellular metabolism.
3) Because of the large fluctuations in cerebral oxygenation and haemodynamics that may occur during labour, single measurements may be misleading. *Continuous* measurements of fetal cerebral haemodynamics from the start of labour to detect the already compromised fetus entering labour would be of more value.
4) It is obvious that any fetal monitoring technique has to be acceptable to the mother. This means that it should be relatively *non-invasive, safe* and cause a minimum of discomfort to both mother and fetus.
5) In order to achieve widespread clinical use the technique needs to be *cheap* and *easy* to use.
6) Finally, it is vital that any new technique be subjected to rigorous testing, in the form of randomised trials, to establish it's value as a means of fetal surveillance.

The development of the technique of near infrared spectroscopy for measuring intrapartum fetal cerebral oxygenation and perfusion will be considered against this background ideal method of fetal surveillance.

CHAPTER 2

NEAR INFRARED SPECTROSCOPY (NIRS)

This chapter provides background information on the technique of near infrared spectroscopy and discusses how the technique has been applied to clinical practice. The limitations of current NIRS monitoring for intrapartum use and the development of a new technique of intensity modulated optical spectroscopy (IMOS) will be discussed. Finally the aims of the thesis will be presented.

2.1 THEORETICAL BACKGROUND

The use of NIRS for intrapartum fetal use was based on two fundamental physical properties of near infrared light (Jobsis 1977).

a) **Biological tissue is more transparent to light in the near infrared (650-900nm) part of the spectrum.**

Photons in the near infrared part of the spectrum can therefore penetrate into biological tissue for distances of up to 8-9 cm (Cope1988). This is in contrast to pulse oximetry, which uses light in the visible part of the optical spectrum that has a high attenuation in tissue. It is therefore only possible to study peripheral arterial saturation with pulse oximetry.

b) **Oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (Hb) have characteristic absorption spectra in the near infrared light region.**

The absorption spectra of both forms of haemoglobin are shown in Figure 2.1a. It is now possible to study the absorbing properties of haemoglobin alone by taking normal human blood, lysing the red cells and removing the cell membrane to leave a clear solution of haemoglobin. If the solution is reduced by equilibrating with 100% N_2 and then fully oxygenated by bubbling with oxygen the different absorption spectra of HbO_2 and Hb can be elucidated (Wray 1988).

There are several important points to notice in the absorption spectra. Both HbO_2 and Hb absorb far more light in the visible than in the near infrared part of the spectrum and it is this that makes biological tissue so much more transparent to near infra red light. Secondly, both HbO_2 and Hb have different absorption characteristics in the near infrared region that enables them to be independently measured. They are also the only light-absorbing compounds or ' chromophores' present in the brain in variable concentrations. Those present at fixed concentrations (e.g. water, lipid) merely add to the overall absorption properties of the brain tissue whilst those with concentrations are

oxygen dependent (e.g. haemoglobin) provide potentially useful information on tissue oxygenation.

There are other forms of haemoglobin which have specific absorption spectra in the near infrared region. Carboxyhaemoglobin may constitute up to 10% of haemoglobin content but its optical effect is negligible. Another form of haemoglobin, met-haemoglobin, which has a significant absorption in the near infrared is present in only small amounts at physiological pH and so may only contribute 1% of the haemoglobin signal.

During the course of fetal observations with NIRS a fixed amount of light will be lost due to absorption by chromophores whose concentration does not alter. This means that any change in near infrared light absorption will be due to a change in the concentrations of HbO_2 and Hb.

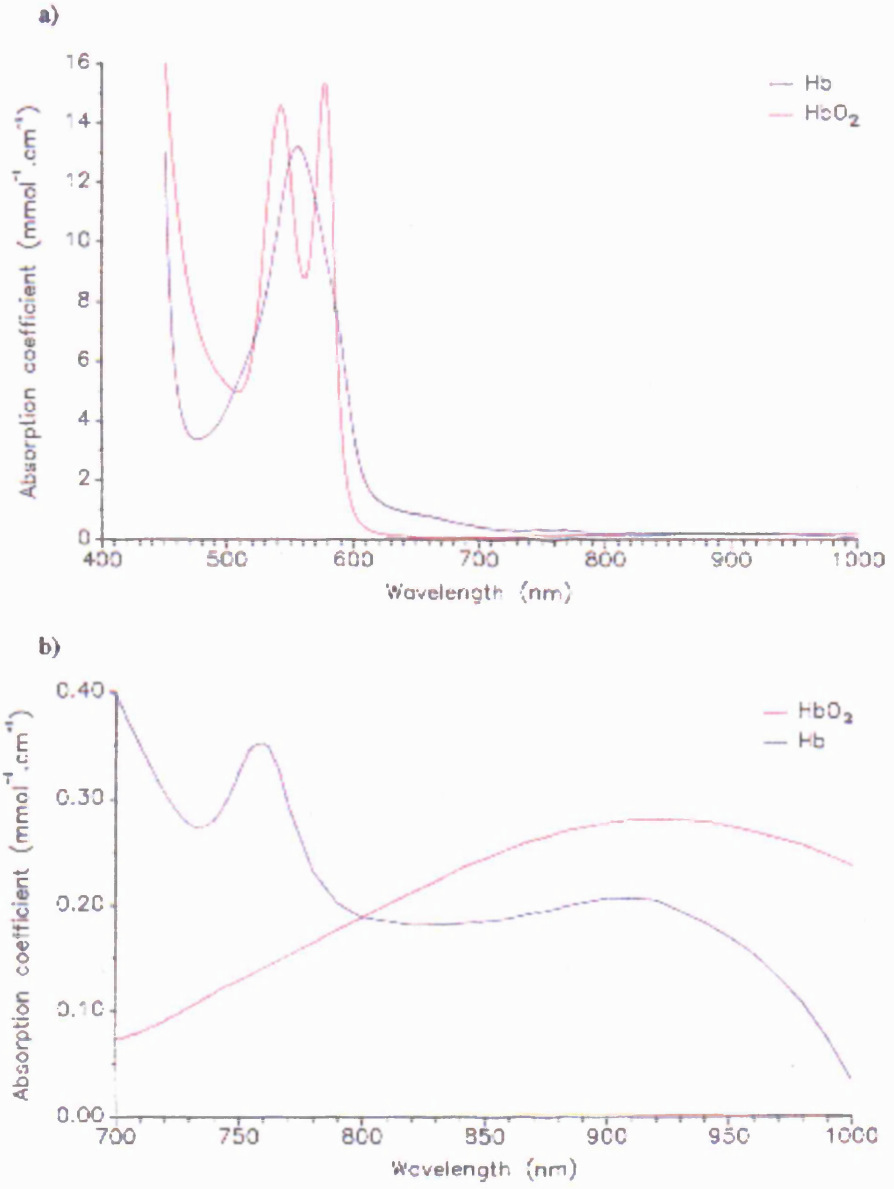


Figure 2.1 The visible and near infrared specific extinction coefficient spectra of HbO_2 and Hb are shown in a). The spectra in the near infrared region are depicted in a magnified form in b).

2.2 CLINICAL APPLICATION

It was Jobsis, at Duke University, Carolina who in 1977 first put this information together using a sensitive spectroscopy system, which used only near infrared wavelengths, to measure changes in brain oxygenation in the cat (Jobsis et al 1977). Although most of the work from the Jobsis group was on the application of this technique to physiological studies in laboratory animals it was his system which formed the basis of clinical NIRS.

2.2.1 Neonatal use

Before the technique of NIRS was applied for use on the fetus during labour, most of the publications described measurements of cerebral oxygenation and haemodynamics in the neonate. It is possible to transmit near infrared light through the head of a term or preterm infant and so make spectroscopic measurements across the entire thickness of the brain. This is because the surface tissue and skull are thin and so contribute little to the overall signal.

The first work on the clinical application of near infrared spectroscopy was published in 1984 by Ferarri and Giannini and in 1985 by Brazy and Jobsis. These clinical data were mainly from preterm infants and described changes in the cerebral concentrations of oxyhaemoglobin and deoxyhaemoglobin during induced fluctuations in cerebral oxygenation. Subsequently, quantifiable changes in neonatal cerebral concentrations of HbO_2 and Hb have been reported (Wyatt 1986, Edwards1988). Wyatt et al (1990) then reported on a method for measuring cerebral blood volume (CBV) using NIRS. In infants undergoing intensive care, but were thought to have normal brains, mean CBV was 2.2 +/- 0.4 $ml.100g^{-1}$. In contrast, infants who had evidence of brain injury (mostly hypoxic-ischaemic) had a significantly elevated CBV of 3.0+/- 1.0 $ml.100g^{-1}$, suggesting that cerebral vasodilatation leading to elevation of CBV is a frequent occurrence following hypoxic-ischaemic brain injury. However, these methods involve eliciting small changes in cerebral blood volume by changing pCO_2 concentration through alterations in the ventilation rate.

2.2.2 Fetal use

As discussed in chapter 1 the perceived need to measure cerebral oxygenation and perfusion led several groups to investigate the potential role of NIRS to provide information on fetal brain haemodynamics during labour.

The two main centres that used NIRS during labour were University College, London and Keele University, Stoke. Commercially available NIRS monitors were (Hamamatsu Photonics KK (Japan), Radiometer/ Keele University (Denmark/ UK), and Critikcon (UK)).

Specially designed optical fibres have been attached to the fetal head either hand-held through an amnioscope (Schmidt 1990), as a hinged optode (O'Brien 1993) or mounted in silicone rubber (Peebles 1993) to transmit and receive light to and from a NIRS spectrophotometer.

Early studies on human fetuses by Peebles et al (1992a) used the NIRO 500 spectrophotometer (Hamamatsu Photonics KK, Japan, see figure 2.2). This provides a light source comprising four laser diodes, emitting near infrared light at 775,825,850 and 904nm wavelengths (sequentially pulsed at 1.9kHz, with a pulse width of 100nsec). The power output of the lasers varies with their operating temperature. Although it is recommended that 30 minutes should elapse after switching on the lasers, before measurements are made, accurate measurements can be made from the time that the optodes are in place if the lasers are turned on many hours before the study.

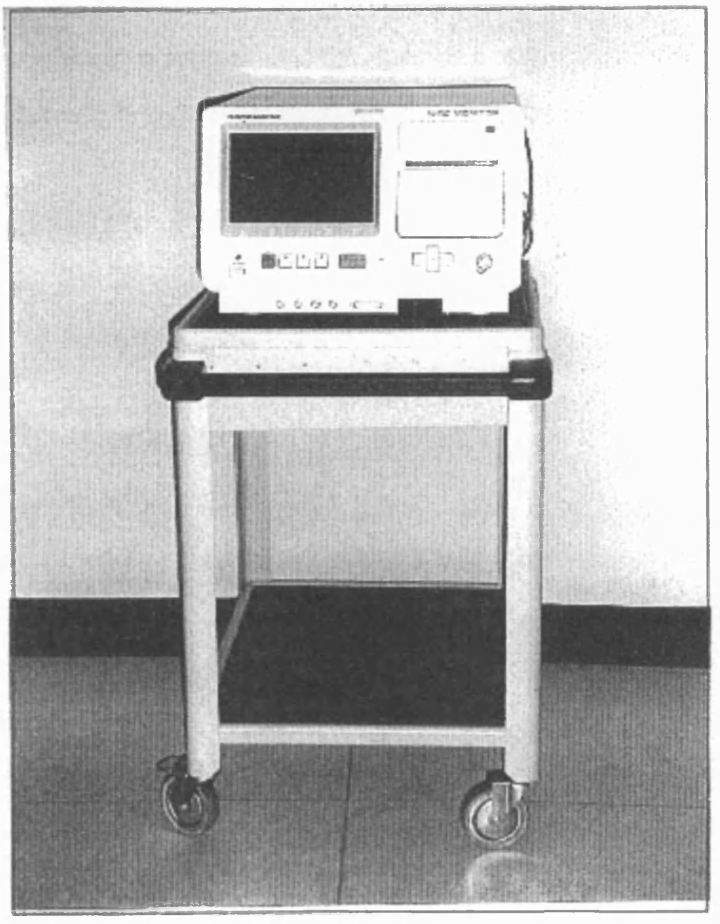


Figure 2.2 NIRO 500 on an equipment trolley

A photomultiplier tube within the spectrometer detects transmitted light. A controlling computer calculates a mean value for the amount of light detected at each wavelength over the chosen sample time (operator selective, ranging from 0.5 s to 60 seconds).

Following baseline measurements the change in light intensity (or attenuation) for each sample, relative to the baseline attenuation is then derived.
The change in intensity of the near infrared light can be subsequently used to calculate changes in Hb and HbO_2 concentration. This calculation can be done in real time to give a visual indication of the relative changes in oxygenation at the bedside.

Changes in the concentrations of Hb and HbO_2 are calculated from the changes in attenuation (defined as the logarithm (base e) of the ratio of incident and detected intensities) using a modification of the Beer-Lambert Law (Delpy et al. 1988):

$$\Delta c = \Delta OD/(a.B.L)$$

where OD is the tissue attenuation of the infrared light (units of optical density), a is the extinction coefficient of the chromophore (/mmol per cm), c is the concentration of chromophore (mmol/l), L is the distance between the point of light entry and exit (cm), and B is a pathlength factor that takes account of the scattering of light in tissue, which causes the optical pathlength to be greater than L. The extinction coefficients of HbO_2 and Hb are known (Wray 1988) and a mean value for B of 4.99 was used (Duncan 1995).

Using the NIRO 500 spectrophotometer, a silicone rubber probe and the above method of calculation of Hb and HbO_2, Peebles et al at University College, London applied the technique of NIRS to study changes in human fetal cerebral oxygenation during labour.

2.2.2.1 Effect of uterine contractions

The first report on changes in human fetal cerebral haemoglobin concentration and oygenation during labour measured by NIRS was in 1992 by Peebles (1992b). He

compared the effects of uterine contractions with and without fetal heart rate decelerations in eight singleton term fetuses. In six of eight fetuses normal uterine contractions were associated with proportional decreases in both oxyhaemoglobin and deoxyhaemoglobin and a fall in cerebral blood volume without desaturation of cerebral haemoglobin. Mean cerebral haemoglobin oxygen saturation calculated during normal contractions was 43% +/-10% (SD). Contractions with fetal heart rate decelerations produced different results in that oxyhaemoglobin fell but deoxyhaemoglobin rose, indicating cerebral desaturation. Figure 2.3 illustrates these two different patterns. From these data, Peebles suggested that the change in total haemoglobin concentration observed during normal uterine contractions probably reflected a balance between direct pressure effects, reducing cerebral blood flow and obstruction of venous drainage. The usual change in cerebral blood volume observed with normal contractions implied a fall in total cerebral blood volume of 30%, assuming neonatal values for cerebral blood volume (Wyatt 1990). Peebles hypothesised that the reciprocal changes in oxyhaemoglobin and deoxyhaemoglobin during contraction associated with fetal heart rate decelerations indicated transient desaturation of haemoglobin within the brain. This may have been due to a transient desaturation in the umbilical venous supply which was transmitted to the brain, or conversely it may have indicated a reduction in cerebral blood flow, secondary to fetal bradycardia, leading to increased oxygen extraction within the brain.

2.2.2.2 Effect of frequency of uterine contractions

Peebles (1994) found that a significant fall in HbO_2 with a rise in Hb occurred with a short contraction interval (< 2 minutes). This again suggested there was a transient desaturation of the fetal brain. Furthermore, the changes in HbO_2 were positively, and in Hb negatively, correlated with the time interval between contractions. A mean contraction interval of 2.3 minutes was found below which the HbO_2 concentration usually fell and that of Hb rose, indicating a fall in cerebral haemoglobin saturation. Conversely, longer contraction intervals were associated with findings indicative of a rise in cerebral haemoglobin saturation.

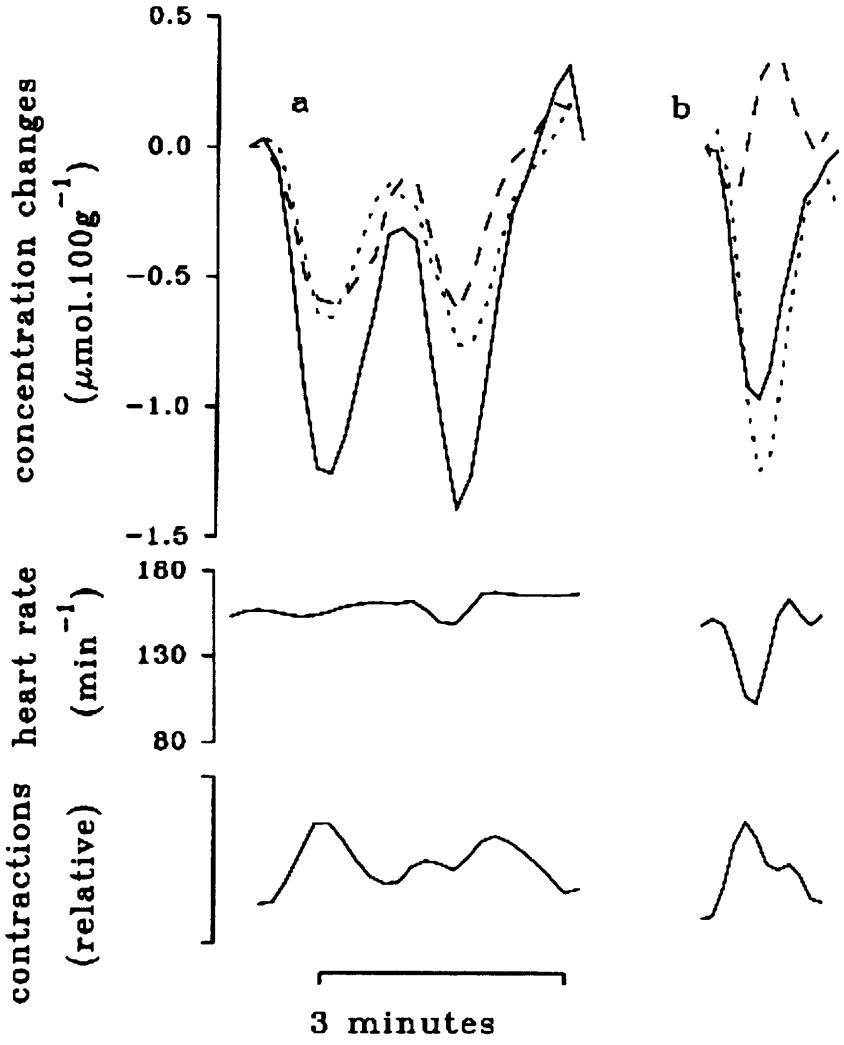


Figure 2.3 Changes from an arbitrary baseline in the cerebral concentrations of HbO_2 (*dotted line*), Hb (*dashed line*) and total haemoglobin (*solid line*) in the same fetus during a) 2 normal contractions and b) a contraction during a FHR deceleration. Reproduced with permission from Peebles (1992b).

2.2.2.3 Changes with FHR decelerations

Subsequently, Aldrich (1995a) using the same spectrometer and fetal probe as Peebles set out to determine the relationship between contraction related changes in fetal heart rate and cerebral oxygenation. The changes in fetal cerebral HbO_2 and concentrations that occurred during contractions were quantitatively similar, irrespective of the fetal heart rate changes. However, late fetal heart rate decelerations were associated with a significantly greater fall, after the uterine contraction, in the mean concentration of HbO_2 of 0.52 mmol/100 g and a significantly greater rise in the mean concentration of Hb of 0.36 mmol/100 g.

2.2.2.4 Effect of second stage of labour and delivery

Aldrich et al (1995c) then used NIRS in the second stage of labour to show that the onset of pushing was associated with a significant decrease in $SmcO_2$ and a rise in HbT (and thus cerebral blood volume). Following the onset of maternal pushing, the mean Hb concentration increased by a mean of 0.79 mmol.100 g-1 without any consistent change in the HbO_2 concentration. These changes were associated with a significant decrease in the calculated mean cerebral oxygen saturation from a mean of 46.8% to 38.1%. Pushing was also associated with a significant increase in the mean cerebral blood volume, which rose, by a mean of 0.33 ml.100 g-1.

Further studies during delivery, showed that with the onset of respiration, HbO_2 rose rapidly with reciprocal changes in Hb, indicating a rapid rise in cerebral oxygenation. In response, cerebral blood volume fell, suggesting the onset of cerebral vasoconstriction (Peebles1992a, Schmidt 1994).

2.2.2.5 Acid-base correlation

In relation to clinical end-points, $SmcO_2$ measurements thirty minutes prior to delivery correlated with pH in both umbilical vessels (Aldrich 1994a) and HbO_2 correlated with pO_2 measured by FBS (Schmidt 1990a).

2.2.2.6 Other fetal studies

Changes in fetal cerebral oxygenation have also been described in response to changes in maternal posture during labour, maternal oxygen administration and in fetuses with a nuchal cord (Aldrich 1994b,1995b, D'Antona 1995). A case report by Peebles (1991) demonstrated a fall in HbO_2 and a rise in Hb, occurred in response to uterine hyperstimulation from the inappropriate use of oxytocin.

2.2.2.7 Animal studies using NIRS

These reports on the changes in cerebral haemodynamics in human fetuses appear to be similar to those found in the sheep fetus to investigate the cerebral response to hypoxia/ischaemia in a controlled manner. Induced hypoxia, in near-term fetal sheep, is associated with a sustained increase in cerebral blood flow and CBV (Bennet 1998). This is associated with a decrease in the metabolic rate, with no change in oxygen delivery and a fall in oxygen consumption (Newman 2001). CBV also increases in response to acute moderate asphyxia, induced by occlusion of the maternal common internal iliac artery, with a fall in brain oxygen delivery and consumption (Newman 2000). Conversely, acute umbilical cord occlusion, to induce an asphyxial state, is associated with a fall in cerebral blood flow but without a decrease in CBV (Bennet 1998). Post-asphyxia NIRS data suggests that there is a significant secondary reduction in CBV along with a reduction cerebral blood flow and oxygenation despite normal perfusion pressure and heart rate (Bennet 1999).

2.3 Limitations of conventional NIRS

NIRS therefore has the unique ability to provide measurements of cerebral saturation and changes in CBV from within the brain itself. As mentioned in section 2.2.2.7, data from animal studies suggest that measurement of CBV may provide a better reflection

and sensitivity to the type, severity and timing of the insult to which the fetus is exposed.

On the basis that the initial fetal response to acute hypoxia is cerebral vasodilatation and an increase in cerebral blood flow, knowledge of absolute blood volume might help distinguish the normoxic from the hypoxic fetus. By providing this information during labour, NIRS has the unique potential to detect the fetus at greatest risk of irreversible brain injury during labour.

Although NIRS is a promising new method of fetal surveillance, the technique used to date is limited in the clinical setting. This is because, firstly, interpretation of results using intrapartum NIRS is hampered by the fact that the technique provides information about changes in CBV, derived from changes in the concentration of HbO_2 and Hb which are calculated from an arbitrary baseline set at the beginning of the study. It is therefore not currently possible to quantify absolute CBV, and therefore the degree of cerebral vasodilatation, at the beginning of the study. Furthermore, the method for measurement of cerebral saturation ($SmcO_2$) relies on parallel changes in HbO_2 and Hb, which do not always occur during labour.

Secondly, the measurements of cerebral saturation that have been described may be affected by changes that occur within the fetal probe used, in response to uterine contractions. This was suggested by Hamilton et al (1995) at North Staffordshire Hospital. They reported that $SmcO_2$ measurements using NIRS were possible from the brain of a dead fetus during labour. Hamilton argued that because there was no possibility of a change in cerebral oxygenation in a dead fetus, the apparent concentration changes recorded were likely to be due to movement artefact, resulting from changes occurring induced by contractions within the fetal probe.

2.4 INTENSITY MODULATED OPTICAL SPECTROSCOPY (IMOS)

The main aim of this study was to try to overcome these limitations of NIRS. Recent advances in optical technology means that the infrared signal can be manipulated, using a technique called Intensity Modulated Optical Spectroscopy (IMOS).

This section explains the materials and methods that were used in this thesis to provide measurements of absolute concentrations of Hb and HbO_2 in biological tissue using the new infrared technology of IMOS. The theoretical background to IMOS to derive these measurements are described and then the materials required for the collection and analysis of data using this new optical technique.

2.4.1 Theoretical background to IMOS

The conventional NIRS monitor (Hamamatsu Photonics KK, Japan) used by Peebles (1993) and Aldrich (1994c) to measure changes in fetal cerebral blood volume and saturation, used near infrared light that was emitted from laser diodes in short pulses. When the laser is switched on the brightness, or intensity, is constant.

When the light was passed through the fetal head, the detected change in intensity by the spectrometer allowed calculation of changes in the concentrations of fetal cerebral oxy and deoxyhaemoglobin. These measurements were derived from the measured changes in the attenuation of near infrared light (defined as the logarithm (base e) of the ratio of incident and detected intensities) using a modification of the Beer-Lambert Law (Delpy et al. 1988), previously described in section 2.2.2.

Using newer infrared technology it is now possible to modulate the near infrared light source into an ultra-high frequency sinusoidal waveform. The waveform has AC and DC components as illustrated in figure 2.4a.

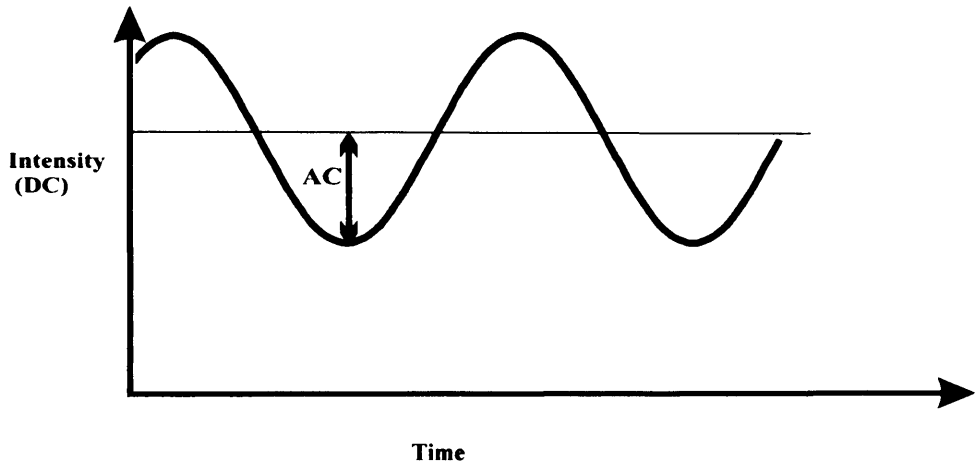


Figure 2.4a Schematic representation of an intensity modulated light source.

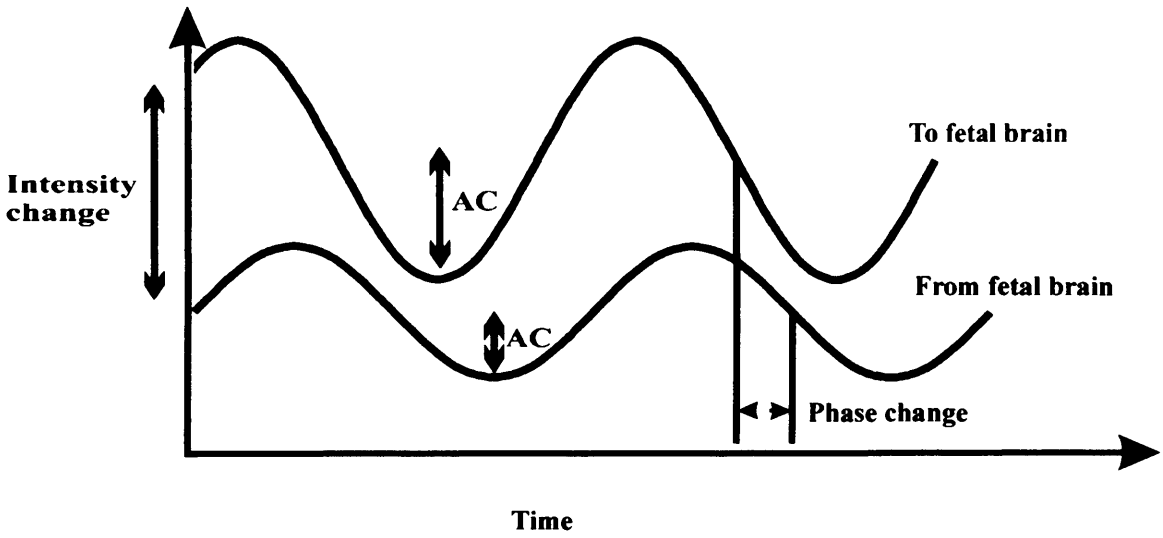


Figure 2.4b Simplified diagram of the changes in an intensity modulated light source before and after passing through the fetal brain.

If this light source is passed through the fetal brain changes in the waveform will occur. The intensity will change similar to conventional NIRS. In addition, as can be seen in figure 2.4b, the waveform is out of step or 'out of phase' with the original incident light and the ac and dc component (the ratio of which is defined as the modulation depth) of the modulated light wave will change.

Therefore in addition to **attenuation** (derived from intensity), changes in the **phase** and **modulation depth** occur.

2.4.2 Absolute fetal Hb and HbO_2 derivation with intensity modulated light source.

In simple terms, the absorption properties (termed the absorption coefficient (μ_a)) of the fetal brain at near infrared wavelengths is composed of the absorption properties of its constituent components. As previously described in section 2.1, the absorption properties of biological tissue are largely composed of HbO_2 and Hb. Variations in refractive index are responsible for the scattering of light in tissue. Biological tissue also has scattering properties (defined as the scattering coefficient) and is dependent on variations in the collective refractive indices of the tissue constituents. These optical properties of scattering media (μ_a and μ_s) determine the attenuation of light diffusely reflected from a medium as well as the phase and modulation depth of an intensity modulated light wave. If there are changes in the tissue absorption coefficient changes in light intensity (attenuation), phase and modulation depth will occur.

From these changes it is possible to derive the absolute absorption coefficient of a medium the measurement of which is independent of scattering.

These measurements are possible because the ratio of the attenuation and phase (defined as Q in this thesis) is a linear function of μ_a (and hence concentration) only.

This is also true for the ratios of attenuation with modulation depth and phase with modulation depth (similarly, defined as R and V). This linear function is based on diffusion theory, which is described in detail in appendix 1. (Kohl 1997)

From these measured absolute absorption coefficient (μ_a), absolute concentrations of Hb and HbO_2 can be derived in biological tissues as the extinction coefficients of these chromophores for the laser wavelengths are known.

Thus

$$\mu_a^{\lambda} = \varepsilon_{HbO2}^{\lambda}[HbO_2] + \varepsilon_{Hb}^{\lambda}[Hb]$$

where μ_a^{λ} is the absorption coefficient at wavelength of NIR light λ of the tissue under investigation, and ε_{HbO2} and $\varepsilon_{Hb}^{\lambda}$ are the known extinction coefficients at wavelength λ for HbO_2 and Hb respectively. The product of the extinction coefficient (e.g. ε_{HbO2}) and absolute concentration (e.g. HbO_2) at a certain wavelength is the absorption coefficient of a chromophore. Hence, if two or more wavelengths (λ_1 and λ_2) are used, absolute concentrations of HbO_2 and Hb can be derived by solving two simultaneous equations as follows:

$$[HbO_2] = (\mu_a^{\lambda 1}\varepsilon_{Hb}^{\lambda 2} - \mu_a^{\lambda 2}\varepsilon_{Hb}^{\lambda 1})/ (\varepsilon_{Hb}^{\lambda 2}\varepsilon_{HbO2}^{\lambda 1} - \varepsilon_{HbO2}^{\lambda 2}\varepsilon_{Hb}^{\lambda 1})$$

$$[Hb] = (\mu_a^{\lambda 2}\varepsilon_{HbO2}^{\lambda 1} - \mu_a^{\lambda 1}\varepsilon_{HbO2}^{\lambda 2})/ (\varepsilon_{Hb}^{\lambda 2}\varepsilon_{HbO2}^{\lambda 1} - \varepsilon_{HbO2}^{\lambda 2}\varepsilon_{Hb}^{\lambda 1})$$

If absolute concentrations of HbO_2 and Hb are calculated, then the following simple equations can derive absolute total haemoglobin (HbT) and cerebral saturation ($SmcO_2$):

$$HbT = [HbO_2] + [Hb]$$

$$SmcO_2 = 100\% * [HbO_2] / [HbO_2] + [Hb]$$

Cerebral Blood Volume is directly related to HbT by the following equation and therefore an estimate of absolute CBV can be derived:

$$CBV = HbT \times MW_{Hb} \times 10^{-6} / tHb \times 10^{-2} \times D_t \times 10 \text{ (Elwell 1995)}$$

where MW_{Hb} is the molecular weight of haemoglobin (64500), tHb is the mean umbilical vein Hb concentration (16.8g/dl) (Wintrobe 1997) and D_t is the brain tissue density (1.05g/ml).

2.4.3 Optical Pathlength measurement

As previously described in section 2.2.2, conventional NIRS assumes that the optical pathlength remains constant. In these measurements, based on the Beer Lambert law, optical pathlength is a product of the interoptode spacing (IOS) and a differential pathlength factor.

However, IMOS has the unique ability to provide information on the changes in the optical pathlength during studies in biological tissue. This is because the change in phase (that can be measured by an intensity modulated optical spectrometer) is inversely related to the change in optical pathlength, which can be calculated, from the following equation:

$$\Delta d = - \Delta \Phi c / 2\pi n \nu_m.$$

where $\Delta\Phi$ is the change in phase of a lightwave, intensity modulated at a frequency v_M (200MHz) c is speed of light in a vacuum and n is the refractive index of tissue (1.4).

Thus the technique of IMOS has the unique ability to provide absolute concentrations of HbO_2 + Hb in biological tissue *and* provide information on the optical pathlength.

2.4.4 Clinical equipment

In order to put the theory of IMOS in to practice, the principle equipment required are a spectrometer that modulates at least two different wavelengths of infrared light sources and optical fibres to transmit and receive the light via a specially designed probe.

The spectrometer

In 1991 the Wellcome Trust funded the development of the intensity modulated optical spectrometer at Department of Medical Physics and Bioengineering, University College, London. (Wellcome Trust Ref033126/Z/91,Duncan et al 1993). This spectrometer was based on an original design by Chance et al (1990).

This spectrometer has a purpose built unit that holds the laser diodes (Delta Development Ltd), of which there are four lasers at the wavelengths 741nm, 787nm, 815nm and 856nm (Seastar Ltd) with a variable mean output powers between 6 and 40Mw. The NIR light is provided by two phase locked frequency synthesisers (PTS Ltd model 500). A second modulation frequency offset from the first, typically by 10kHz, is also available to facilitate cross-correlation of later signals within the mixers (see figs 2.5 and 2.6). The signals are mixed, filtered (Mini Circuits, ZAD-1 WSH & BLP-10.7) to produce a modulation frequency of 200mHz. This is the frequency in which the optical pathlength derived from the phase will be equal to that derived from time of flight measurements (Arridge 1992).

A counter card provides timing pulses, enabling each of the lasers to be turned on in sequence for 20ms at 100ms intervals. Four separate optic fibres are then required to form a bundle, which can be sheathed in Kevlar, to carry light to and from a sample. The photomultiplier tube (PMT, R4998, Hamamatsu Phototonics KK), detects the signal that reaches the spectrometer from a sample. This signal consists of two components, DC intensity and the AC amplitude which are filtered and amplified. By simple arithmetic, using the X and Y co-ordinates of the AC amplitude and the DC intensity, the attenuation, phase shift and modulation depth can be derived. Readings can be taken every half-second during which each laser is fired five times. An analogue card in the PC allows up to eight external analogue channels to be monitored and these may also be displayed.

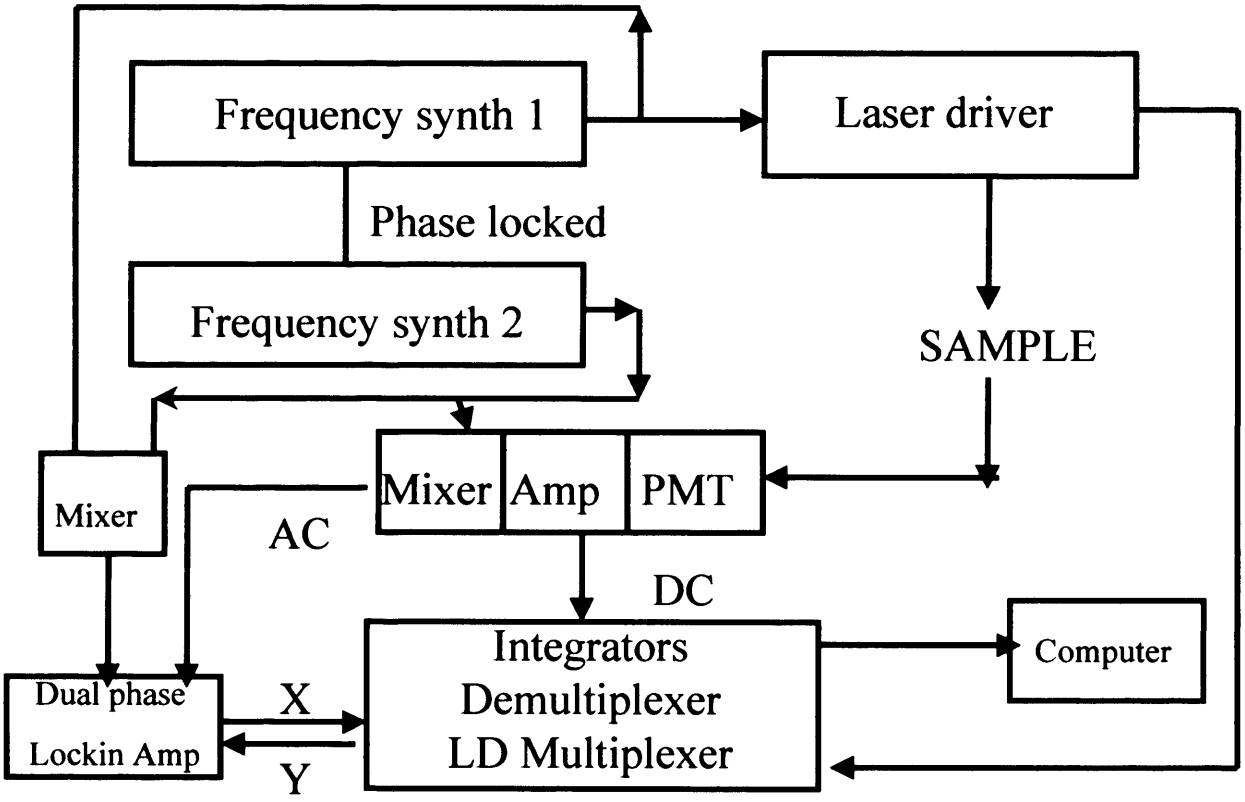


Figure 2.5 Schematic diagram of the intensity modulated optical spectrometer.

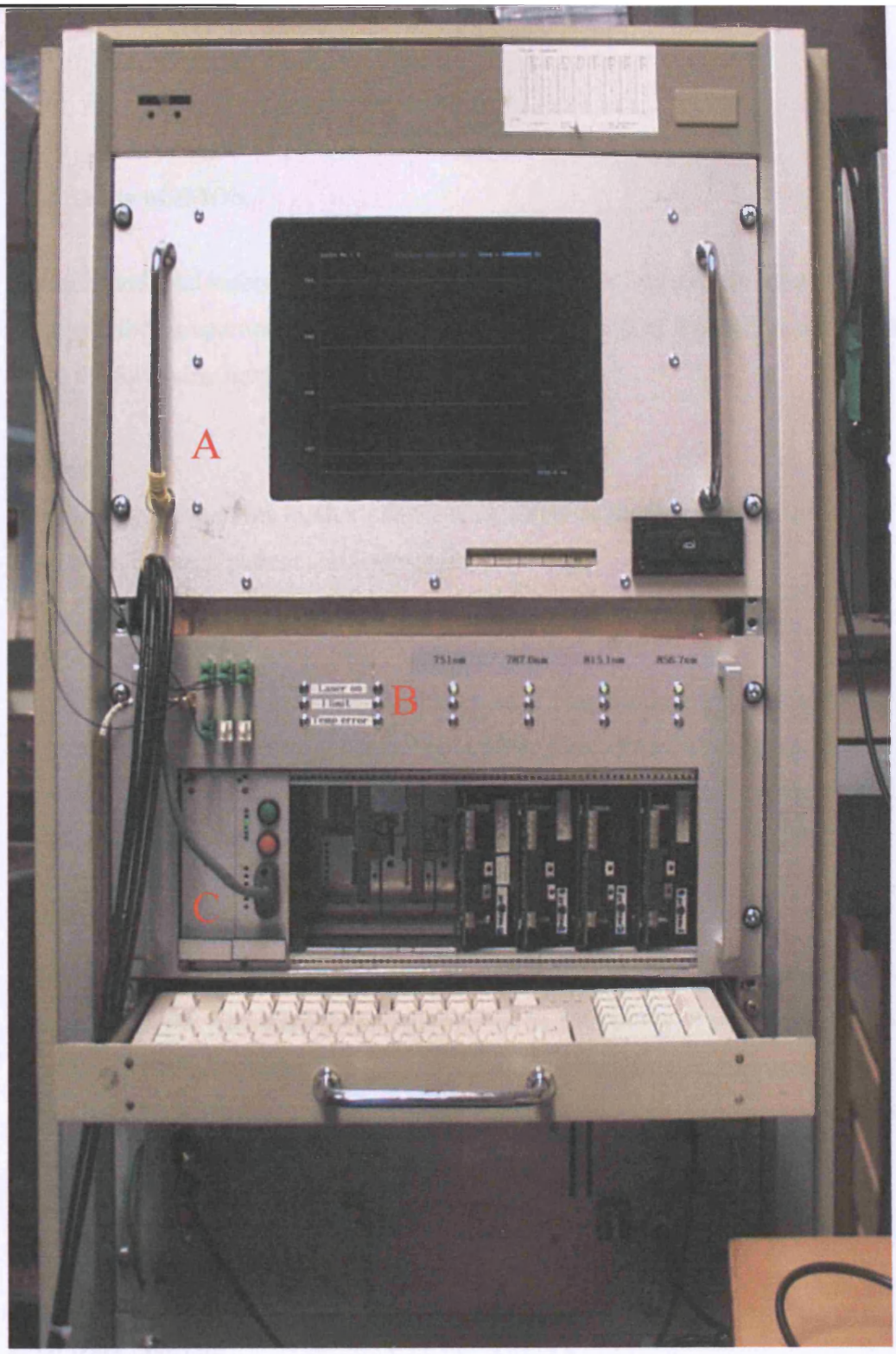


Figure 2.6 The components of the intensity modulated optical spectrometer (A is the visual display, B is the laser compartment also containing the synthesisers and mixers and C is the PMT compartment).

2.4.5 Safety of IMOS

Maternal and fetal safety is of obvious prime importance when applying a new technique for intrapartum surveillance in the research setting. Safety can be considered under the following headings:

Electrical

Electrical isolation of the mother and fetus is simple as glass optical fibres are the only connection between patient and instrument.

Laser safety

Output of the lasers is approximately one order of magnitude below the safety limits recommended by the electro-technical committee regulations. There is therefore no possibility of light-induced changes to the brain, skin or eye, even if the transmitting optode was placed accidentally directly over the orbit.

Mechanical safety

Any object placed against the fetal head and maternal tissues during labour will be forced against the fetal scalp by pressures of up to 150mmHg (Svenningsen et al 1988). It is therefore important that a probe should not have any sharp edges or corners that could cause local trauma. The only hard elements in the probes are the resin potted glass prisms. However, these are almost completely encapsulated in silicone rubber, apart from the smooth prism face which made contact to the fetal scalp.

2.4.6 Data analysis

In order to calculate absolute concentrations from the measured changes in attenuation, phase and modulation depth it is necessary to have the equations readily available. These were therefore incorporated into the notebook which was developed at the Department of Medical Physics, UCL, which runs in the Mathematica™ version 3.01 programme (Wolfram Research Inc, Champaign, Illinois, USA) (see appendix 1).

This programme displays

a) continuous changes in attenuation, phase and modulation depth for each laser wavelengths

b) changes in HbO_2 and Hb (calculated from the changes in attenuation using conventional NIR techniques)

c) Q, R and V ratios (expressed as first order regression lines, graphically and numerically)

d) Measured absolute absorption coefficients and absolute concentrations

e) Data from collected from the external analogue channels (such as FHR and contractions)

The display of c), d) and e) are obtained when an observer selects two different time points in the data in a) and runs the programme.

2.4.7 Measurements with IMOS to date

Duncan (1995) used the spectrometer and specially designed probes to measure optical pathlength on the adult head, calf and forearm and the head of the newborn infant. These correlated well with more established methods of measurement. Duncan also investigated its preliminary use on a light-absorbing tissue simulating medium with known optical properties. The absolute experimental values of the absorption coefficient were found to be approximately twice that of the known values. It was thought that more accurate measurements could be made with improved design and operation of the spectrometer. Further technical improvements allowed Kohl et al (1997) to undertake a similar study. He was able to calculate absorption coefficients of a light absorbing compound with uncertainty of only 4-6%.

2.5 CONCLUSION

Based on the described studies using conventional NIRS and the preliminary results using IMOS, it is clear that IMOS has the remarkable potential to

a) overcome the limitations of conventional NIRS found during intrapartum studies and
b) provide measurements of absolute CBV and cerebral saturation in human fetuses during labour.

Thus, the hypothesis of this thesis was that these measurements were different in normoxic fetuses compared with hypoxic fetuses during labour. The objective was to compare CBV and cerebral saturation in normoxic and hypoxic fetuses using IMOS.

The principle aim of the work on which this thesis is based was to obtain *absolute* concentrations of $Hb0_2$ and Hb in human fetuses during labour, using the described technology of IMOS.

If IMOS is capable of providing this information from the onset and the duration of labour, it has the unique potential to detect the chronically compromised hypoxic fetuses entering labour and to define those intrapartum events that require obstetric intervention to prevent irreversible brain injury and subsequent long-term neurological handicap.

CHAPTER 3

OPTICAL PHANTOM STUDIES

Previous experience at UCL with NIRS and IMOS showed that the recruitment of mothers in early labour into studies were easier with the commercially available NIRO 500 clinical instrument compared with the IMOS instrument. This was attributable to the necessity of having an extra (clinically non-qualified) person in the delivery suite to operate the IMOS system. Furthermore, the IMOS system was a prototype, the components of which were extremely fragile. Components such as the PMT and a higher resolution analog to digital converter to reduce system noise were updated before the start of fetal studies. It was therefore necessary to study the optical performance of the current IMOS system before fetal studies were undertaken. It was also hoped that my experience gained with the IMOS system during these studies would negate the need for an extra person to be present during the proposed fetal studies.

This chapter describes the optical phantom studies that I performed within the Department of Medical Physics at UCL. The aims of these studies were to (a) assess the current optical performance of the spectrometer and compare this with that found by Kohl (1997) using light absorbing tissue-simulating media (optical phantoms) and (b) to provide information to optimise the data collection and analytic process of IMOS during studies on human fetuses during labour.

3.1 STUDY 1- MEASUREMENT OF ABSORPTION COEFFICIENT IN AN OPTICAL PHANTOM.

3.1.1 Introduction

As described in chapter 2, the new optical technique of intensity modulated optical spectrometry has the potential to provide absolute measurements of fetal cerebral haemoglobin concentrations. However, the technique relies on the absolute measurement of the light–absorbing properties of the medium under investigation. By using the spectrometer and specially designed optical probes, which contain the optical fibres to transmit and receive near infra red light, it is possible to collect data from specially constructed mediums, known as optical phantoms. These are designed so that they have absorbing properties similar to biological tissue. As described in chapter 2, the measurement of absolute concentrations of haemoglobin require the absolute measurement of the absorption coefficient (μ_a) of the tissue. It is therefore possible to obtain measurements of absorption coefficient of optical phantoms in a controlled manner within the laboratory to provide information on the accuracy of the measurements of the spectrometer. By using such light-absorbing, tissue-simulating media that have similar optical properties to biological tissue, and the spectrometer described in this thesis, Kohl et al (1996,1997) reported that the uncertainties of their measurements of absolute absorption coefficient were as little as 4-6%.

However, in contrast to other more established intrapartum monitoring techniques, the spectrometer used in this thesis was a prototype. Technical improvements in the design of the spectrometer's hardware were ongoing. Since the reporting by Kohl, the spectrometer had been updated with a new PMT, a higher resolution analog to digital converter card, laser diodes and the specially designed Mathematica^{TM} notebook for data analysis had been developed.

This study therefore set out to determine the accuracy of the measurements of the updated spectrometer in a controlled manner using an optical phantom which had

known absorption properties. The aim was therefore to derive measurements of the absorption coefficient of the optical phantom and compare these to the known absorption coefficients.

3.1.2 Methods

Data collection

The optical phantom used in this study was an intralipid emulsion. This consisted of phospholipid micelles (soybean oil, glycerine and lecithin) that was diluted to 1 part in 100 with water (1 % lipid, and 99% water) to a volume of 450 ml. This optical phantom has previously been described by Staveren (1991) and Flock (1992) and hence it's absorption properties are known. Within the emulsion two optodes were placed 35mm apart just under the surface and held in place by a specially designed clamp (see figure 3.1).

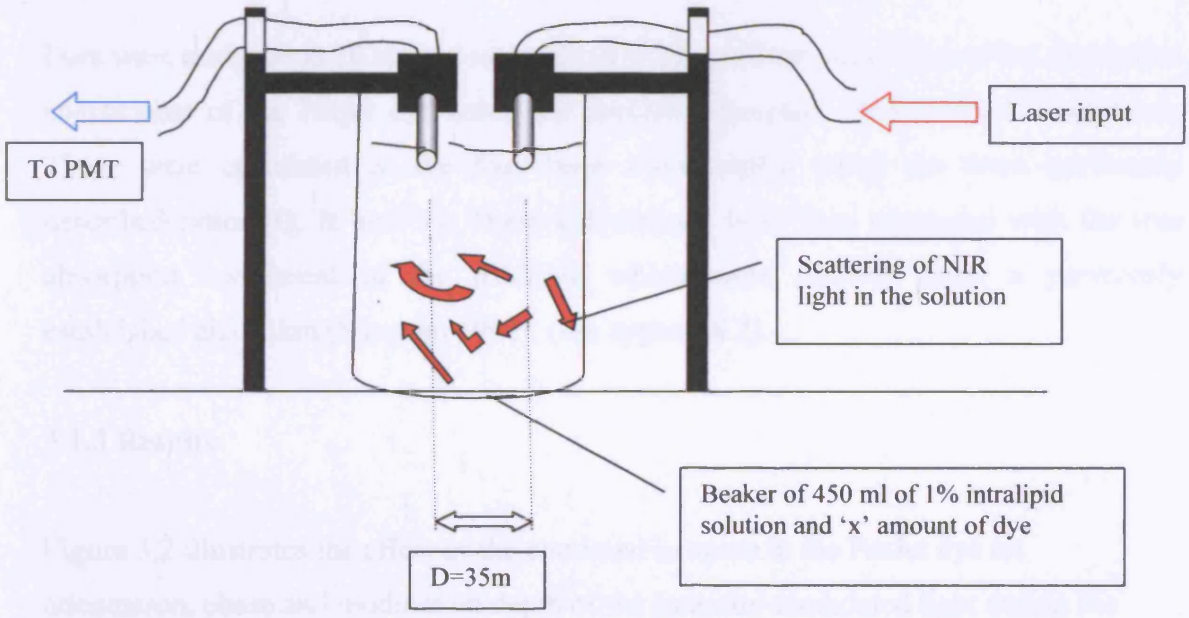


Figure 3.1 Diagram to show experimental set up for study 1.

Near infrared light, generated by the semiconductor lasers at the four discrete wavelengths and modulated at 200 MHz, was carried to the intralipid emulsion by four single mode optical fibres. Light emitted from the emulsion was carried by a bundle of approximately two thousand 50μm diameter multi-mode fibres to the new photomultiplier tube housed within the spectrometer. The optodes were covered with black cloth to avoid interference from background light.

An infrared absorbing dye (Pro-Jet, Zeneca), which has absorbing properties similar to that of biological tissue, was added to the intralipid emulsion in a continuous fashion via a syringe pump.

To allow a continual increase in the concentration of the dye (which increases the absorption coefficient) within the medium, a specially designed peristaltic pump was used. This re-circulated the solution in the container at a rate of 0.22 litres every minute to provide efficient mixing of the contents to produce a homogenous solution with respect to infused dye and intralipid. Data was collected over a period of 120 minutes as the concentration of the dye, and thus absorption coefficient, was continually increased.

Data analysis

Data were analysed at 10 second intervals (n = 22) to allow calculation of the absorption coefficients of the Projet dye using the specially designed Mathematica™ notebook. These were calculated at the four laser wavelengths, using the three previously described ratios (Q, R and V). These calculations were then compared with the true absorption coefficient of the medium, which were derived using a previously established algorithm (Staveren 1991) (see appendix 2).

3.1.3 Results

Figure 3.2 illustrates the effect of the continual increase in the ProJet dye on attenuation, phase and modulation depth of the intensity-modulated light during the study at laser wavelength 787nm. Similar data were obtained at the other three laser wavelengths.

As can be seen, an increase in dye concentration is associated with an increase in all three variables. The sudden 'dips' in the data represent manual increases of the PMT sensitivity to increase the detection of light as the absorption properties of the medium increased. Figures 3.3 illustrate the relationship between the calculated absorption coefficient by the Mathematica notebook and true absorption coefficient measured at the four different wavelengths and the ratios with regression lines, coefficient of regression and correlation coefficients (R^2).

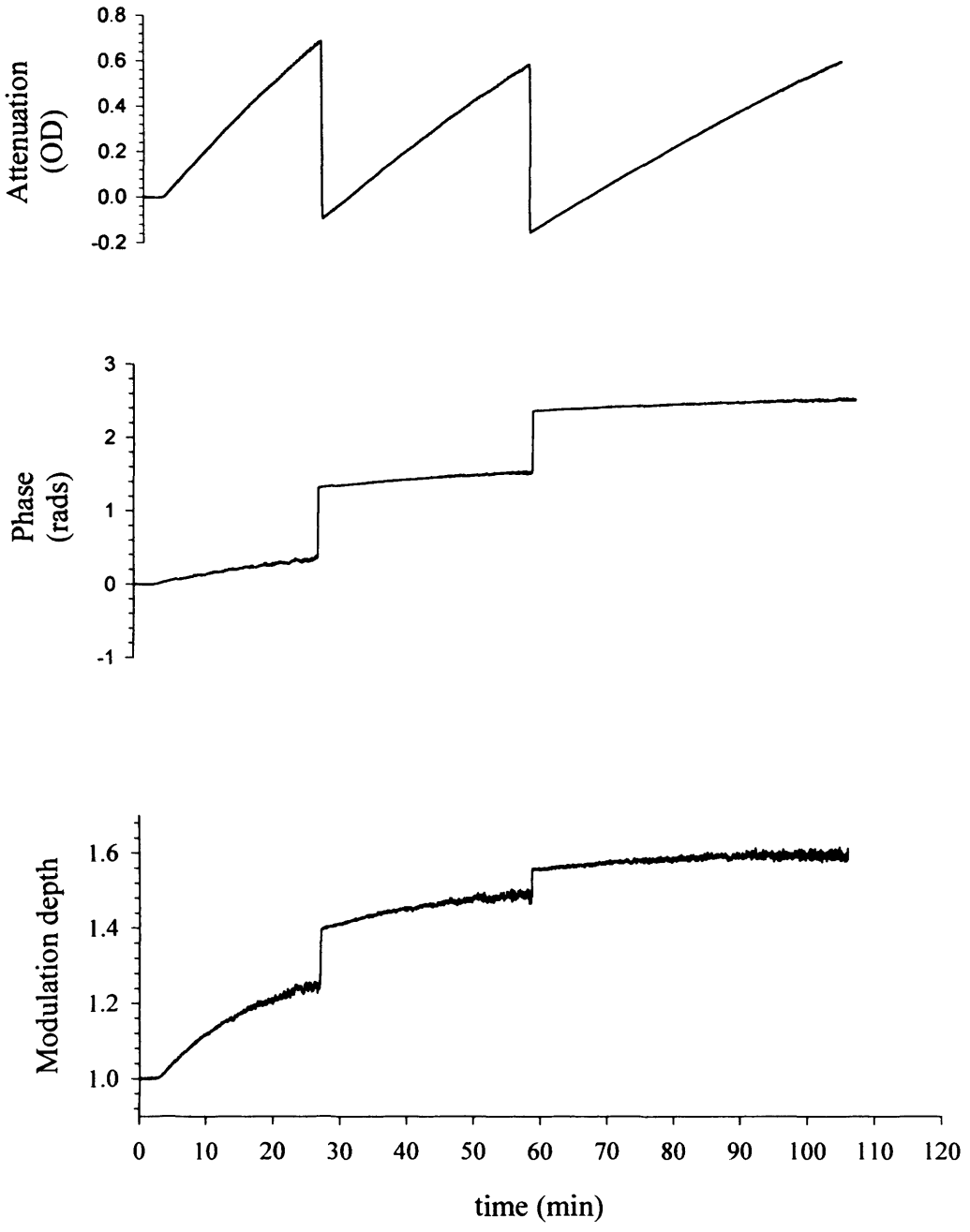


Figure 3.2 Effect on attenuation, phase and modulation depth of a continuous increase in the absorption coefficient in the liquid optical phantom at wavelength 787nm.

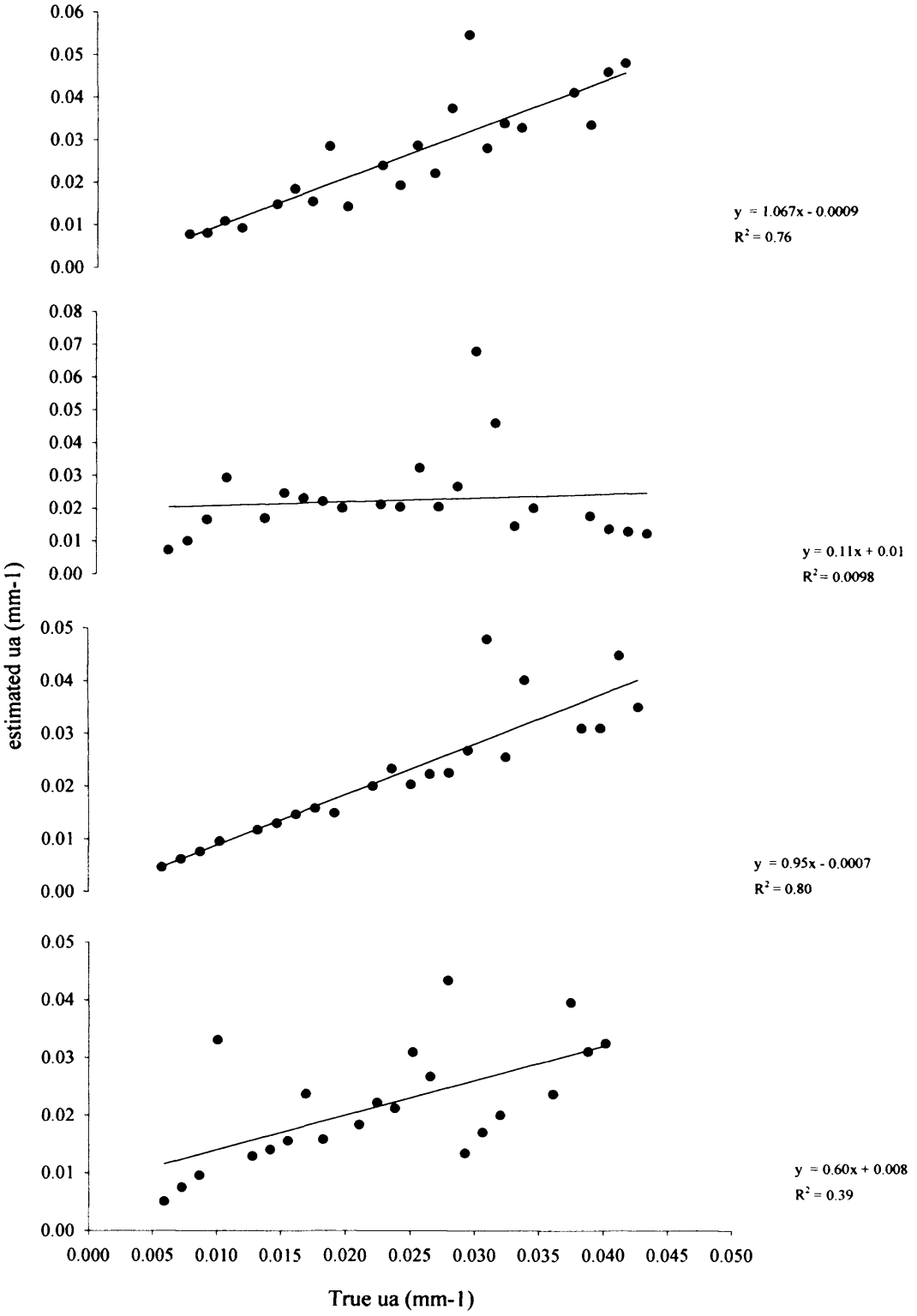


Figure 3.3. Calculated absorption coefficients by IMOS versus the true absorption coefficients using attenuation and phase (Q) at the four wavelengths (856,815, 787,741nm,above to below) with regression lines, coefficient of regression and correlation coefficients (R^2)

Table 3.1 summarises the calculated error of these measurements of absorption coefficients using the different ratios described in chapter 2.

Table 3.1 The error of the calculated mean absorption coefficient (% of the mean true absorption coefficient) at each laser wavelength for each ratio.

Wavelength	Q	R	V
856nm	3.0	26.0	21.7
815nm	7.7	58.0	58.0
787nm	4.8	40.1	28.4
741nm	8.3	11.1	20.7

3.1.4 Discussion

This study shows that the updated spectrometer and the new specially designed Mathematica notebook allow calculation of the absolute absorption coefficient of a compound within an optical phantom, the optical properties of which are similar to biological tissue. Absolute absorption coefficients were calculated from the different ratios of attenuation, phase and modulation at all four laser wavelengths. It is this calculation of the absolute absorption coefficient of a compound, such as haemoglobin in the fetal brain that is required to provide the absolute concentration.

However from these data there appears to be a wide discrepancy in the accuracy of these measurements between the different lasers and between the different ratios. Correlation of the calculated measurements with the true values of absorption coefficients suggests that the most accurate method is the Q (attenuation and phase) ratio at 856 and 787nm wavelengths of light. The errors of measurement were less than 10% at all four wavelengths. Using modulation depth and phase (V) gave the least

reliable results. This may reflect the quality of the individual optical fibres transmitting the infrared light but is more commonly due to the high signal noise on the modulation depth data, which has also been reported by Kohl (1996,1997).

STUDY 2- SIGNAL-NOISE CHARACTERISTICS OF THE SPECTROMETER.

3.2.1 Introduction

It is clear from study 1 that even in a controlled laboratory manner the accuracy of IMOS measurements is variable. When applied to the human fetus, the unpredictability of labour and the intrapartum events that may occur not only have an effect on data collection but also reflects the available time in which a new intrapartum method of fetal surveillance can be potentially employed. The commercially available NIRS monitors (Hamamatsu Photonics KK (Japan), Radiometer/ Keele University (Denmark/ UK), and Critikcon (UK)) required a 'warming-up' period of approximately two minutes (Elwell 1995). This allowed Peebles (1993) and Aldrich (1994) monitored fetuses in labour with NIRS from 35 to 765 (mean 196) and 7 to 666(mean 132) minutes, respectively. To achieve similar results, the performance and the stability of the spectrometer needed to be optimal at the beginning of observations.

The sensitivity of a photomultiplier tube (PMT) to NIR light is temperature dependent, especially near the long wavelength region. The light level received by a PMT is also a strong function of optode spacing and tissue type. To allow for a wide range of intensities and to ensure the optimum working temperature is achieved, a settling period maybe required for the PMT gain to be optimised at the beginning of fetal measurement.

Furthermore, the signal to noise ratio may have an influence on the performance of a near infrared spectrometer. Peebles (1993) and Aldrich (1994) found that this was particularly the case when monitoring fetuses with thick, black curly hair.

This study therefore set out to define the time required to ensure optimal performance of the new PMT in the spectrometer and once signal stabilisation had occurred, to define the background signal-noise characteristics of the attenuation, phase and modulation depth.

3.2.2 Methods

The fetal probe, which is described in detail in chapter 4, was applied to a solid conical epoxy resin phantom. This phantom was used as it was initially constructed by Firbank(1995) to replicate the absorbing properties of biological tissue. The probe was secured with black adhesive masking tape and the probe and the phantom were covered with black cloth. The optical fibres of the probe were connected to the spectrometer and data were collected from the time that the lasers and PMT of the spectrometer were switched on. To reflect the potential time that data could be collected from human fetuses during labour, data were collected up to 900 minutes. Signal stability and noise characteristics were monitored using all four lasers. The modulation frequency was set at 200Mhz. Data were collected at half-second intervals. After stabilisation had occurred, the standard deviations of the signals of attenuation, phase shift and modulation depth were calculated to represent the signal noise for a period of 60 minutes.

3.2.3 Results

Figure 3.4 shows representative data of attenuation, phase and modulation depth data collected by the spectrometer at laser wavelength 856nm. This shows that the time taken for the signal to stabilise is in the order of approximately 600 minutes (10 hours). Similar results were obtained in the other 3 lasers. Figure 3.5 illustrates the noise within the attenuation, phase and modulation depth data after this period of stabilisation and table 3.2 shows the noise characteristics represented by the standard deviations of the data in these figures.

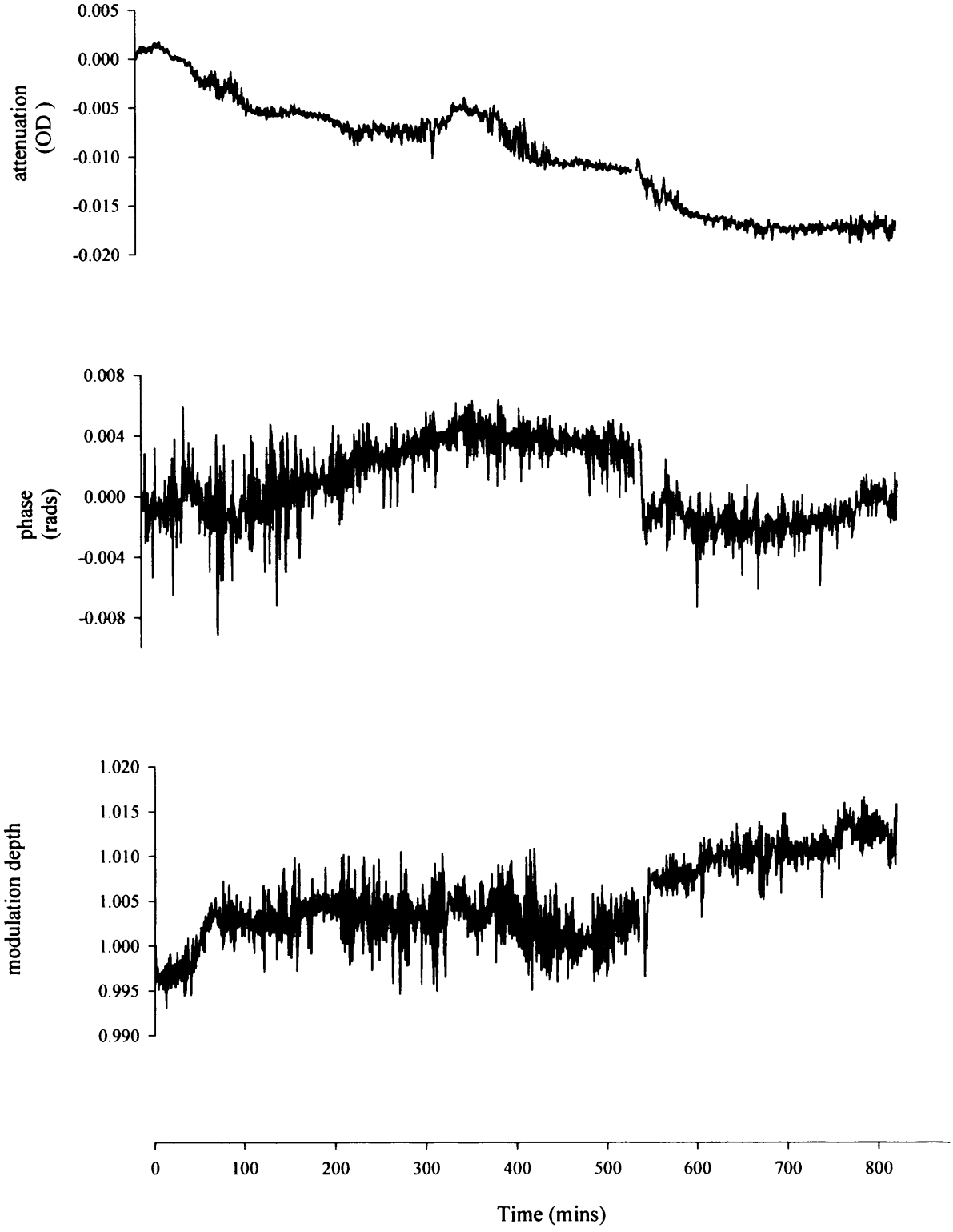


Figure 3.4 Attenuation, phase and modulation depth data at wavelength 856nm on a solid phantom with the fetal probe.

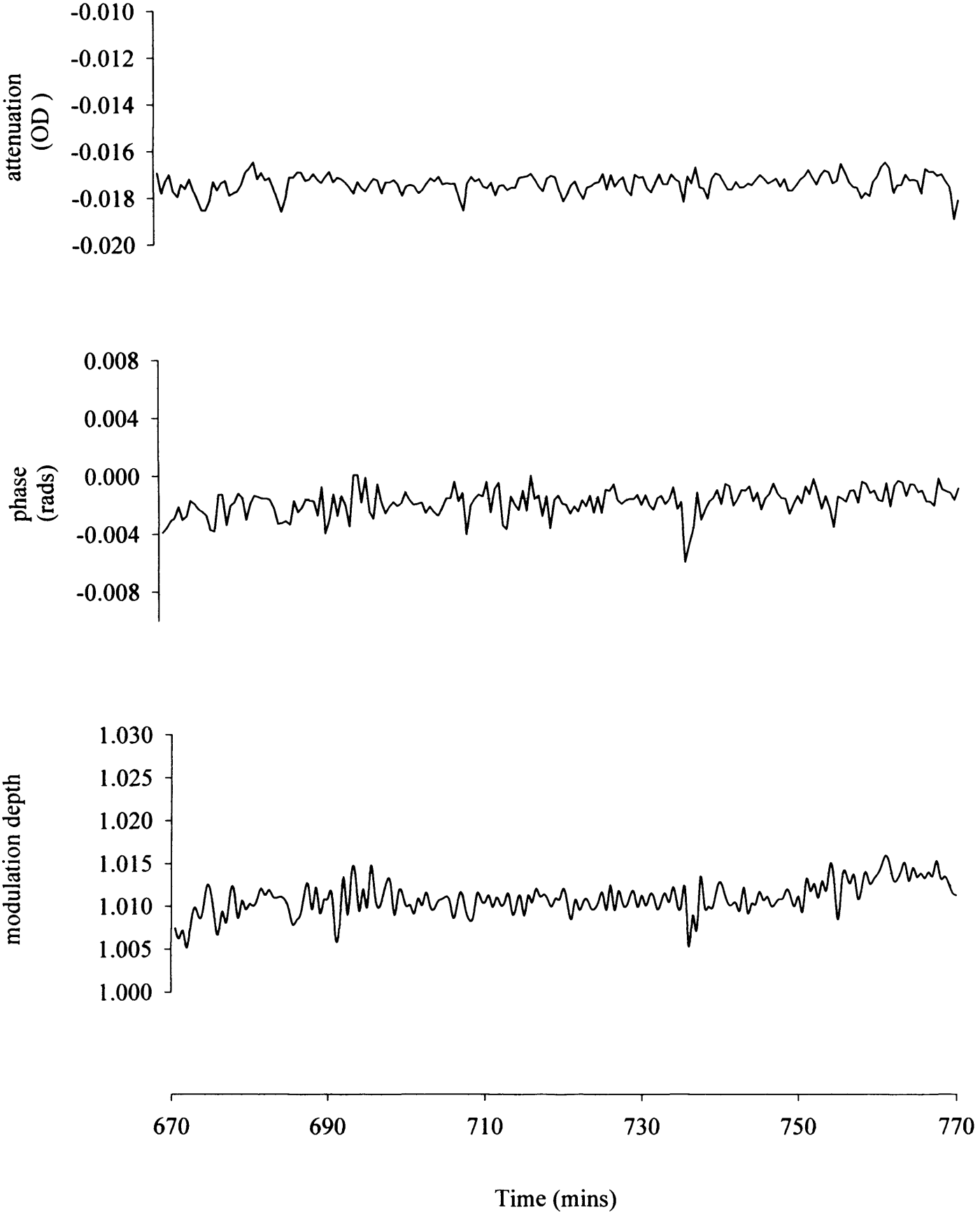


Figure 3.5 Noise characteristics for attenuation, phase and modulation depth

Table 3.2. Signal noise characteristics at the four laser wavelengths, represented by standard deviations of the signals during the total data collection and the time from 670 to 770 minutes.

	856nm	815nm	787nm	741nm
Attenuation (OD)				
SD (total)	0.0006	005	0.01	0.006
SD (670-770 min)	0.0004	0.01	0.003	0.0005
Phase (RADS)				
SD (total)	0.003	0.003	0.006	0.002
SD (670-770 min)	0.0009	0.0003	0.0002	0.0009
Modulation depth				
SD (total)	0.005	0.002	0.004	0.002
SD (670-770 min)	0.0002	0.001	0.0003	0.002

3.2.4 Discussion

This study shows that firstly, the time for signal stabilisation is much longer than for the conventional NIRS monitors and secondly the magnitude of the signal noise of the current spectrometer is similar to previous published results. Using a similar phantom model, Duncan (1993) found similar magnitudes of noise in all three signals (0.0006OD, 0.0011 RAD and 0.0008 for attenuation, phase and modulation depth respectively).

Encouragingly, other studies suggest that this background signal noise level may not have an effect on the data that was to be collected on fetuses for this thesis.

Kohl (1997) found that a small increase (15%) in the absorption properties of a liquid phantom, similar to biological tissue, results in a much larger change in the three signals (attenuation of 0.2 OD, phase of 0.8 RADS and modulation depth of 0.2). From these changes it was possible to calculate the absolute absorption properties.Watson (1996) found a similar magnitude of attenuation change (0.3OD) in one human fetuses during labour. It was therefore likely that the signal noise defined in the study described would not have a marked effect on the data collected from fetuses.

STUDY 3 SIGNAL INTERFERENCE

3.3.1 Introduction

As previously described in chapter 2, the conventional NIRS monitors calculate changes in haemoglobin concentration from the changes that occur in the attenuation of near infrared light. The sensitivity of the PMT of these spectrometers could not be adjusted manually during a study and therefore potentially the PMT may not be able to detect changes in intensity of light at low light levels. The commonest reason for failure to acquire a good quality signal with these monitors was the inability to transmit sufficient light through the tissue. This was observed most frequently if there was a combination of thick black curly hair and dark skin (Peebles 1993, Aldrich 1994). Melanin significantly reduces the amount of near infrared light transmitted through tissue because of its high attenuation. Tissue with a high melanin content, such as black skin or hair will therefore effectively screen out transmitted light.

Conversely, the sensitivity of the PMT of the IMOS system used in this thesis could be manually adjusted and therefore potentially has the ability to overcome this problem by being able to detect changes in attenuation, phase and modulation depth at a far greater range of intensities. These measurements are theoretically all independent of each other, throughout a range of light intensities and the signal/noise ratio of the phase and modulation depth should remain stable and not be affected at extreme ranges of light intensities.

This study set out to detect the presence of signal interference (cross-talk) between the three parameters throughout a range of intensities that may be encountered during studies on human fetuses.

3.3.2 Methods

A circular disk, whose thickness and thus it's attenuation properties varied continuously as a function of angle, was placed between an optical fibre attached to the spectrometer and an optode attached to a solid conical epoxy resin phantom. The spacing distance between the incident NIR light on the phantom and the detector optode was set to 3.0 cm. The whole of the set-up was covered in dark cloth to exclude as much ambient light as possible that could otherwise damage the PMT of the spectrometer. The circular disc was rotated smoothly through its central axis manually, so that light passing through it was increasingly attenuated. Figure 3.6 illustrates the experimental set-up.

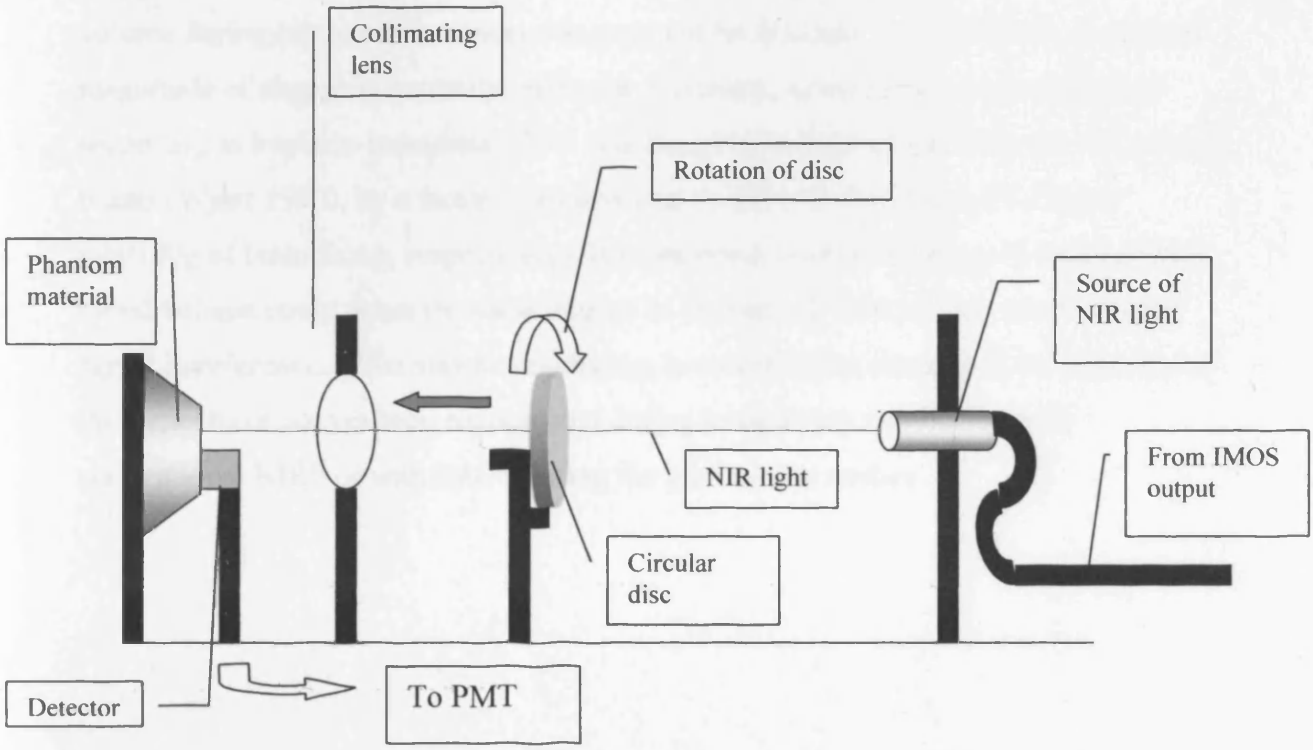


Figure 3.6. Experimental set-up of the signal interference study

3.3.3 Results

Figure 3.7 illustrates the effect of varying the intensity of detected light on the data collected by IMOS at the four laser wavelengths. This shows that as the light signal is increasingly attenuated (lower intensity of light reaching the PMT), the phase and modulation depth data become affected (cross-talk) and the signal/noise ratio decreases.

3.3.4 Discussion

Ideally the IMOS system should be capable of measuring a wide range of absolute concentrations of Hb and HbO_2 from fetuses over a wide range of light intensities. However, data from the third study suggest that at low intensity detected by IMOS there is increasing interference between the three signals. This may have importance therefore when the system is applied to fetuses, as wide range of changes in cerebral blood volume during intrapartum manoeuvres may not be detected. Unfortunately, the actual magnitude of change is currently unknown. Certainly, in neonates with brain injury secondary to hypoxia-ischaemia, CBV was found to be higher than in those with normal brains (Wyatt 1990), by a factor of approximately 50% (3.00 ± 1.04 and 2.22 ± 0.4 mls/100g of brain tissue, respectively). It is unknown whether changes in fetal cerebral blood volume could cause the same change in intensity (2 ODs) which would induce signal interference. What may be reassuring, however is that changes in the intensity of this order have not yet been encountered during intrapartum monitoring with conventional NIRS or with IMOS during the preliminary studies

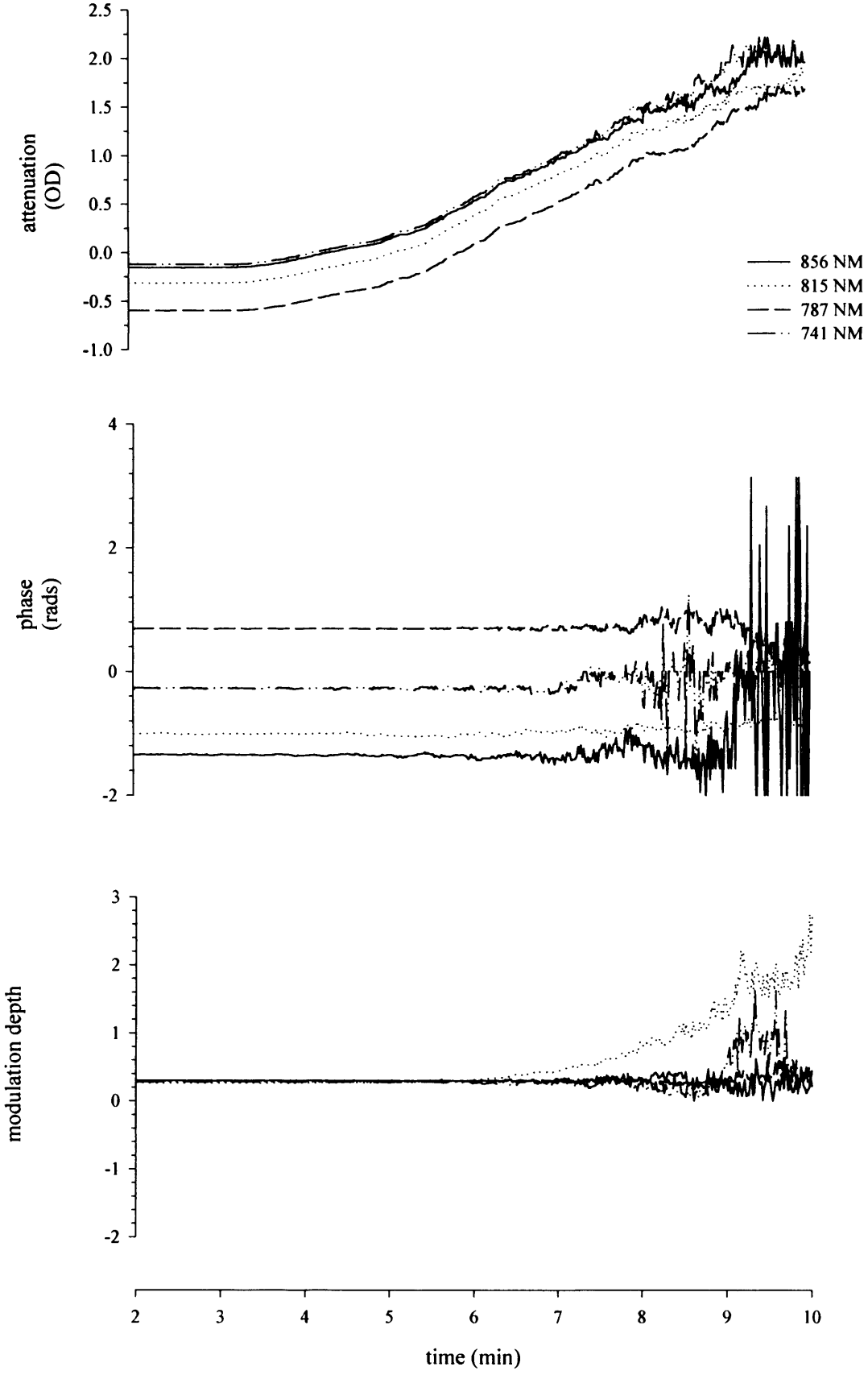


Figure 3.7 The effect of increasing the attenuation of light (decreasing intensity) on the phase and modulation depth data (cross-talk).

3.4 CONCLUSION

These optical phantom studies provide useful information in order to optimise the process of data collection during subsequent intrapartum studies. Despite the spectrometer being a prototype, the data from study 1 suggest the error of its measurements may be as little as 3%. This compares favourably with reported errors of conventional NIRS (Elwell 1995), pulse oximetry (Dassel1997) and fetal ECG (van-Wijngaarden 1996). Due to the long period of initialisation suggested by study 2 and to overcome the potential signal interference suggested by study 3, plans were made to keep the spectrometer on the delivery suite with the lasers switched. A device to enable manual change of the PMT, and therefore reducing the effect of signal interference, was to be employed.

CHAPTER 4

EXPERIENCE WITH IMOS ON DELIVERY SUITE

This chapter describes my experience on the delivery suite at University College London Hospital using the intensity modulated optical spectrometer described in section 2.4.4 and a specially designed fetal probe. A summary of this experience is presented, the fetal probe is described and details of the observations obtained from fetuses during labour are discussed. Table 4.1 provides a summary of the outcome of these studies.

Table 4.1 Summary of my experience using the spectrometer and fetal probe on delivery suite at UCL.

No of women approached	63
No(%) of women consented	49 (78)
No of fetuses to which the probe was not applied	20
-Reasons	
- Probe in use	10
-Technical malfunction	5
-Patient request	5
No of fetuses to which the probe was applied	29
Failure in data collection	11
Timing mechanism	3
No light source	3
PMT failure	5
No of fetuses in which data was collected	18
Total time (minutes(range))	2280(16-440)
No of uterine contractions	97
No of fetuses(contractions) with absolute Hb calculated	10 (60)

4.1 THE FETAL PROBE

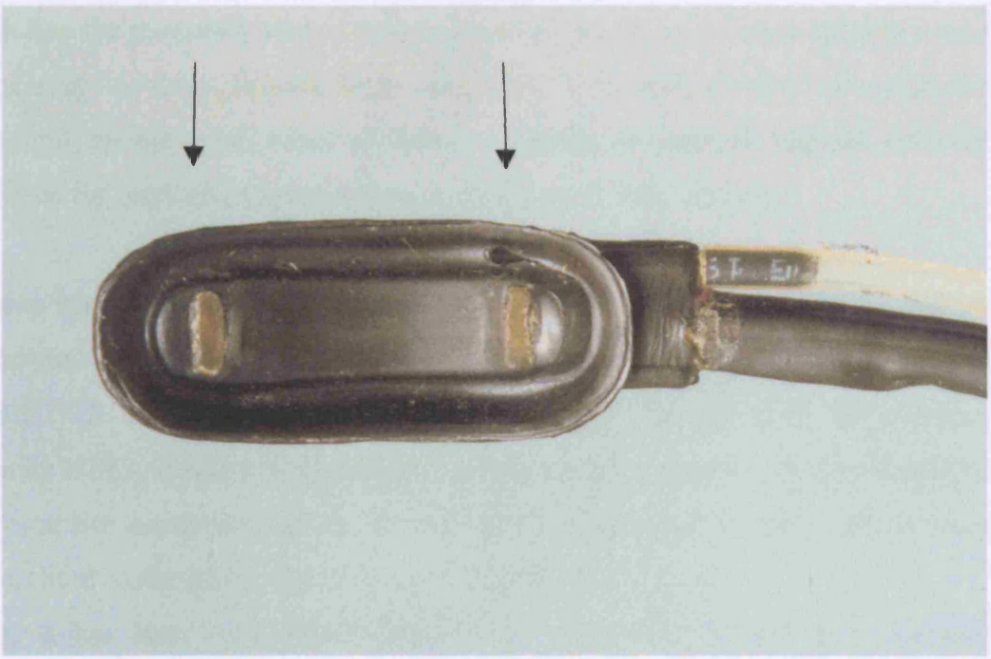
The design of the fetal probe was based on those used previously for NIRS intrapartum fetal monitoring at UCL by Peebles and Aldrich as they were simple to use, effective and not associated with any significant tissue trauma (Peebles et al. 1992).
The probe was designed within the Department of Medical Physics, UCL and manufactured by a commercial company (Gorritt plc, Wales). The probe incorporates glass prisms that are the ends (optodes) of the fibreoptic bundles (external diameter

5mm, length 3m) and suction tubing (3mm diameter). A transmitting optode reflects the light through 90 degrees to pass through fetal head and the light emerging from the head is returned to the PMT in the spectrometer via the receiving optode. The spacing between the optodes was 3.5 cm (see figure 4.1). The four optical fibres that conveyed the light are encased in Kevlar, which in turn was encased in polvinylchloride tubing. This was encased in a heat-shrink sleeve in order to reduce the effect of background light and this sleeve terminated 10 cm from the fetal end of the probe to allow flexibility during application to the fetal head.

4.2 PATIENT CONSENT

Women were approached on the delivery suite at UCL, both before and during established labour, after discussion with their midwife and the medical team on duty. If the woman and her fetus were considered suitable for study, the parents were given additional written and verbal information about the research project. Consent was only sought from mothers who were considered capable of understanding the given explanation and preferably only in the presence of her partner and midwife. This was obtained from each woman prior to commencement of the study using a specially prepared consent form (see appendix 3), which had been approved by the local committee on the ethics of human research at University College London.

a)



b)

Figure 4.1 a) The optodes (shown by arrows) held at fixed separation from one another (3.5cm) in a silicon rubber suction cup and b) the fetal probe in profile.

4.3 STATISTICS

In order to define the proposed normal ranges for absolute values of haemoglobin it was thought necessary to study fetuses from singleton, term pregnancies with cephalic presentation and spontaneous onset of labour, leading to normal vaginal delivery without the need for oxytocin augmentation or evidence of fetal distress.

With a normal vaginal delivery rate of at University College, London of 65% and an augmentation rate of 15% it was estimated that the entry criteria would be met in approximately 50% of this group by the end of labour. On the basis of previous experience with NIRS, it was anticipated that data from 50 successful studies would be required to establish normative values. It was therefore intended to study 100 women who were admitted to the labour ward over the course of two years.
Furthermore, it has been previously discussed in section 2.2.2 that many normal obstetric practices are associated with a transient fall in cerebral oxygenation. On this basis it was anticipated that approximately 25% of the fetuses enrolled in the normal group would experience at least one episode during a study.

Power calculations based on previous data suggest that in order to detect changes in total haemoglobin concentration occurring during intermittent fetal hypoxaemia, with a power of 80% and at a 0.05 significance level, 60 fetuses would be needed to be studied.

It was therefore proposed to study a further 50 labours where the fetus was thought, on clinical grounds, to be at increased risk of intrapartum hypoxia. These therefore would include pregnancies complicated antenatally by intrauterine growth restriction, oligohydramnios, antepartum haemorrhage and diabetes and those with intrapartum complication such as meconium staining of the liquor and/or an abnormal tochograph.

Due to technical delays with fetal probe and the spectrometer (see chapter 7) there was a total of eight months available in which potential intrapartum fetal observations could occur.

4.4 FETAL AND MATERNAL OBSERVATIONS

Despite this, during these eight months sixty-three women were approached to take part in the study. Of these, forty-nine women agreed and gave written informed consent to the study. The recruitment rate was therefore approaching 80%.

The reasons given from the women who declined to take part in the study were that they requested minimal medical interference, others requested water births, while other women thought that the probe would restrict maternal movement. These reasons for failing to recruit women are similar to those previously described by Peebles and Aldrich.

The majority of women approached were Caucasian women as difficulties in obtaining consent from women from ethnic minorities were encountered due to communication difficulties and cultural differences. Similarly, from previous experience with NIRS monitoring women who were primiparous and who had an epidural anaesthesia were studied. These groups of women were of particular interest as potentially they provided more satisfactory data over a longer period of time

Of the forty-nine women who agreed to take part in the study, twenty of the fetuses were not studied due to a failure of labour induction, failure of the membranes to rupture, withdrawal from the study during labour or the probe was in use on another fetus.

For those women who agreed to take part in the study, it was necessary for the membranes to have ruptured before the probe could be applied. Artificial rupture of the membranes was only performed where clinically indicated, not in order to commence measurements. The probe was applied only during routine vaginal examinations by JC and was inserted through the dilated cervix (minimum 2-3 cm) after rupture of the amniotic membranes. This occurred either spontaneously or following artificial rupture for clinical indications. The probe was introduced between the fetal head and cervix and applied against the fetal head and maintained in position by both maternal tissue

pressure (from either lower uterine segment, cervix or vagina) and controlled continuous negative pressure (150 mmHg), provided by the wall suction apparatus.

Once the probe was applied, the optical fibres were connected to the laser output of the spectrometer. A Hewlett Packard CTG monitor was connected to the spectrometer via the external analogue channels and suction tubing was connected to the fetal probe. Data collection was commenced from the time the lasers and the PMT of the spectrometer were switched on by DK. Data were collected at 0.5 second intervals and the computer screen displayed the number of photons being back-scattered to the PMT for all four lasers and the elapsed time in seconds (see figure 2.2). JC or DK recorded events during labour in logbooks with the corresponding elapsed time.

If there was sufficient light being detected by the spectrometer, as assessed by the number of photons detected displayed on the computer screen data collection continued. If there was too much light being detected, the voltage of the PMT was reduced to avoid oversaturation and possible damage to the PMT. If there was no data being recorded due to technical difficulties with the lasers or PMT, the study was abandoned.

Subsequent vaginal examinations, during data collection, were performed by JC. The probe position was checked at each vaginal examination, which occurred every two to four hours. Where feasible, observations were continued until the point of delivery. After completion of a study the raw data obtained was saved on the hard drive of the computer system in the spectrometer along with the CTG data. At the end of the study, the raw data were averaged over 5 seconds intervals and saved on to a 3 1/2-inch floppy disc for subsequent analysis.

On completion of a study, the probe was washed thoroughly in hot water and chlorhexidine 0.1 % solution to remove all organic material, and then sterilised in gluteraldehyde solution for 10 hours within the Medical Physics Department. It was rinsed with sterile water, before being repackaged in a sterile drape. This method for disinfecting the probe was approved by the Department of Microbiology at University College, London.

Of the forty-nine women who gave written consent, the probe was applied to the head of twenty-nine fetuses during the first and second stage of labour (see table 4.2). As previously mentioned the probe was either in use on another fetus or more commonly the spectrometer malfunctioned before the probe could be applied which preventing data being collected in twenty of the women.
Of the 29 fetuses in which the probe was applied, it was not possible to collect data in eleven of these. This was due to unpredictable intermittent technical problems with the spectrometer. Initially this was due to failure of the timing mechanism (n=3) and then in subsequent studies it was due to the spectrometer failing to generate a light source (n=3) and failure of the PMT (n=5).
In eighteen of the twenty-nine fetuses, the individual components of the spectrometer functioned properly and data was collected that could be used for subsequent analysis.

Continuous observations in these eighteen fetuses were obtained for periods lasting from 16 to 440 minutes (mean 127) with a total number of hours of 38. The details of these 18 studies are given in table 4.2.

The probe was applied to the fetal head when the cervix was as little as 3cm dilated. It was then possible to collect data for long periods, extending through the first stage of labour, although due to the inflexibility of the probe, occasionally it would detach during a maternal movement. In two fetuses monitoring was continued through to the point of delivery. None of the mothers found that the actual process of optical monitoring was uncomfortable. Although the technique did not interfere with routine clinical management, the majority of women, their partners, and the attending medical and midwifery staff commented on the space taken up in the delivery room by the spectrometer, DK and JC during a study (see figure 4.2).

Table 4.2. Maternal details and outcome of labour in the 18 studies from which data was collected.

Study details		No.
No of women	Primigravida	11
	Multiparas	7
Race	Caucasian	15
	Afro-Caribbean	3
Gestation (weeks)	Mean(S.D.)	39.7 (+/-1.0)
Analgesia	Epidural	10
	Entonox/pethidine	8
Labour	Spontaneous onset	14
	Induction	4
Mode of delivery	NVD	10
	Instrumental	4
	LSCS	4
Indication for operative delivery		
	Distress	1
	Failure to progress	7
Umbilical pH < 7.2		2
Apgar <7 (1 min)		2
Apgar <7 (5 min)		0
Cervical dilatation at application	3-5 cm	7
	6-9 cm	9
	10 cm	1

There were no intrapartum or postpartum complications related to probe insertion in any of the mothers and no fetal injuries or infections were observed. Only one fetus required an emergency Caesarean section for fetal distress, following an ante-partum

haemorrhage in the first stage of labour. Although the fetus required resuscitation at birth, there was no development of hypoxia-ischaemic encephalopathy.

In the 18 fetuses there were contraction-related changes in light attenuation data, which allowed calculation of changes in Hb and HbO_2. Of these 18 fetuses, there were contraction-related changes in phase shift and modulation depth in 10 of these and therefore the Mathematica™ notebook (see chapter 7) could calculate absolute measurements of Hb and HbO_2. In the other eight fetuses the signal/noise ratio in the phase and modulation depth data was too low. This was thought to be because insufficient light was detected arising from either the presence of dark curly hair under the probe. Melanin significantly reduces the amount of near infrared light transmitted through tissue because of it's high attenuation. Tissue with a high melanin content, such as black skin or hair will therefore effectively screen out transmitted light. It is likely that this was the explanation for the noisy signal obtained from these fetuses, although it is not known in how many successful cases black hair was also present. This was also the reason given for failing to obtain a signal using conventional NIRS (Peebles 1993). Occasionally this problem was overcome by practical measures such as sweeping the hair aside, by moving the optodes across the fetal scalp, or positioning them over an area with less hair. If data was not obtained after a maximum of two attempts at positioning the probe, the study was abandoned.

The maternal and labour details of the 10 fetuses are shown in Table 4.3. There were no fetal blood samples performed during observations. In all ten fetuses, the cord pHs were above 7.2 and there were no admissions to the neonatal intensive care unit.
Data were obtained during a total number of 97 uterine contractions with related changes in the three signals. Of these, 60 contractions the Mathematica programme calculated absolute absorption coefficients within the range of biological tissue at more than one laser wavelength which used to calculate measurements of absolute concentrations of Hb and HbO_2.

Table 4.3. Details of labour and delivery in the 10 fetuses in which contraction related data in phase, modulation depth were obtained

Maternal age	34 (22-42)
Gestation	39 (37-41).
Race	
Caucasian	9
Afro-Caribbean	1
Parity	
Primiparous	7
Multiparous	3
Onset of labour	
Spontaneous	9
Induced	1
Epidural	5
Mode of delivery	
Normal vaginal	5
Instrumental	2
Caesarean	3
Cord pH < 7.2	0

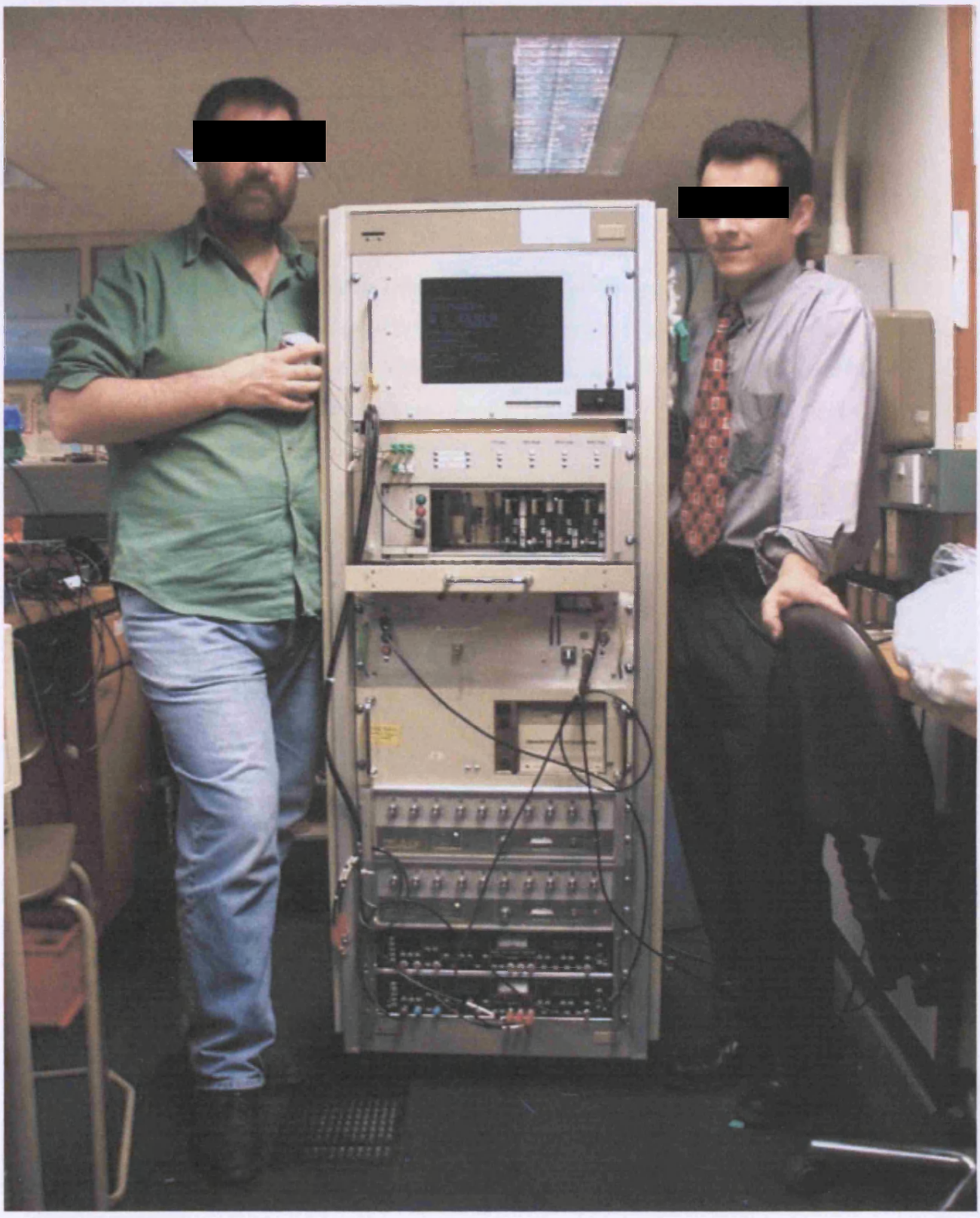


Figure 4.2 Photograph to illustrate the dimensions of the spectrometer with JC and DK.

4.5 DISCUSSION

The total number of fetuses studied and the number in which satisfactory data were collected using IMOS were relatively small, compared with previous experience at UCL using commercially available NIRS monitors.

Peebles designed and manufactured a fetal probe and applied this to 40 fetuses. Peebles, using a similar probe to the one used in this study, investigated fetuses with a success rate of approximately 75% (Peebles 1993). Following on from Peebles, Aldrich studied a total of 75 fetuses and obtained satisfactory data in 68 (90%) fetuses (Aldrich 1994c). Watson et al (1996) studied the changes in the optical pathlength during uterine contractions with IMOS. Data was satisfactory for analysis in four of the 10 fetuses studied. Insufficient light detected has been cited as the main reason for obtaining poor quality data with NIR intrapartum monitoring and is usually due to the presence of thick black hair and skin underneath the probe.

In this study the recruitment rate was relatively high and primiparous Caucasian women were particularly targeted to in an attempt to prolong the period of observation and reduce the problem of black hair. However technical problems with the spectrometer and probe reduced the number of satisfactory fetal studies. These problems reflected the fragility of the individual components of the instruments. Usually, these problems were not resolvable at the time of the study and therefore the studies were abandoned. Despite these practical problems, overall raw data were obtained during labour with IMOS in approximately 62% of women studied and of these, 55% provided data suitable for analysis.

CHAPTER 5

INTRAPARTUM MEASUREMENT OF FETAL CEREBRAL HAEMOGLOBIN CONCENTRATION AND OXYGEN SATURATION WITH IMOS.

INTRODUCTION

As discussed in chapter 1, direct information on cerebral haemodynamics during uterine contractions (Peebles 1992a), fetal heart rate decelerations (Aldrich 1995a) and obstetric manoeuvres (Aldrich 1994b) have been reported using the conventional NIRS monitoring system at UCL.

However, interpretation of the results is hampered by the fact that the technique used to date provides information about changes in the concentration of HbO_2 and Hb calculated from an arbitrary baseline set at the beginning of the study. Thus it is only possible to obtain relative rather than absolute quantification of total haemoglobin and therefore cerebral blood volume.

A method has been described for measurement of cerebral saturation (Peebles 1992), but this relies on parallel changes in HbO_2 and Hb, which do not always occur. This problem reduces the clinical value of NIRS as it is not currently possible to quantify cerebral blood volume, and therefore the degree of cerebral vasodilatation, at the beginning of the study. On the basis that the initial fetal response to acute hypoxia is cerebral vasodilatation and an increase in cerebral blood flow, knowledge of absolute blood volume might help distinguish the normoxic from the hypoxic fetus.

This chapter therefore describes the studies undertaken using IMOS to define normal values for fetal cerebral total haemoglobin concentration (HbT) and mean cerebral oxygen saturation ($SmcO_2$) during the first stage of labour.

5.1 METHODS

Twenty-nine pregnant women were recruited into the study during uncomplicated labour at term, the details of which are described in chapter 4. All had a singleton cephalic presentation and were studied after either spontaneous or artificial rupture of membranes.

During routine vaginal examination the silicon rubber probe, especially designed to hold two optic fibre bundles at a constant separation of 3.5cm, was inserted through the cervix after 3cm dilatation and placed against the side of the fetal head.
This separation was used as it was the maximum size possible because of the sheathing and the prism size and Peebles (1993) found this to be the optimal distance for obtaining fetal data.
The standard, unmodified wall suction apparatus provided controlled, continuous negative pressure (150 mmHg below atmospheric) to maintain contact between the probe and the head. This level of suction was used to maintain good probe apposition was similar to that used in other studies (Peebles 1993, Aldrich 1994c) where no evidence of fetal trauma was observed. The fetal heart rate and uterine contractions were monitored continuously using a cardiotocograph (Hewlett Packard 80300A, Hewlett Packard, Massachusetts, USA).

Off-line evaluation of the raw IMOS data was performed using the Mathematica™ notebook. This produced changes in **Attenuation, phase and modulation depth** at the four different wavelengths.
First, changes in light attenuation during the course of a study were used to calculate changes in cerebral Hb and HbO_2 concentration using a similar method to those previously described (Peebles 1992).

In addition, the novel analytic method was used to quantify absolute tissue absorption coefficients from the ratio of any two of the changes in A,P, and M. These were then be used to calculate absolute haemoglobin concentrations (μmol/l).

Data were analysed from the first three uterine contractions for each fetus that fulfilled the following criteria: a) tocographic evidence of uterine activity associated with changes in optical parameters, b) occurring during the first stage of labour, c) a normal FHR pattern d) and no gross change in maternal posture, and e) no maternal oxygen therapy or drug administration.

Summing of Hb and HbO_2 provides the total haemoglobin concentration (HbT in umol/l), and the ratio of HbO_2/ HbT provides the cerebral saturation ($SmcO_2$) expressed as a percentage (%).
Statistical analysis was performed on commercially available software (Sigmaplot V4.0. SPSS Inc.1997).

An estimate of Cerebral Blood Volume (mls/100g) was derived using the following equation:

$$CBV = HbT \times MW_{Hb} \times 10^{-6} / tHb \times 10^{-2} \times D_t \times 10$$

where MW_{Hb} is the molecular weight of haemoglobin (64500), tHb is the mean umbilical vein Hb concentration (16.8g/dl) (Wintrobe 1997) and D_t is the brain tissue density (1.05g/ml).

RESULTS

Figures 5.1 and 5.2 illustrate the method of deriving absolute concentrations of Hb and HbO_2 from the contraction-related attenuation and phase data using the Mathematica™ programme. Figure 5.1 shows typical changes, from one fetus, in attenuation and phase shift during a contraction. If change in attenuation at one wavelength (856nm), measured between the start and peak of a uterine contraction, is plotted against a

corresponding change in phase shift (shown in Figure 5.2), the gradient of the first order regression line (Q, 7.84 OD/RAD) can be calculated, giving an absolute absorption coefficient (μ_a) of 0.018 mm^{-1}. Using the same method, the absolute μ_a calculated at wavelength 787nm was 0.016 mm^{-1}. Using these calculated absolute μ_as it was possible to calculate absolute concentrations of Hb and HbO_2, using previously measured extinction coefficients for Hb and HbO_2, and these were 42 and 26 μmol/l respectively.

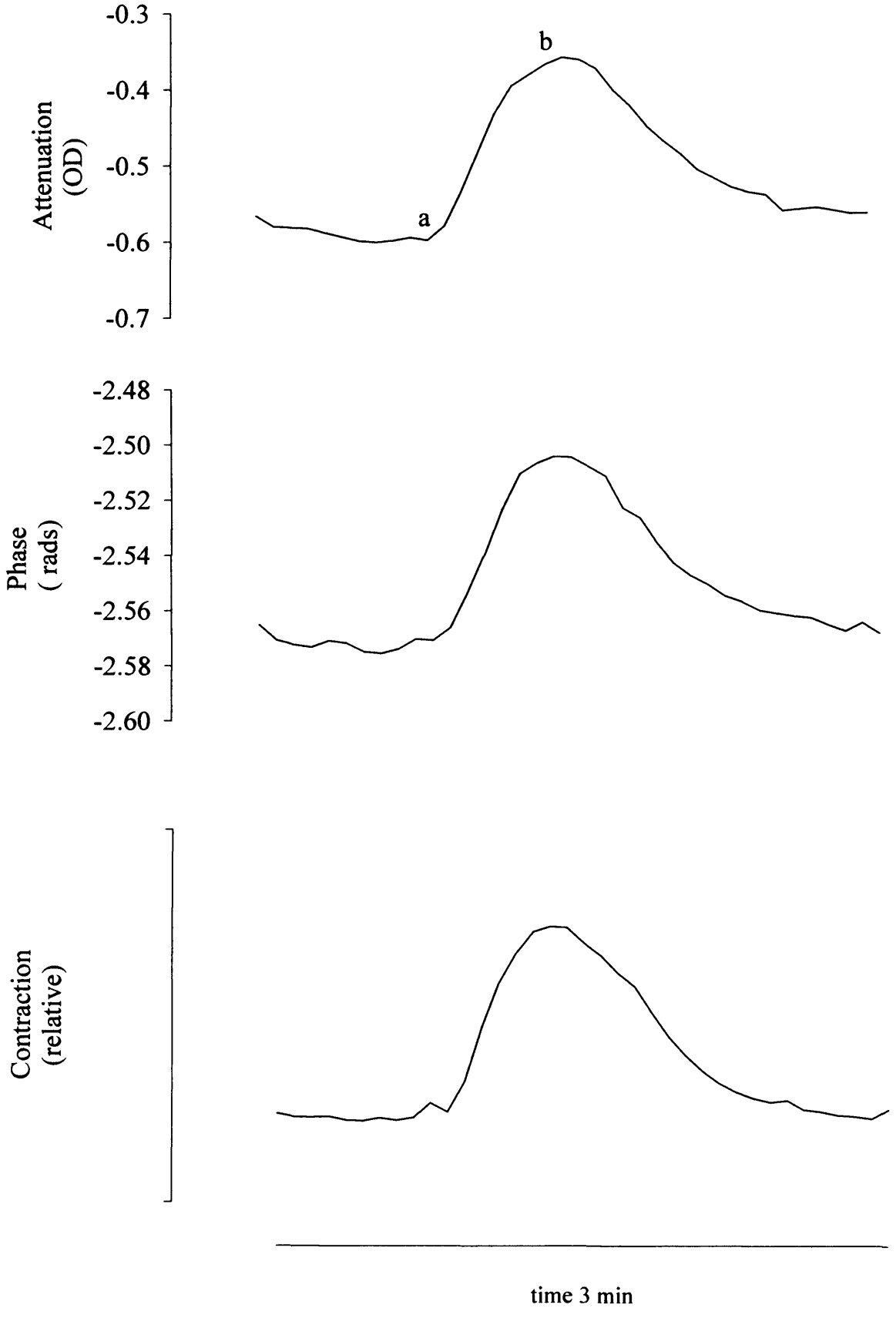


Figure 5.1 Changes in attenuation and phase at 841nm over one uterine contraction in a fetal study.

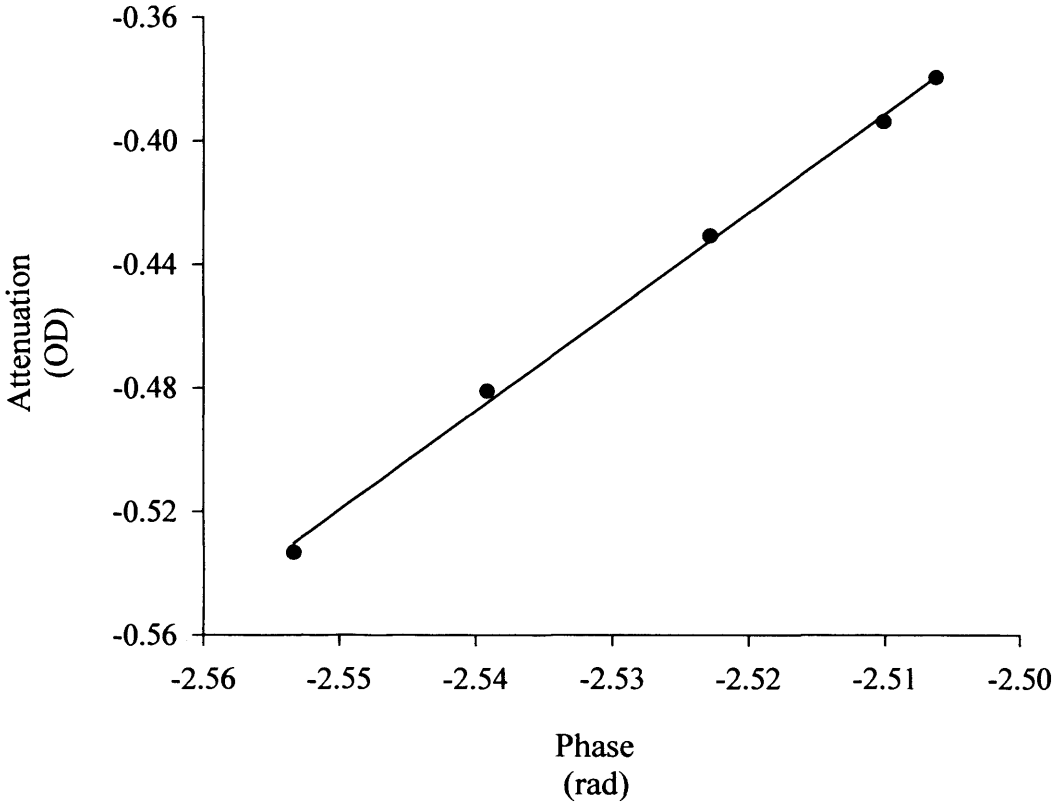


Figure 5.2 Scatter plot and first order regression line obtained from the changes in attenuation and phase during the start (point a) and peak of the contraction (point b) in fig 5.1.

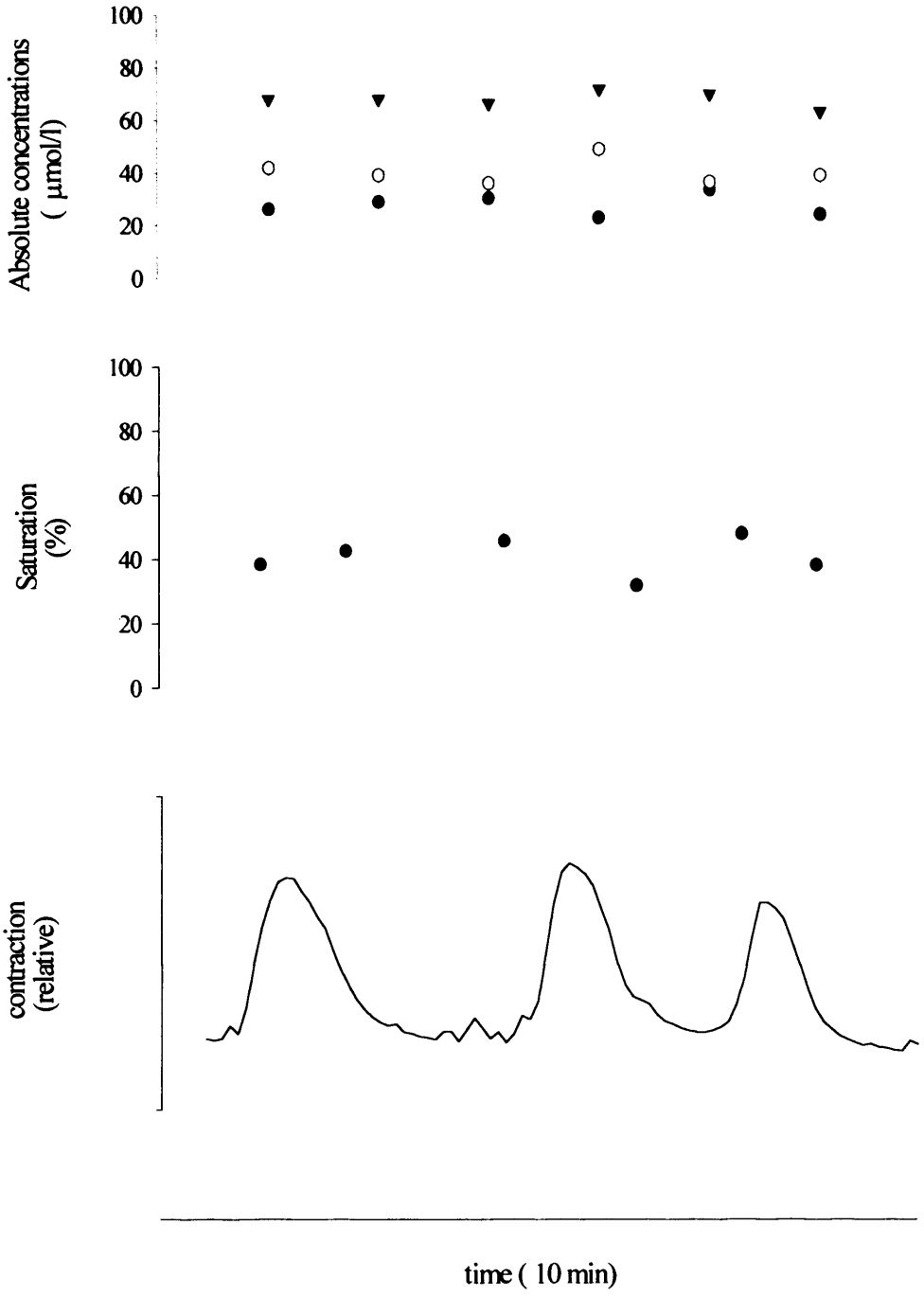


Figure 5.3. Absolute concentrations of cerebral Hb (open circles), HbO_2 (closed circles) and HbT (closed triangles) (umol/l), and cerebral saturation, calculated over 3 uterine contractions in the same fetus are shown in Figure 5.1 and 5.2.

Absolute concentrations of Hb and HbO_2 were obtained during the up- and down-slopes during three consecutive contractions (see Figure 5.3). These gave a mean value of absolute concentrations of cerebral Hb and HbO_2 of 40 +/-4μmol/l and 28 +/-4 (mean ± SD) respectively, giving a mean absolute total haemoglobin of 68 +/-3 μmol/l and $SmCO_2$ of 41 +/-5%.

Using the same methods on the data from the 10 fetuses, the mean +/-SD absolute concentration of Hb and HbO_2 calculated were 30 +/-18 and 46 +/-21 μmol/l, respectively. This gave a mean cerebral HbT of 77 +/-29 μmol/l and a mean $SmcO_2$ of 59 +/-12 %. Assuming a large vessel mean haemoglobin concentration for the term fetus of 16.8g/dl the calculated mean HbT concentration equates to a mean fetal cerebral blood volume of 2.8 +/-1.0 mls / 100g.

Table 5.1 Data from the individual fetuses (n=10).

Study	HbO_2 (μmol/l)	Hb (μmol/l)	HbT (μmol/l)	$SmcO_2$ (%)
1	28 (4)	40 (4)	68 (3)	41 (5)
2	74 (14)	29 (13)	103 (25)	73 (7)
3	42 (16)	31 (11)	73 (26)	57 (5)
4	88 (42)	53 (24)	141 (48)	62 (16)
5	52 (26)	13 (12)	65 (35)	81 (10)
6	29 (16)	27 (8)	56 (18)	49 (16)
7	47 (21)	45 (37)	93 (56)	57 (23)
8	37 (23)	30 (29)	67 (49)	58 (19)
9	19 (6)	18 (11)	37 (5)	53 (23)
10	44 (22)	22 (12)	66 (31)	67 (7)

Data are expressed as mean +/-1SD. HbO_2 = oxyhaemoglobin. Hb = deoxyhaemoglobin HbT = total haemoglobin. $SmCO_2$ = saturation.

In four fetuses it was possible to calculate $SmcO_2$ over these uterine contractions using the conventional method of analysis, as there were parallel changes in HbO_2 and HbT. This gave a calculated mean $SmcO_2$ of 58 +/-19%. In these four fetuses the mean $SmcO_2$ was 59 +/- 17 % calculated by the novel IMOS analytic method ($p = 0.84$).

Figure 5.4 shows that if absolute measurements of Hb, HbO_2 and HbT concentration are used as a starting point on the continuous data collected by the conventional NIRS method (which starts at arbitrary baseline), absolute measurements of Hb, HbO_2, HbT and cerebral saturation can be represented in a continuous fashion.

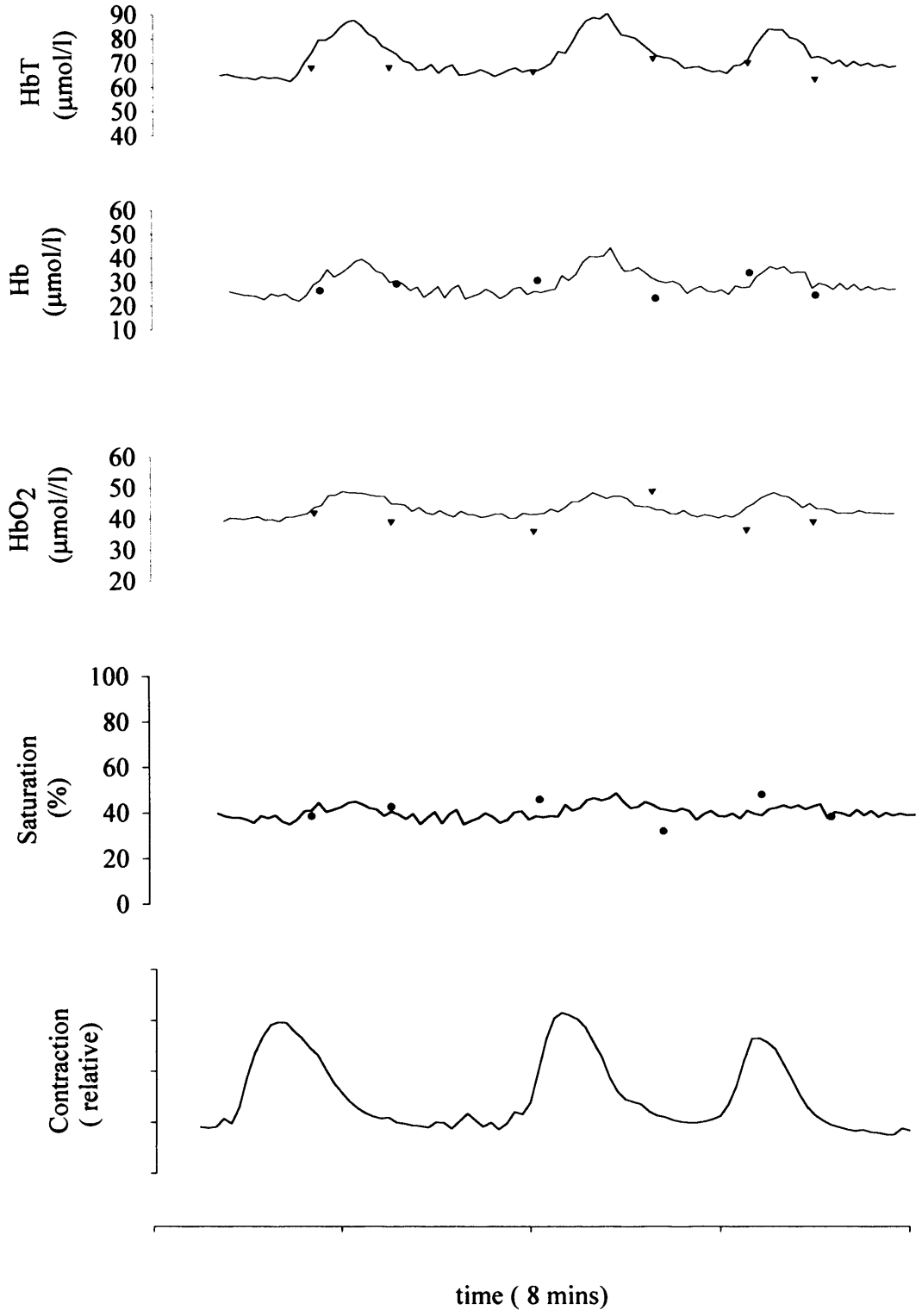


Figure 5.4 Measurements of absolute concentrations of Hb, HbO_2, HbT (µmol/l) and cerebral saturation (%) by IMOS (a) using the novel method of analysis (dots) and (b) using an absolute measurement on the changes in Hb, HbO_2, HbT to provide continuous absolute measurements (line), over 3 contractions from a fetus during labour.

5.3 Measurements during obstetric events

5.3.1 Early deceleration

Of the 10 fetuses that were analysed during the first stage of labour, nine were identified as having a 'normal' cardiotocograph in that they were not associated with a significant change in the fetal heart rate (FHR). The remaining fetus had 6 uterine contractions that were associated with early FHR decelerations. Absolute Hb, HbO_2 and HbT were determined for each uterine contraction during the FHR deceleration and during the preceding and subsequent contraction (see figure 5.5). When the uterine contractions were associated with early FHR decelerations, the mean (S.D.) absolute cerebral HbO_2 fell by 5 (+/- 0.3) μmol/l and absolute Hb rose by 8 (+/- 0.2) μmol/l. Absolute HbT rose by 3μmol/l (+/- 0.3). These results indicate that there was a transient desaturation of the blood within the fetal brain, which occurred simultaneously with the deceleration in FHR. This is confirmed as there was a fall in cerebral saturation of 8% (+/-0.5%).

5.3.2 Fetal bradycardia

In one fetus, it was possible to obtain measurements during a fetal bradycardia secondary to an antepartum haemorrhage (APH) (see fig 5.6). During the fetal bradycardia, absolute HbO_2 fell consistently from 27μmol/l to 9μmol/l. There was an initial fall in absolute Hb from 15umol/l to 12umol/l and then a subsequent rise up to 24μmol/l. Absolute HbT calculated from the sum of HbO_2 and Hb fell from 42μmol/l to 27μmol/l and then rose to 33μmol/l. This corresponded to a fall in CBV from 1.5 to 0.98 mls/100g then a rise to 1.2 mls/100g. Absolute cerebral saturation was initially maintained at 63 and 56% but then dramatically fell to 25%. These data are consistent with an impairment in placental function secondary to the APH. The secondary rise in HbT (and therefore CBV) may be due to impairment in cerebral venous drainage, but it is more likely to be due to vasodilatation of the cerebral vasculature, in an attempt to maintain cerebral blood volume. This suggests that the fetus studied had adequate amount of reserves to avoid the permanent sequelae of ischaemia, in response to increasing fetal hypoxia. The fetus was delivered by emergency caesarean section and although it was born with Apgar scores of 3 at 1 minute and 5 at 8 minutes and the

umbilical artery cord pH was 7.19, after resuscitative measures, the neonate did not require SCBU admission and there was no short or long term neurological deficit.

5.3.3 Effects of delivery

In one fetus it was possible to continue observations on the brain throughout the process of delivery and the initiation of spontaneous respirations. Figure 5.7 shows the changes in Hb, HbO_2 and HbT calculated in the conventional manner (from an arbitrary baseline) and absolute HbT and cerebral saturation (using absolute measurements of Hb and HbO_2 as a starting point). With the onset of respirations, HbO_2 rose rapidly by 6μmol/l and Hb fell by15μmol/l. Simultaneously HbT fell by 11μmol/l (from 55μmol/l to 44μmol/l) corresponding to a fall in CBV of 0.4mls/100g (from 2.0 to 1.6mls/100g) and cerebral saturation rose from 72% to 98%. The fetus was born with normal Apgar scores and umbilical cord gases.

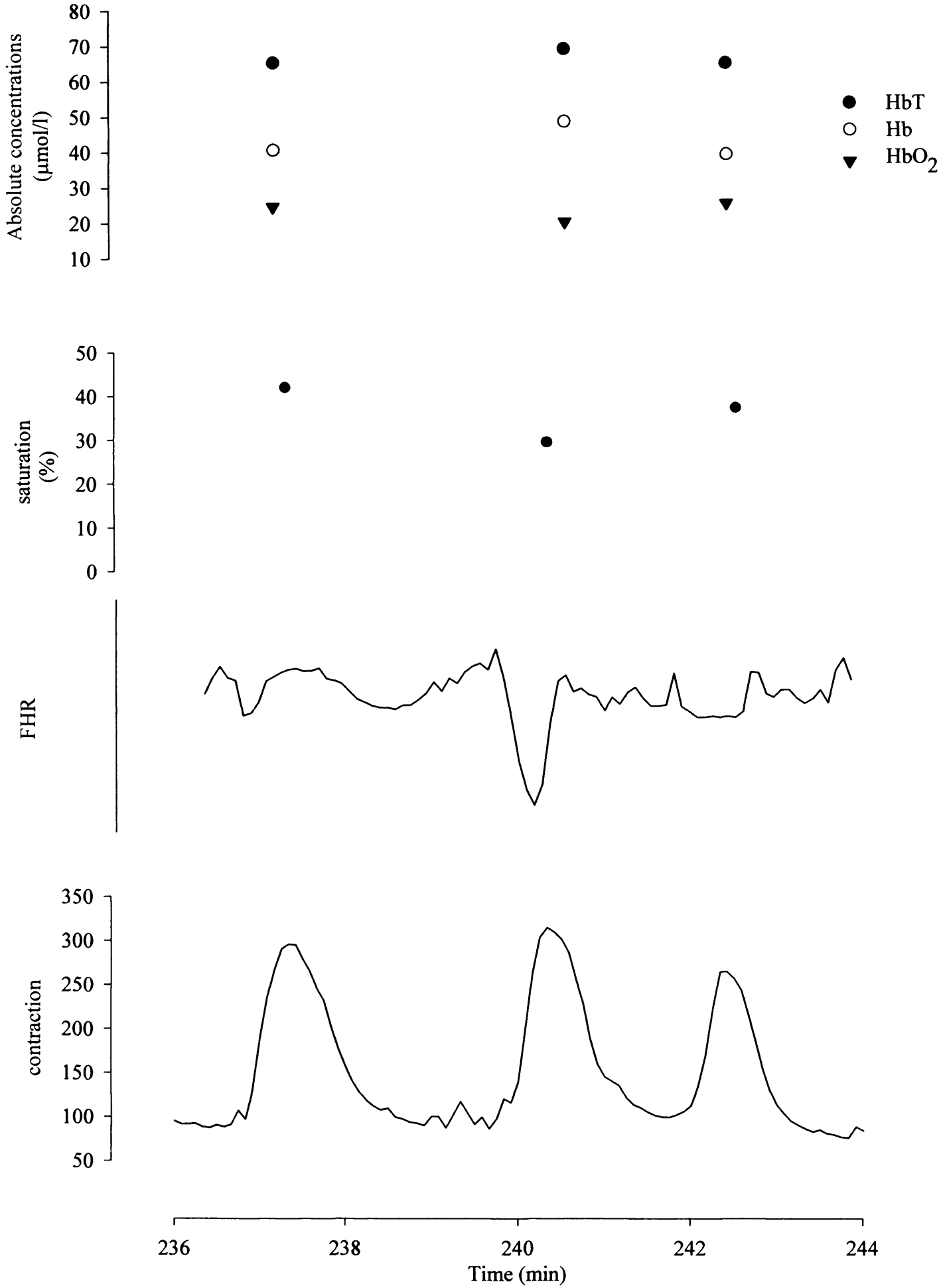


Figure 5.5 Measurements of absolute concentrations of Hb, HbO2, HbT and cerebral saturation during an early FHR deceleration.

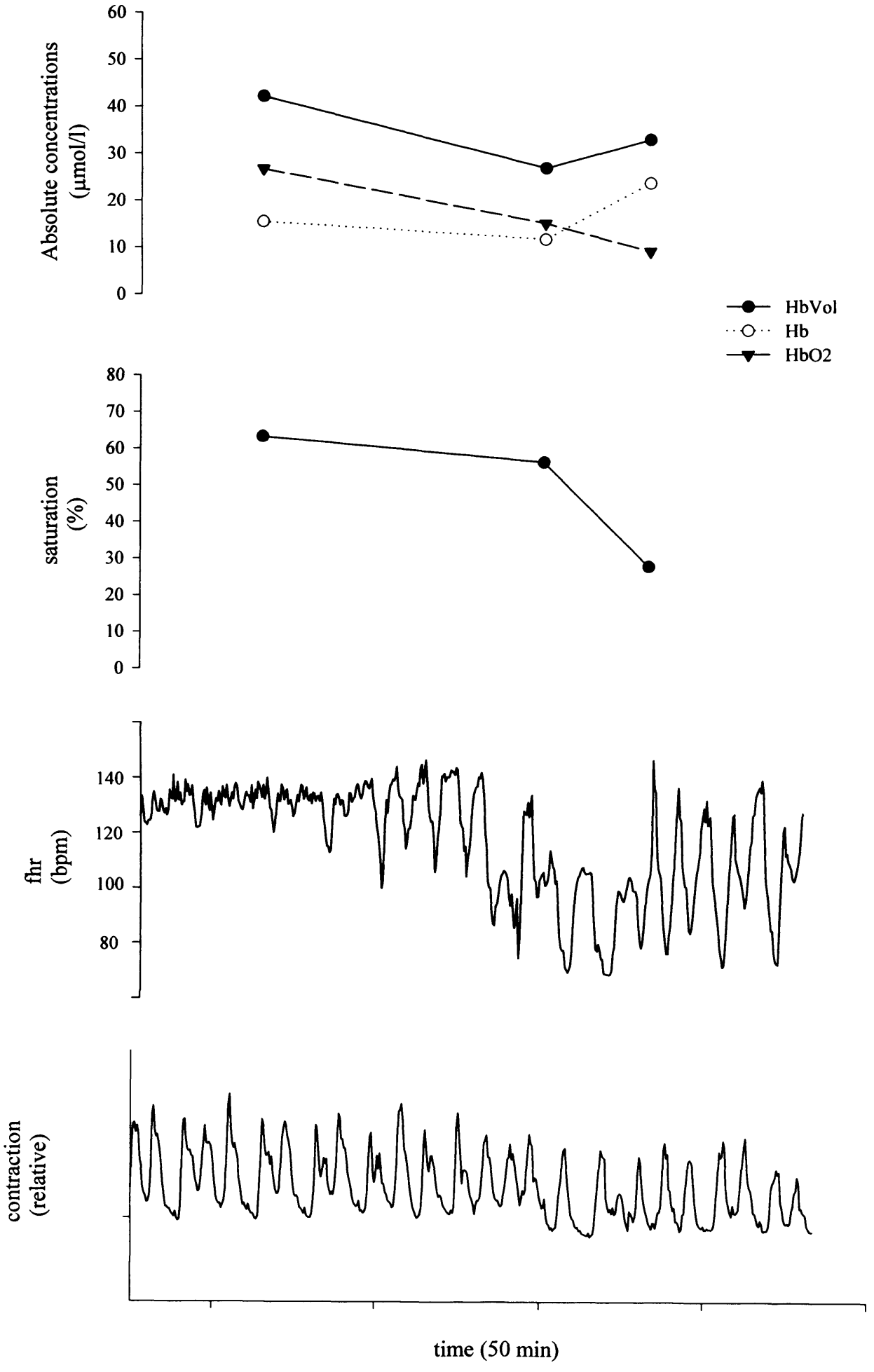


Figure 5.6 Measurements of absolute haemoglobin and cerebral saturation during a fetal bradycardia secondary to an a antepartum haemorrhage

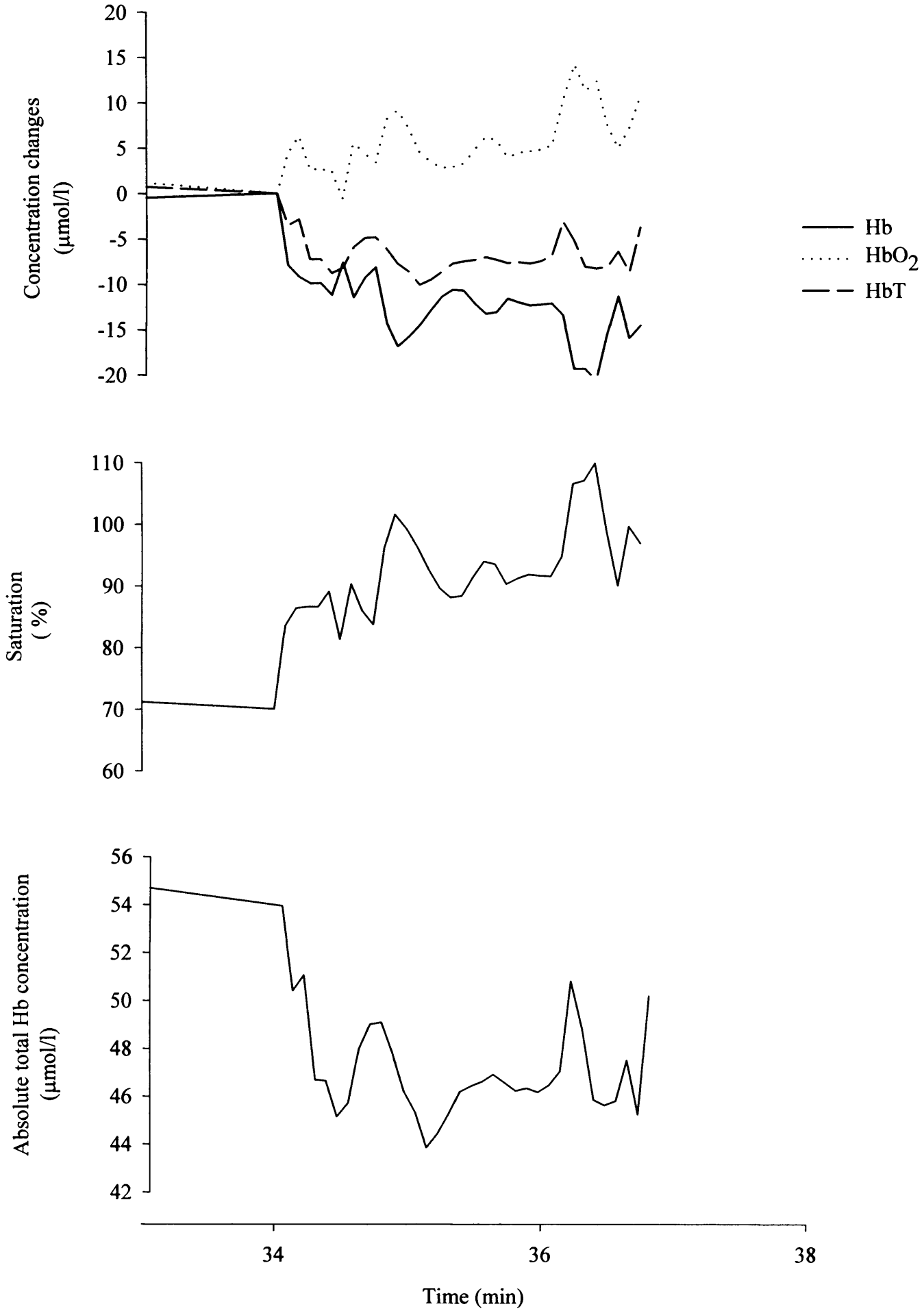


Figure 5.7 Measurements of changes in cerebral Hb and absolute cerebral saturation and HbT during delivery

5.4 Discussion

The data presented in this chapter are the first to describe absolute quantification of oxyhaemoglobin and deoxyhaemoglobin concentrations within the brain of the human fetus during labour. The techniques described in this study allow absolute quantification of cerebral concentrations of total haemoglobin (HbT), by summing Hb and HbO_2, and therefore provide information on fetal cerebral blood volume (CBV) as HbT is related to CBV.

In the absence of any published fetal values the most relevant values with which to compare the CBV measurements reported here are those obtained in the human neonate shortly after birth (Wyatt 1990). These were calculated using parallel changes in peripheral oxygen saturation, measured by pulse oximetry, and cerebral haemoglobin concentration, measured by NIRS, in response to an alteration in the inspired oxygen fraction in ventilated neonates. In neonates with brain injury secondary to hypoxia-ischaemia, cerebral blood volume was found to be higher than in those with normal brains (3.00 ± 1.04 and 2.22 ± 0.4 mls/100g of brain tissue, respectively). The value calculated during the first stage of labour in this study is closer to that seen in neonates post hypoxia-ischaemia than in those with normal brains and suggests that normal fetal values may be higher than normal neonatal values.

There are several possible explanations for a raised CBV during the first stage of labour. Mean fetal PO_2 is known to fall during normal labour and baseline fetal levels are lower than those in the neonate. In animal studies hypoxia leads to a redistribution of cardiac output with an increase in adrenal, cardiac and cerebral blood flow and a fall in blood flow to carcass muscle, gut and kidneys. Similar changes have been noted in the human fetus during labour with an increase in middle cerebral artery blood flow, measured by Doppler, in fetuses with a low peripheral oxygen saturation (<30%) measured by pulse oximetry (Sutterlin 1999).

Another mechanism that could increase CBV during labour is hypercapnia, a potent vasodilator of the cerebral circulation and a decrease in venous return to the heart because of thoracic compression during uterine contractions.

The observations during delivery indicate the magnitude and rapidity of the changes in cerebral oxygenation and perfusion during delivery. These changes indicate a rapid rise in cerebral oxygenation, reflecting the rise in arterial saturation. A fall in CBV of 0.4 mls/100g is approximately 20% if the previously published mean value of 2.22 ml/100g for normal neonates is used (Wyatt 1990). There are several reasons why this may occur. A change in thoracic pressure could cause blood to withdrawn from the extremities, including the brain, and therefore a fall in CBV. This effect would probably occur rapidly, during the first few seconds after delivery. A more gradual change may occur as the result of changing blood gases. Both hypoxia and hypercapnia are potent vasodilators of the cerebral circulation. The data presented here show that show that cerebral saturation increases rapidly after birth and it is reasonable to suppose that $PaCO_2$ will fall with the onset of respiration. These changes will lead to vasoconstriction and a fall in CBV.

The ability to quantify the cerebral concentrations of HbO_2 and Hb using these techniques also make it possible to calculate cerebral oxygen saturation. Previous studies using NIRS have described values for $SmcO_2$, calculated by defining the fraction of a measured change in HbT due to a change in HbO_2. This assumes no change in oxygen saturation during measurements and requires that both Hb and HbO_2 change in parallel. As these conditions are not always met the use of this technique is limited. Given values ranged between 43 and 47% (Peebles 1992b, Aldrich 1994a). Although these are slightly lower than the mean value given here, obtained by IMOS, the standard errors are large. In the four fetuses in which it was possible to use both methods of measuring $SmcO_2$ there was almost complete agreement between values. It should be repeated that the saturation values given are for haemoglobin within all three vascular compartments and so should lie between expected arterial and venous values. Certainly they are higher than mean arteriolar saturation measured by pulse oximetry which is 42.2 % (Goffinet 1997).

The transient fall in cerebral saturation in response to FHR decelerations is consistent previously published data (Peebles 1992) using conventional NIRS that are described in section 2.2.2.1. Similarly, the changes that occurred in cerebral oxygenation and

perfusion in response to a fetal bradycardia are consistent with data during inadvertent hyperstimulation of the uterus with oxytocin (Peebles 1991). In both of these cases it the IMOS system allowed calculation of absolute oxy- and deoxyhaemoglobin from which absolute changes in cerebral saturation and CBV were derived.

There is a wide variation in the observed values in the 10 fetuses as shown in table 5.1. It is possible that this reflects the complexities that occur within the different vascular compartments within the fetal brain during labour that potentially could have an effect on the sensitivity of the IMOS system and analytic process. Watson (1996) has also reported the sensitive nature of IMOS during fetal studies.

These data suggest that IMOS has the potential to provide the first continuous measurements of both fetal cerebral blood volume and saturation during labour. Of the two parameters it is likely that changes in CBV, which reflects vascular tone, will be the best predictor of potential neurological damage occurring due to intrapartum hypoxia. As mentioned previously, hypoxia is associated with cerebral vasodilatation in both human and animal studies, which leads to an increase in both cerebral blood flow and volume. It is thought that this is a compensatory mechanism that maintains cerebral oxygen and substrate delivery. It is only during severe oxygen deprivation that cerebral perfusion falls (Bennett 1998), endogenous protective mechanisms are overwhelmed and brain injury ensues. Certainly, in the ovine fetus the severity of brain injury correlates better with the degree of hypotension and cerebral hypoperfusion than oxygenation (Gunn 1992). This may be why intrapartum pulse oximetry, which measures peripheral arteriolar saturation, but provides no direct information on cerebral perfusion, has yet to show a beneficial effect on either the rate of obstetric intervention or neurological outcome. Similarly, the clinical use of fetal ECG waveform analysis, which provides no direct information on cerebral oxygenation or perfusion, may be limited (Strachan 2000). In contrast the data presented here suggest that using IMOS it may be possible to assess both the degree of fetal cerebral hypoxia and, more importantly, the haemodynamic response to it.

CHAPTER 6

THE CONTRIBUTION OF ARTEFACT TO NIRS MEASUREMENTS.

INTRODUCTION

Having obtained data from a dead fetus during labour using a conventional NIRS monitor and a transparent fetal probe which relied on pressure from maternal tissue alone, Hamilton (1995) was able to reproduce similar results to Peebles (1992b). This data questioned the validity of conventional NIRS measurements. Of a total of 70 contractions that were suitable for analysis, they found that in 28 there were similar changes in HbO_2 and Hb that had previously been described in viable fetuses (Peebles 1992) and illustrated in chapter 2 of this thesis. From these changes it was possible to calculate a mean cerebral saturation of 71.9%. Hamilton argued that because there was no possibility of a change in cerebral oxygenation in a dead fetus, the apparent concentration changes recorded by the NIRS monitor were likely to be a result of changes in the geometry in the fetal probe, which were occurring in direct response to the pressure exerted during a uterine contraction.

Theoretically this may be possible. Conventional NIRS monitors (used by Peebles and Hamilton) have been programmed such that in the analysis of data, which is based on the modification of the Beer-Lambert Law (see chapter 2), the space between the optodes (or interoptode spacing (IOS)) and hence the optical pathlength is assumed to be constant.

If there were changes that occurred in the IOS during the uterine contractions, this would have led to a change in optical absorption and thus wrongly interpreted as changes in chromophore concentration by the conventional NIRS monitors. This could theoretically account for some or all of the contraction-related changes in concentration of oxy- and deoxyhaemoglobin documented by Peebles.
Although Peebles used optodes that were held securely at a fixed separation from one another within a black silicone moulding, the possibility remains that small changes in optode position during uterine contractions may alter the interoptode space and hence lead to erroneous calculation of changes in fetal cerebral haemoglobin concentrations.

IMOS has the unique ability to provide more information on changes in the optical signal compared with the conventional NIRS monitors. This is because in addition to measuring attenuation changes, which enables calculation of changes in haemoglobin, IMOS measures the changes in phase of the optical signal. If changes in phase are measured it is possible to calculate changes in optical pathlength (Δd), which is directly related to the IOS. Phase and optical pathlength are inversely related by the following equation (Kohl 1996).

$$\Delta d = - \Delta \Phi c / 2\pi n \nu_m.$$

where $\Delta \Phi$ is the change in phase of a lightwave, intensity modulated at a frequency ν_M (200MHz) c is speed of light in a vacuum and n is the refractive index of tissue (1.4)

To replicate biological tissues such as the fetal brain, optical phantoms have been developed. A s previously described in chapter 3, these can be designed so that they have similar optical properties to biological tissue and changes that occur in the optical signal can be manipulated in a controlled manner. It was therefore thought by using a specially designed optical phantom, it was possible to provide information on the changes that occurred in the optical signal during the previously described fetal studies.

The study in this chapter therefore had two aims (a) to identify changes in the optical signal (attenuation and phase), using an optical phantom, in response to controlled

changes in IOS and chromophore concentration and (b) to compare these to the data observed in response to uterine contractions during intrapartum fetal monitoring. It was hoped that this would therefore provide information on the contribution of IOS changes, and hence 'artefact' to near infrared fetal studies.

MATERIAL AND METHODS

To represent the fetal brain, the optical phantom employed in this study consisted of Opaque Polyester Pigment Super White 407-220 liquid held within an electronic component box (dimensions 114 x 89 x 51 mm,). Within this liquid the ends of two optical fibres (optodes) were placed 25mm apart just under the surface (Figure 6.1). The ends of the optodes were held securely in specially designed vices, whose separation could be measured and adjusted manually. The optical fibres were connected to the spectrometer.

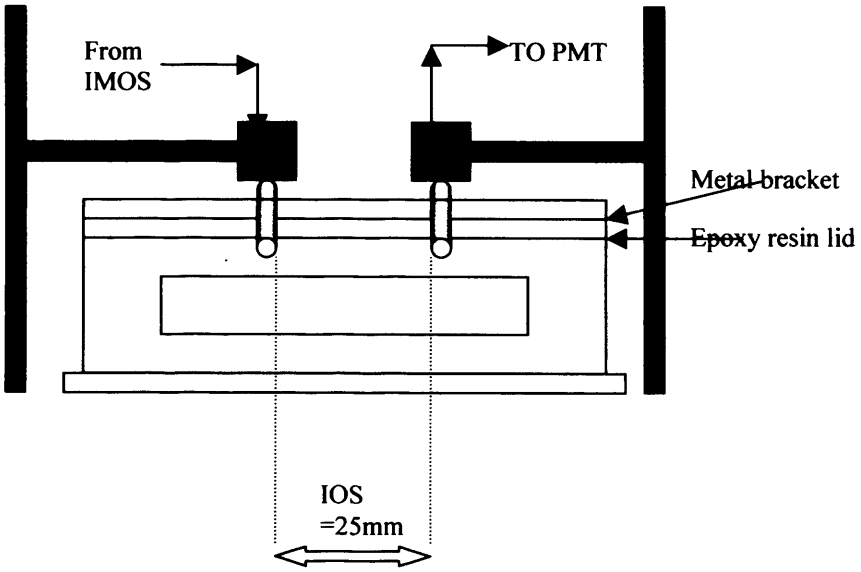


Figure 6.1 Diagram of the experimental set up to change IOS and absorption coefficient in an optical phantom.

In order to represent changes in chromophore concentration, such as haemoglobin in biological tissue, the container had a small hole on its superior aspect, which allowed the addition of light- absorbing liquids. The liquid used in this study was Zeneca Pro Jet 900NP. At two-minute intervals, 10 microlitres of dye were added to the optical phantom, via a micropipette to increase the absorption properties of the optical phantom. A total of 100 microlitres was added to the phantom.

To represent changes that may occur due to pressure on the fetal probe during uterine contractions the distance between the optodes was increased by 10mm over 30 seconds (n=11). This distance was then decreased back to the original starting point. These changes in the IOS were made in between the additions of the dye.

The changes in attenuation, phase and modulation depth data were collected by the spectrometer and analysed using the Mathematica^{TM} programme previously described in chapter 3. These data were then compared with the data collected from the ten fetuses described in chapter 5 and data collected during a routine vaginal examination during which the position and the application of the fetal probe was checked by JC.

Results

Qualitative

Figure 6.2 shows the effect on the attenuation and phase data of increasing the concentration of the dye within the optical phantom and the effect of changing the interoptode spacing at wavelength 856nm. Similar effects were found at all laser wavelengths.

It can be seen that increasing the concentration of dye resulted in an *increase* in both attenuation and phase (and therefore a decrease in optical pathlength, from equation 6.1). The small peaks seen are as a result of mixing the dye within the solution. Conversely, increasing the IOS resulted in an increase in attenuation and a *decrease* in phase. The changes from increases in concentration are in the same direction as figures, 6.3 and 6.4, which illustrate typical changes in the data, obtained from fetuses during a uterine contraction. Figure 6.5 illustrates the changes in attenuation and phase during a

vaginal examination in which the fetal probe was palpated to check its correct position and application. It can be seen that the changes in attenuation and phase are in the same direction as the changes that occur during a change in IOS.
Table 6.1 provides a qualitative summary of the data obtained.

Table 6.1 Qualitative representation of the changes in optical parameters during the described physical changes.

	attenuation	phase	pathlength
↑ dye concentration	↑	↑	↓
↓IOS	↓	↑	↓
uterine contraction	↑	↑	↓
Vaginal exam	↓	↑	↓

Quantitative
Table 6.2 shows the mean (+/-S.D.) changes in attenuation, phase and optical pathlength calculated during this study and compared with the changes during uterine contractions from the 10 fetuses.

Table 6.2 Qualitative representation of the changes in optical parameters during the described physical changes.

	attenuation	Phase	optical pathlength

	(OD)	(RADS)	(mm)
dye concentration	0.071 (+/-0.028)	0.02 (+/- 0.003)	3.4 (+/-0.05)
IOS (10mm change)	1.7 (+/- 0.08)	0.51 (+/-0.04)	85 (+/-0.7)
Uterine contraction in 10 fetuses	0.49 (+/- 0.027)	0.06 (+/-. 0.002)	10.2 (+/- 0.032)

It can be deduced from the data in table 6.2 that a 1mm increase in IOS would lead to an increase in attenuation of 0.17 OD and a mean *decrease* in phase of 0.051 RADS with a corresponding increase in pathlength of 8.5mm. These figures are of the same magnitude as those obtained from the fetuses during uterine contractions however the pathlength change is in the opposite direction.

Figure 6.6 illustrates what happens if the Mathematica software programmed to calculate changes in total haemoglobin concentration, such as the conventional NIRS monitors, during the optical phantom study. A mean (+/-SD) increase in HbT of 3.1 (+/-0.03) umol/l during an increase of 10mm in IOS can be calculated from these data, (hence 0.31umol/l during a 1mm increase in IOS).

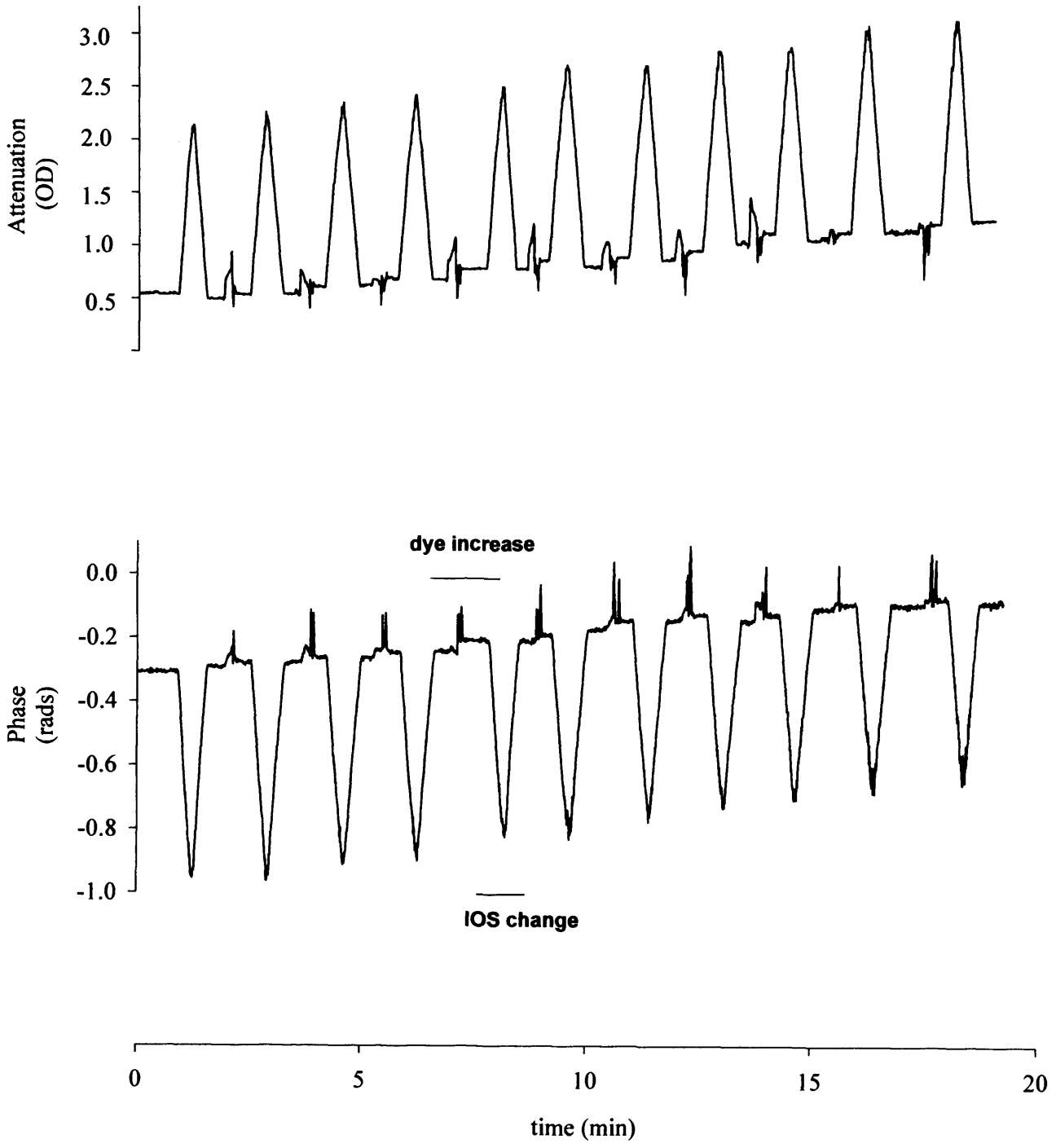


Figure 6.2. The effect of an increase in dye and IOS changes on the attenuation and phase data at wavelength 856nm.

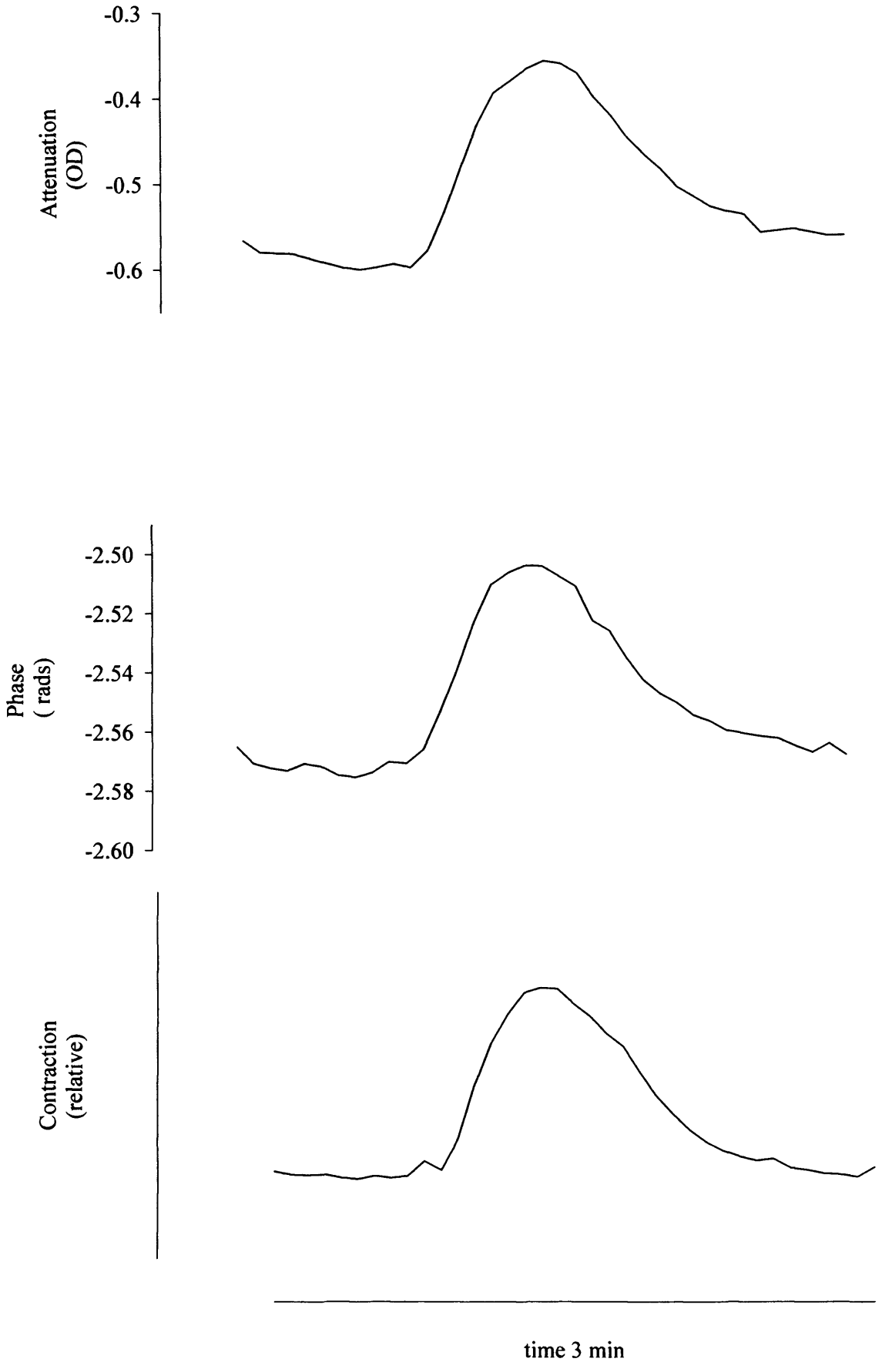


Figure 6.3. Data obtained from a fetus using IMOS showing an increase in both attenuation and phase in response to a uterine contraction.

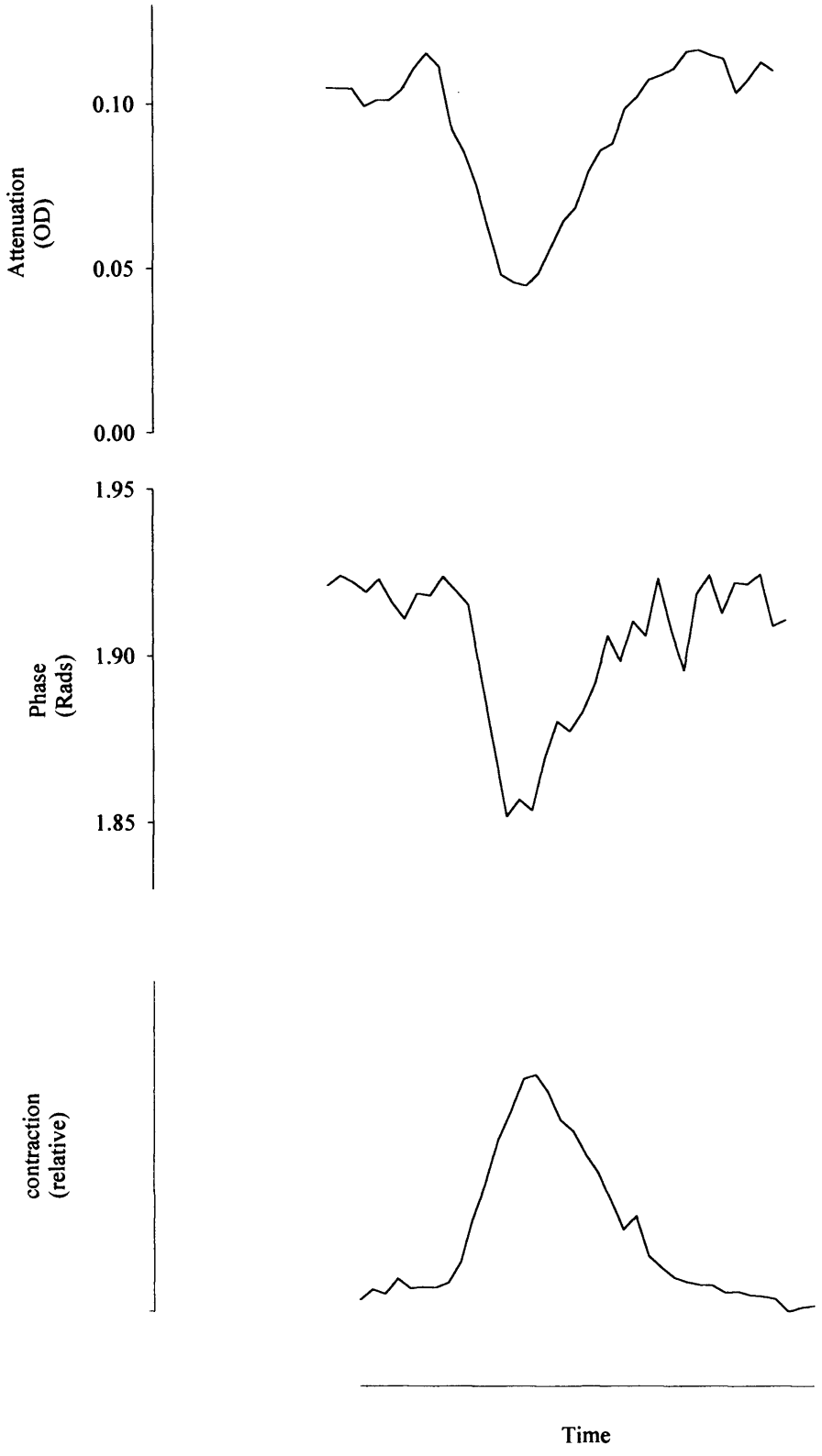


Figure 6.4 Data obtained from a fetus using IMOS showing a decrease in both attenuation and phase in response to a uterine contraction.

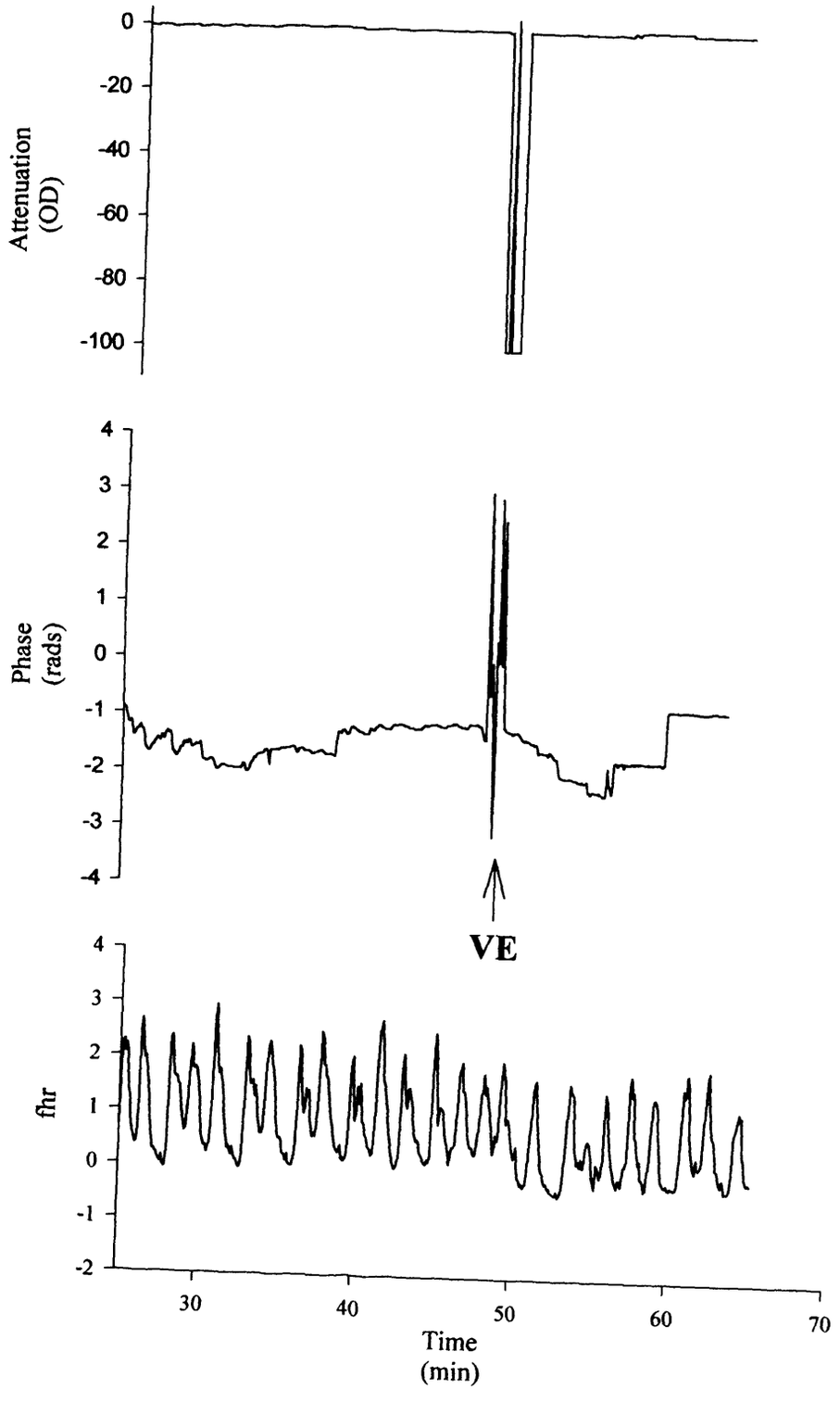


Figure 6.5 Changes in attenuation and phase as a result of a vaginal examination during fetal monitoring.

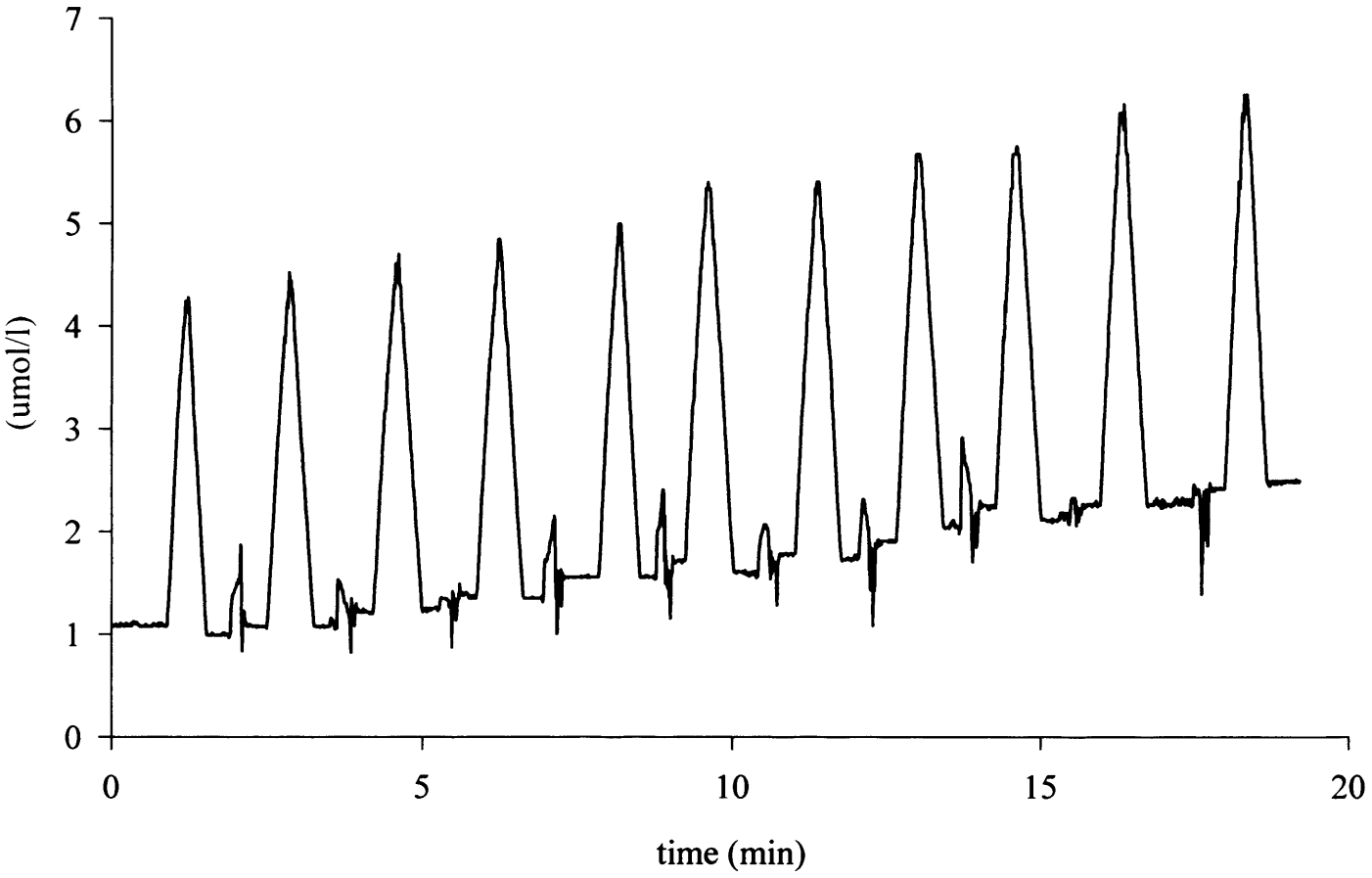


Figure 6.6 The changes in total haemoglobin calculated by the Mathematica software programme for the phantom data.

DISCUSSION

This chapter describes a study to show that IMOS allows calculation of changes in optical pathlength during changes in IOS and changes in chromophore concentration, using media with light-absorbing properties similar to biological tissue. IMOS also allows the display of the raw data from fetuses, so that assessment of the contribution of IOS changes or 'artefact' can be made.

These data show that there are different optical pathlength changes, both in magnitude and direction, in response to changes in IOS and chromophore concentration.

Additionally, the data observed during concentration changes are similar to data derived from human fetuses in response to uterine contractions during labour.

Although, the tissue-simulating medium used can not completely represent the complexities of biological tissue, these data suggest that the observed changes during uterine contractions are dominated by chromophore changes and not by IOS changes.

These data have the potential to describe the argument put forward by Hamilton et al. It is clear that optical pathlength during infrared monitoring *does* change during concentration and IOS changes. However, in viable healthy fetuses concentration changes dominate the changes observed in attenuation. It is likely that in the non-viable fetus studied by Hamilton IOS changes provided the attenuation changes from which the conventional NIRS calculated the haemoglobin concentration changes. It can be seen from this study that even a 1mm change in IOS resulted in the similar magnitude of attenuation change (and calculated haemoglobin) that is similar to that obtained by Hamilton (0.4 umol/l.).

It may possible to use the IOS changes that occur during uterine contractions to provide measurements of haemoglobin concentration. Kohl et al (1997) have used IOS changes to provide measurements of chromophores in tissue-simulating media. However what seems to be important in fetal monitoring as previously described in chapter 1 is the absolute measurement of cerebral oxygenation. It is of more importance that progressive chronic hypoxia during labour is detected rather than the changes that may occur during uterine contraction in cerebral oxygenation.

CHAPTER 7

PRACTICAL PROBLEMS AND FUTURE DEVELOPMENTS

Any direct form of intrapartum fetal monitoring will be affected by limited access, the correct placement of the measuring device and the unpredictability of events that occur in labour. It may therefore be difficult to collect and analyse data from fetuses during the physiological events that occur during the intrapartum period. The problems that were encountered with using the spectrometer and probe used in this thesis and with the subsequent data analysis will be discussed in this chapter.

7.1 DATA COLLECTION

7.1.1 Optode contact

One of the main reasons for failing to acquire data was poor contact between the optodes and the fetal scalp. Any fetal device applied to the fetal head suffers from blind insertion, limited access, and maternal movement affecting the measuring device. Hence, loss of optode contact could occur at any time during a study. Part or the entire probe may become dislodged and this was most frequently observed with rapid descent and/or rotation of the fetal head (especially at the end of the second stage of labour), during difficult vaginal examinations and with rapid changes in maternal position. However, this was sometimes easily recognised by either a break in the suction apparatus (accompanied by amniotic fluid in the tubing) or the display on the spectrometer suggested that too much light was reaching the PMT and oversaturating it. Occasionally the probe became detached completely during a vaginal examination and the probe was simply reapplied. An example of the effect of this on the data is shown in figure 7.1. Large changes in maternal posture especially if they were not predicted were associated with the probe dislodging. The weight and inflexibility of the material

covering the optical fibres probe added to this problem. However, if large changes in posture were predicted then the optical fibres were lifted into a new position. Occasionally it was not possible to maintain adequate suction to the fetal head and the studies were abandoned. A leak was found in the suction tubing of the probe caused by blockage at the probe end from tissue. Therefore care was taken to avoid further blockage of the suction tubing in future studies.

The best way of ensuring stable measurements was by correct initial probe placement. The optimum position appeared to be when the probe was introduced posteriorly, as the suction cup was compressed against the curve of the sacrum by the fetal head, forced down by gravity and uterine contractions. In addition, the fibreoptic bundles were supported by the posterior vaginal wall. Low cervical dilatations in particular limited the positioning and application of the probe and if combined with a high presentation (i.e. unengaged head), then good apposition between the optodes and the fetal scalp was less likely to be achieved.

7.1.2 Contribution of superficial tissue

One of the main concerns with the use of infrared light to investigate blood volume and oxygenation in biological tissue is the contribution of skin and bone to the light signals. Table 7.1 summarises the physical changes that may occur and have a potential effect on intensity modulated light wave if used to investigate changes in biological tissue such as the fetal brain.

Table 7.1 Possible effects on IMOS data during physiological changes in biological tissue

Physical change	Attenuation change	Pathlength change
Absorption of brain ↑	↑	↓
Optode separation ↑	↑	↑
Absorption skin ↓	↓	small
CSF layer ↓	small	↓

Because NIR light is transmitted through these haemoglobin-bearing tissues, as well as brain, any change in their oxygenation or haemoglobin content could have an effect on measurements. Young et al (2000) found a substantial difference in NIR light intensity when between applying a probe to the skin over adult brains compared with a probe applied to the cerebral cortex. However in the fetal and neonatal head this difference is likely to be small (<10%) because the superficial tissues are relatively thin (Wyatt 1990). The size of the effect will depend partly on the distance between the transmit and receive optodes, so that at small interoptode spacings more of the signal will be derived from the scalp and skull, (Grubhofer 1997, Owen-Reece 1996). It is thought that the critical minimum interoptode spacing is probably 2.5 cms (van der Zee 1992). The light transmitted from the fibreoptic bundle is multiply scattered within the cranial compartment and the exact volume of brain tissue interrogated is not yet determined. The mean distance travelled by each photon is known to be 12-15cms and it is therefore probable that a signal is obtained from a substantial proportion of the brain. It is for this reason that exact positioning of the probe on the fetal scalp is unlikely to be important

Caput

It is also possible that caput formation during labour, leading to an increase in the extracellular fluid of the fetal scalp is another potential source of error. Lower values for fetal scalp saturation, measured by pulse oximetry, have been obtained from caput than from surrounding areas (Johnson 1990). The reason for this discrepancy is thought to be because haemoglobin saturation is genuinely lower over caput and also because oedema acts as a preferential absorber of red light, as opposed to near infrared light which has the effect of lowering the measured value for saturation. The effect of caput on near infrared observations remains difficult to determine accurately, although the fact that caput contains mostly serum rather than haemoglobin means that it is probably relatively transparent to NIR light. In practice caput did not seem to significantly alter either the quality or the magnitude of the signal, as similar data were obtained from one fetus when the probe was intermittently repositioned in different regions with and without obvious caput. The problem of caput during NIR monitoring may be avoided by routinely positioning the probe further back on the fetal scalp.

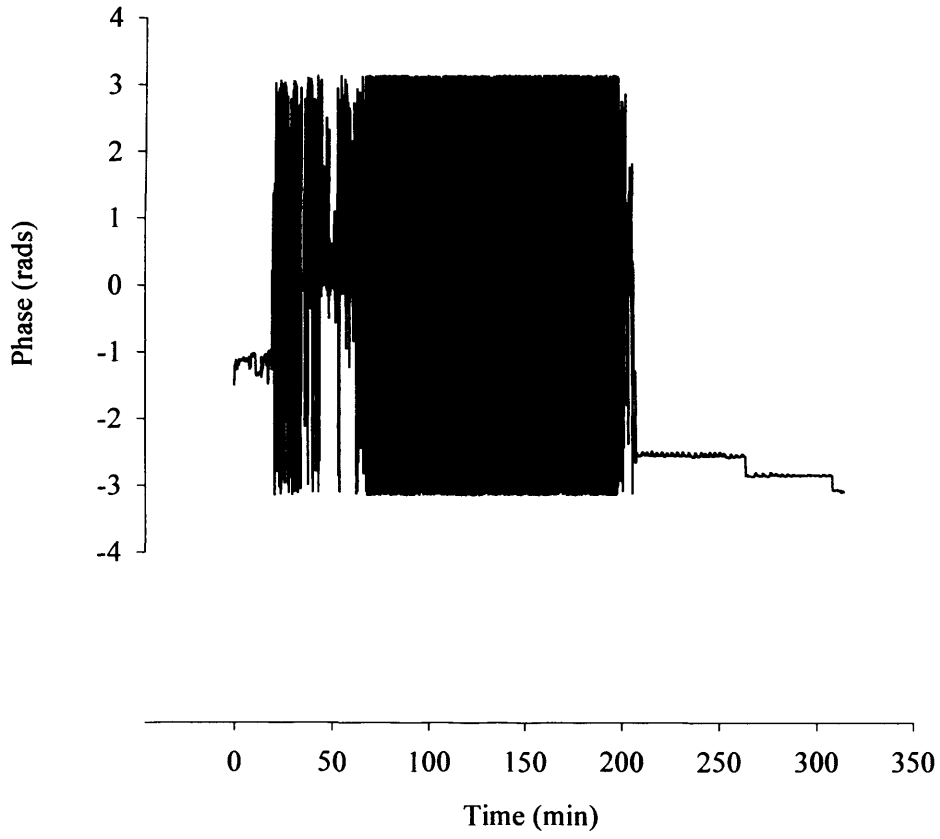


Figure 7.1 Effect on the phase data when the fetal probe becomes dislodged from the fetal head.

7.1.3 Signal quality

In some of the cases in which the probe was applied it was not possible to collect satisfactory data suitable for analysis. This was either due to poor signal quality that provided 'noisy' data but more commonly it was due to technical malfunctions within the spectrometer.

In eight of the cases poor quality signals provided data that was unsuitable for analysis. In six of these, there were identifiable contraction-related changes in the attenuation data but 'noise' dominated the signals in the phase and modulation depth data. An example of this is illustrated in figure 7.2 and the calculations of absolute concentrations are by the analytic programme are shown in table 7.2. As there is no correlation between the phase and modulation depth data in response to uterine contractions, the Mathematica programme would be unable to calculate 'sensible' absolute concentrations of Hb and HbO_2.

It was not possible to detect noisy phase and modulation data during a study as the spectrometer only displayed changes in light intensity, which is related to attenuation. Conversely, the on-line display of the conventional NIRS monitors can give an indication of noise within the signal.

Previous NIRS studies have shown that the commonest reason for failure to acquire data or noise was the inability to transmit sufficient light through the tissue (Peebles 1993). This was observed most frequently if there was a combination of thick black curly hair and dark skin, as melanin significantly increases the attenuation of near infrared light. Although there were few African and Asians in this study, noisy phase and modulation depth data was still encountered despite being able to adjust the PMT sensitivity manually

The reason behind this may be because the three signals (attenuation, phase and modulation) have different sensitivities to spatial variations. Arridge (1995) has demonstrated that attenuation data are especially sensitive to changes in absorption close to the surface of a medium, whereas phase and modulation are more sensitive to deeper tissue layers. Although NIR light travels through the brain to a depth of several centimetres, changes in blood volume or oxygenation in a blood vessel close to the surface could theoretically induce large attenuation changes while phase and modulation depth remain largely unaffected.

Table 7.1 Calculations of $SmCO_2$ and haemoglobin concentrations by the Mathematica programme when the data is dominated by noise.

Laser combination	$SmCO_2$	HbT	Hb	HbO_2
"{1, 2}"	-1.99875	8470.6	25401.2	-16930.6
"{1, 3}"	-0.101894	4.45983	4.91426	-0.454432
"{1, 4}"	0	0	0	0
"{2, 3}"	5.18735	2250.92	-9425.39	11676.3
"{2, 4}"	0	0	0	0
"{3, 4}"	0	0	0	0
"{1, 2, 3}"	1.26084	1183.31	-308.651	1491.96
"{1, 2, 4}"	0	0	0	0
"{1, 3, 4}"	0	0	0	0
"{2, 3, 4}"	0	0	0	0
"{1, 2, 3, 4}"	0	0	0	0

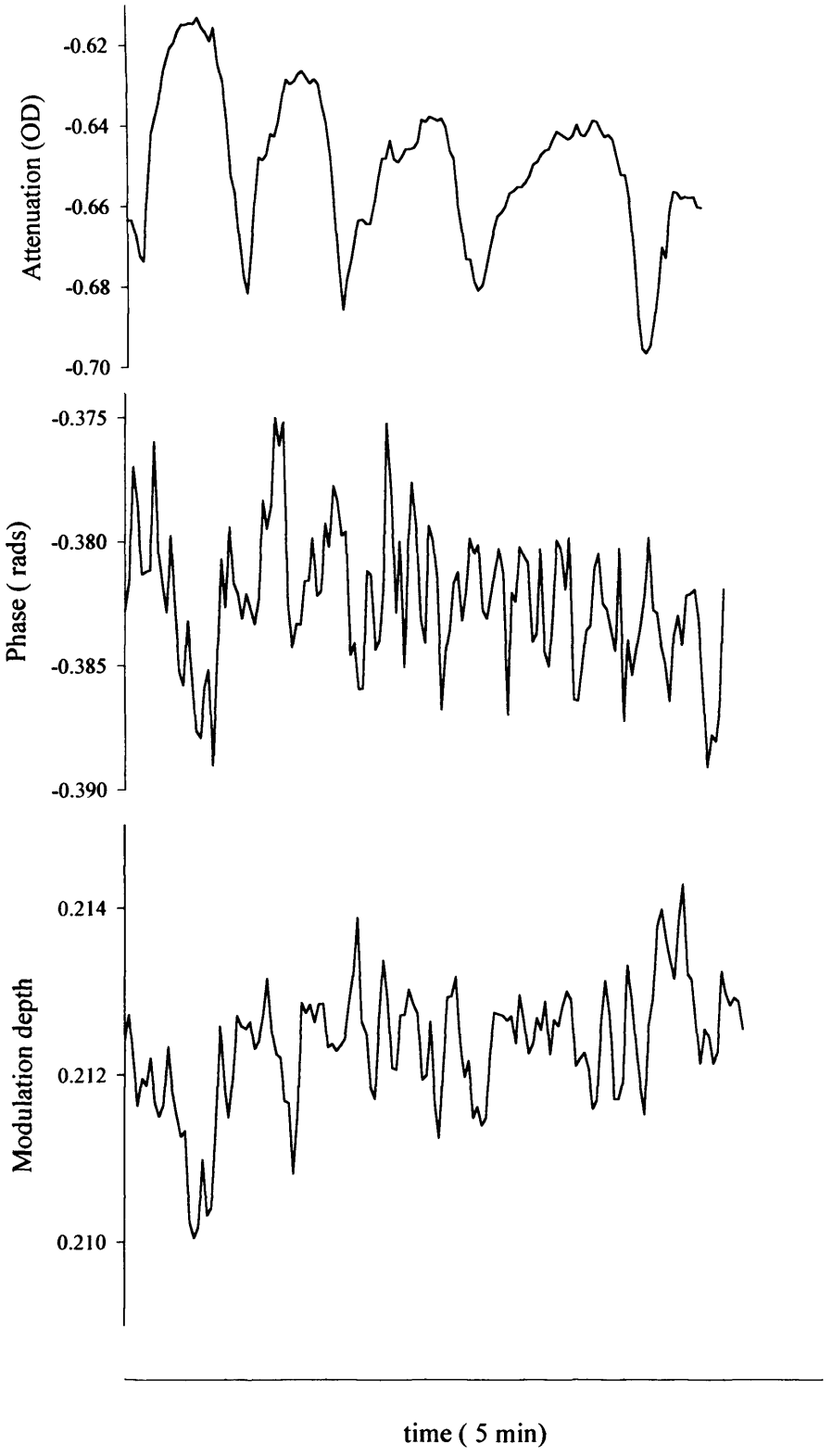


Figure 7.2. Data collected from a fetus using IMOS in which the phase and modulation depth data is dominated by 'noise'.

7.1.4 Technical problems

The spectrometer and the fetal probe used in this thesis were prototypes, and so developmental problems were encountered. Despite satisfactory application of the fetal probe to the fetal head, it was not possible to collect data in eleven of the twenty-nine fetuses in which the probe was applied. In the first three intrapartum studies this was due to a problem with the timing mechanism of the spectrometer. In three further studies in which data was unobtainable the spectrometer failing to generate a light source. In 5 cases the PMT failed.

Unfortunately these technical malfunctions were not resolvable at the time of the studies and so the studies were abandoned. It was thought that these fragile components were damaged during transportation of the spectrometer from the Medical Physics Department to the delivery suite. Further proposed studies were also abandoned until any new components that were required were fitted and tested on optical phantoms within the Medical Physics department. To avoid further damage of the components of the spectrometer, it was kept on the delivery suite as much as possible.

Other minor practical problems with the spectrometer were encountered during it's time on delivery suite. During the initial studies, the acoustic noise of the fan system of the spectrometer, which kept the lasers at a constant temperature, was too much for four women who declined to be monitored after having consented. Removing one of the fans for future studies reduced this acoustic noise.

The fetal studies required both JC and DK to be present for manoeuvring the spectrometer close to an electrical socket and a wall suction apparatus and also required DK to be present to provide technical assistance. The dimensions of the spectrometer are approximately twice that of a CTG monitor and of the conventional NIRS spectrometer and because of the large area taken up by the JC, DK and the spectrometer, three women declined to be part of the study after giving consent. All of the women, who were entered into the study, their partners and the midwifery and medical staff involved in their care, commented on the size of the spectrometer.

Currently the monitor of the spectrometer has the capability of displaying the number of photons back-scattered to the PMT for the four laser wavelengths. Changes in this

number reflects the changes in light absorption during a study. However, it is only after a study is completed and data are passed through the analytic programme that changes in phase and modulation depth can be displayed. Furthermore, the spectrometer records data from the start of a study but it does not have the capability of displaying the number of photons in real–time. Additionally, the spectrometer does not have the facility of marking events that occur during data collection. These problems were overcome by strict recording of events both by JC and DK during the studies.

Faults in the design and manufacture of the first fetal probe that was made available were encountered before intrapartum studies were commenced. The optodes of this probe were manufactured in such a position within the silicone rubber moulding that the receiving optode collected all the light that was transmitted from the emitting optode. Thus its use for fetal studies was not appropriate. The second probe optodes were in a more satisfactory position (see figure 4.1) however the covering of the optical fibres was transparent to background light. A further heat-shrink covering was therefore required. As previously discussed, this covering made the probe less flexible and heavier and therefore during fetal studies the optical fibres were attached to the woman's thigh to avoid displacement of the probe during a maternal movement. Lastly, the optical fibres are extremely fragile and therefore extreme care with the probe was necessary to ensure these fibres were not broken during intrapartum studies.

7.1.5 Analytic method

IMOS measurements of absolute haemoglobin concentration rely on changes in the three measured signals (attenuation, phase and modulation depth). However Arridge (1995) has shown that these three signals have different sensitivities to spatial variations in the absorption properties of media. He demonstrated that attenuation data are especially sensitive to changes in absorption near the light source or the detector i.e. close to the surface of the medium, whereas phase and modulation depth are more susceptible to changes in deeper tissue layers. These spatial sensitivity differences are of no consequence in homogenous media in which A/P and P/M can derive the same measurements of absorption characteristics. However, in inhomogeneous media, such as

biological tissue the differences may be substantial. For example, any change in blood volume or oxygenation in a blood vessel close to the surface may induce large changes in attenuation while the other two signals remain largely unaffected. Consequently, in highly inhomogeneous media, it might be advantageous to base absolute concentrations on phase and modulation depth. Furthermore the deeper penetration depth of phase and modulation depth might be required for reducing artefact arising from the skin and skull. In practice however it was clear from the data in this thesis that the modulation depth data on the current spectrometer had a low signal/noise ratio throughout the fetal studies and consequently its use was limited in the absolute measurement of haemoglobin concentrations.

Finally, the analytic process of the raw data with the Mathematica programme is currently not incorporated into the software of the spectrometer. This is because analysis with the Mathematica programme requires an observer to select two data points to calculate the absolute Hb and HbO_2 concentrations. The process of computing the measurements from the raw data is time-consuming. For a single set of observations approximately 30 minutes are required. This length of time would therefore be impractical even if the analytic programme was incorporated within the spectrometer.

7.2 FUTURE DEVELOPMENTS

It is clear that technical developments are required. However, design and manufacture of the fetal probe for intrapartum infrared monitoring remains time-consuming and expensive. However, in the short term increasing the size of the fibreoptic bundles and prisms, particularly the receiving bundle and/or converting the prism holder into a similar sensor to a pulse oximetry may improve the performance. Unfortunately, because the probe has to create a vacuum, the fibreoptic bundles, which are currently very expensive (approximately £500 a pair) and not particularly robust, have to be sealed in place and handled very carefully.

Because of the risk of infection and difficulties in cleaning, the probe would ideally be disposable. A smaller probe would also be beneficial for studying fetuses at lower cervical dilatations, however at the present time; this is not practical, as a minimum interoptode spacing of about 3cm is required. The probe moulding also has to support the optodes and allow a vacuum to form, which require it to be at least 1.5 cm wide. Making the probe moulding flatter and thinner similar to pulse oximetry sensors may be beneficial. In future a fetal probe for IMOS use would have to be robust enough to avoid damage to the prisms and the optical fibres, but at the same time be lightweight and flexible for intrapartum fetal monitoring.

Data interpretation remains complex and time-consuming. Attending medical and midwifery staff require measurements displayed online and in real time and require a system that would reject poor quality signals and artefact. Encouragingly, work is already underway at UCL to provide a smaller version of IMOS that is more user-friendly and more acceptable in the clinical setting. This may prove more acceptable to midwives and labouring women. This will incorporate the analytic programme in the hope that it would provide an on-screen display of fetal cerebral oxygenation parameters. This should lead to a large improvement in the usefulness of IMOS as a clinical tool. This would also provide online absolute pathlength measurement, which would be useful in determining the magnitude of changes in interoptode spacing induced by alterations in uterine contraction pressure.

CHAPTER 8

CONCLUSION

In the conclusion to chapter 1, the requirements for the ideal method of intrapartum fetal surveillance were defined. It is now possible to compare IMOS in practice with this theoretical ideal technique.

1) The technique of IMOS allows the measurement of *absolute* fetal cerebral blood volume and saturation, rather than the peripheral saturation. These measurements are derived form absolute fetal cerebral oxyhaemoglobin and deoxyhaemoglobin. This is in contrast to conventional NIRS and pulse oximetry. Conventional NIRS measures *changes* in cerebral blood volume and therefore it's clinical usefulness is limited.

2) IMOS provides information on fetal cerebral saturation and blood volume. Cerebral blood volume reflects cerebral perfusion which is known to be a crucial factor in the maintenance of cerebral function.

3) The raw data can be collected continuously, to provide measurements of cerebral blood volume from the early stages of labour, during FHR changes and up to and during delivery. It therefore has the potential to detect the already compromised fetus (e.g. IUGR) entering labour from knowledge of the cerebral haemodynamics.

4) The technique appears to be safe for both the fetus and mother and does not require the use of a scalp clip or other invasive means of fetal attachment. The probe is simple to insert, once the amniotic membranes are ruptured. Although this technique is "non-invasive", it does require a vaginal examination, and it also limits the woman's freedom of movement. This may prove to be unacceptable for some women, especially those with fetuses at "low risk" of intrapartum hypoxic-ischaemic encephalopathy. However,

it's use was acceptable to the majority of mothers and causes minimal interference with routine midwifery and medical care on the labour ward. With the current spectrometer, the raw data can be collected during labour in approximately two-thirds of women who are selected for study. Of these, two thirds of fetuses provide data that is suitable for analysis. Furthermore, potential movement of the probe during uterine contractions appears to have little effect on these measurements.

5) The current available IMOS system however is expensive and it's use is not straight forward. The significant number of failed studies due to technical problems led to a small number of fetuses studied. If more fetuses had been studied then it is probable that measurements could have been obtained from hypoxic fetuses. This would have provided further information on the complexities of changes in cerebral blood volume and saturation in response to acute and chronic insults to the fetus.

The future clinical role of fetal IMOS remains open to speculation. Further intrapartum studies are unlikely to take place with the current available tools for intensity modulated optical spectrometry. Technical refinements and validatory studies are required before future intrapartum trials are considered. In the first instance, the reliability, accuracy and reproducibility of the performance of the individual components of the spectrometer will need to be assessed further by laboratory based studies. Clinical studies using neonates and fetal sheep to compare established methods of measurement of cerebral blood volume and saturation with IMOS measurements will then be required. In line with this, the software controlling the data collection needs to be overhauled to provide on-line analysis in real time to provide a more user-friendly system.

It is only after these studies and improvements could IMOS be considered for intrapartum use to define normal ranges further, for the variables described, from a large number of uncomplicated deliveries. These data can then be compared with measurements obtained from fetuses thought to be at high risk of hypoxia-ischaemia, and those with evidence of "fetal distress" during labour. Deviations from "normal" in fetuses, which subsequently develop hypoxic-ischaemic brain injury, may suggest mechanisms by which such injury can occur.

The establishment of normal values and physiological responses will enable abnormalities of cerebral oxygenation and haemodynamics occurring intrapartum to be correlated with neonatal outcome. This would be in the context of a large prospective study with long-term follow up and this would potentially provide a 'cut-off' measurements of cerebral blood volume and saturation below which neurological damage occurs and delivery would be recommended. After this, clinical intervention studies can be performed to ascertain whether knowledge of fetal cerebral circulation and haemodynamics can reduce the number of infants suffering hypoxic ischaemic brain injury. Only after such studies have been completed could the use of IMOS in clinical practice be contemplated.

APPENDICES

Appendix 1.

Derivation of absolute Hb and HbO_2 by the Mathematica based on diffusion theory

Diffusion theory has become established as a versatile tool for describing light intensity, time of flight (phase), and modulation depth in terms of the transport scattering coefficient (μ_s'), the absorption coefficient (μ_a), and the refractive index of a medium (n). Appendix 1 details the specific equations and theory that is required to derive absolute Hb and HbO_2 in biological tissue using diffusion theory.

For a pencil-beam light source on a semi-infinite half-space, the reflectance R (i.e., the number of photons back-scattered to the surface to the medium per unit area) and the mean transit time (time of flight) <t> detected at a distance r from the source can be written as

$$R(r) = z_o(1/\rho + \mu_{eff}).\exp(-\mu_{eff}\rho)/2\pi\rho^2 \qquad (1)$$

$$\langle t\rangle(r) = \rho^2 / 2c[D + \rho.(\mu_a D)^{1/2}] \qquad (2)$$

where $\rho = (r^2 + z_o^2)^{1/2}$, $z_o = 1/\mu_s$'. c is the velocity of light in the medium, μ_{eff} = effective attenuation coefficient = $[3\mu_a(\mu_a + \mu_s')]^{1/2}$ and D is the diffusion coefficient = $[3(\mu_a + \mu_s')]^{-1}$

Analytical expressions for the phase Φ and the modulation depth M have been derived by Patterson et al, such that

$$\Phi = \Psi_r - \tan^{-1}[\Psi_r/(1 + \Psi_i)], \qquad (3)$$

$$M = [(1 + \Psi_o^2 + 2\Psi_i)^{1/2}) / (1 + \Psi_\infty)].\exp(\psi_\infty - \psi_i), \qquad (4)$$

Where $\psi o = \mu_{eff}\rho(1 + \chi^2)^{1/4}$, $\psi_r = -\psi_o\sin(\theta/2)$, $\Psi_i = \Psi_o\cos(\theta/2)$, $\theta = \tan^{-1}(\chi)$,

and $\Psi_\infty = \mu_{eff}\rho$ and $\chi = (2\pi v_M)/(\mu_a c)$.

The derivative of attenuation A and <t> with respect to changes in μ_a can be derived for Eqns (1) and (2) giving

$$\partial A/\partial\mu_a = [\, 3.\rho.(2\mu_a + \mu_s')\,]/[\,(2l).(1/\rho + \mu_{eff})\,] \qquad (5)$$

$$\partial\langle t\rangle/\partial\mu_a = -{}^3/_2.[\,[\,(\rho/2).\mu_s' / \sqrt{(\mu_a D)}\,] - 1\,] / [\,(1/\rho + \mu_{eff})^2.c\,] \qquad (6)$$

From these two quantities the quotient $\mathbf{Q} = (\partial A/\partial\mu_a) / (\partial\langle t\rangle/\partial\mu_a)$ can be derived.
This ratio can be simplified by the use of the diffusion approximation, which states that scattering dominates absorption ($\mu_a << \mu_s'$), and therefore $\mu_{eff} \approx \sqrt{(3\mu_a\mu_s')}$ and $D \approx (3\mu_s')^{-1}$. Using these approximations, the ratio of equations (5) and (6) becomes:

$$(\partial A/\partial\mu_a) / (\partial\langle t\rangle/\partial\mu_a) = \mu_a(-(1 + \rho\mu_{eff})c / [\,(\rho\mu_{eff}/2) - (\mu_a/\mu_s')\,]) \qquad (7)$$

For media with optical properties similar to tissue, ($\mu_s' = 1\text{-}2\ mm^{-1}$; $\mu_a = 0.005 - 0.05\ mm^{-1}$) and typical source detector distances (r = 20 – 40 mm) , $\rho\mu_{eff}/2 >> \mu_a/\mu_s'$, and therefore:

$$(\partial A/\partial\mu_a) / (\partial\langle t\rangle/\partial\mu_a) = \mu_a(-2(1/\mu_{eff} + \rho)c / \rho) \qquad (8)$$

For large source-detector distances, ($\rho >> 1/\mu_{eff}$), equation (8) is to a good approximation, independent of both μ_s' and ρ. Equation (8) can therefore be reduced to:

$$(\partial A/\partial\mu_a) /(\partial\langle t\rangle/\partial\mu_a) = \mu_a(-2c/\ln 10) \qquad (9)$$

which is a linear function of μ_a (and hence concentration) only.
Hence it is the ratio of the attenuation and phase shift that can be used to derive absolute μ_a which is independent of scattering and is relatively insensitive to uncertainties in the source-detector distance of 5mm. Similarly, two further ratios (**V** and **R**) can be

derived, where $\mathbf{V} = (\partial\Phi/\partial\mu_a) / (\partial M/\partial\mu_a.M^{-1})$ and $\mathbf{R} = (\partial A/\partial\mu_a) / (\partial M/\partial\mu_a . M^{-1})$ which are both linear functions of μ_a.

From these measured absolute absorption coefficient (μ_a), concentrations of Hb and HbO_2 can be derived as the extinction coefficients of the chromophores for the laser wavelengths are known:

$$\mu_a^{\lambda} = \varepsilon_{HbO2}^{\lambda}[HbO_2] + \varepsilon_{Hb}^{\lambda}[Hb] \qquad (10)$$

where μ_a^{λ} is the absorption coefficient at wavelength of NIR light λ, and ε_{HbO2} and $\varepsilon_{Hb}^{\lambda}$ are the extinction coefficients at wavelength λ for HbO_2 and Hb respectively. Hence, if two or more wavelengths (λ_1 and λ_2) are used, absolute concentrations of $[HbO_2]$ and [Hb] (in millimolar units) can be derived by solving two simultaneous equations as follows:

$$[HbO_2] = (\mu_a^{\lambda 1}\varepsilon_{Hb}^{\lambda 2} - \mu_a^{\lambda 2}\varepsilon_{Hb}^{\lambda 1})/ (\varepsilon_{Hb}^{\lambda 2}\varepsilon_{HbO2}^{\lambda 1} - \varepsilon_{HbO2}^{\lambda 2}\varepsilon_{Hb}^{\lambda 1}) \qquad (11)$$

$$[Hb] = (\mu_a^{\lambda 2}\varepsilon_{HbO2}^{\lambda 1} - \mu_a^{\lambda 1}\varepsilon_{HbO2}^{\lambda 2})/ (\varepsilon_{Hb}^{\lambda 2}\varepsilon_{HbO2}^{\lambda 1} - \varepsilon_{HbO2}^{\lambda 2}\varepsilon_{Hb}^{\lambda 1}) \qquad (12)$$

From these absolute concentrations of HbO_2 and Hb, HbT and $SmcO_2$ can be derived :

$$SmcO_2 = 100\% * [HbO_2] / [HbO_2] + [Hb] \qquad (13)$$

$$HbT = [HbO_2] + [Hb] \qquad (14)$$

A measurement of Cerebral Blood Volume (mls/100g) (Elwell 1995) can be derived using the following equation:

$$CBV = HbT \times MW_{Hb} \times 10^{-6} / tHb \times 10^{-2} \times D_t \times 10$$

where MW_{Hb} is the molecular weight of haemoglobin (64500), tHb is the mean umbilical vein Hb concentration (16.8g/dl) (Wintrobe 1997) and D_t is the brain tissue density (1.05g/ml).

Appendix 2.

Calculation of the true absorption coefficient of the ProJet dye used in the phantom experiment in chapter 3.

For the solution in the beaker, there are 495ml, of which 99% is water and 1% intralipid.
There is 300μl of dye (0.3ml) already in the phantom before dye is set to continuously infuse into the phantom at 2.5ml/hour. Hence total initial volume is 495.30ml.

At 140s after the start of the experiment, dye is being delivered to the phantom at a rate of 2.5ml/hour.

The total volume of the phantom for a time t beyond $2^1/_3$ minutes (i.e. beyond 140 seconds) is:

$$\Sigma\text{Volume} = 495.3 + [(2.5/60)*(t-2^1/_3)]$$

Hence the absorption coefficient of the phantom for a wavelength λ, and for a time t is given by:

$$\mu_a^{\lambda}(\text{phantom}) = \mu_a^{\lambda}(\text{water}) + [\,[(0.3) + (2.5/60)*(t-2^1/_3)] / [495.3 + (2.5/60)*(t-2^1/_3)]\,]* \mu_a^{\lambda}(\text{dye})$$

Appendix 3
Information sheet for patients;

STUDIES OF THE OXYGEN SUPPLY TO THE BABY'S BRAIN DURING LABOUR

For the past seven years here at UCH we have been using a new kind off scanning technique, called near infrared spectroscopy, to see if we can measure the amount of oxygen getting to the brain of newborn babies. The technique is absolutely safe and does not upset the baby in any way. It has taught us a lot about the normal supply of oxygen and what can go wrong in ill babies.

We have now reached a stage where we believe we can measure the oxygen supply to the babies' brain during labour and birth. This means that we may learn how to spot babies who are at risk of getting short of oxygen. Although we do not expect you to be in a high risk group, this may be of benefit to your baby.

After the waters have broken, a very thin rubber device is gently placed beside the baby's head and invisible infrared light shone into the head. The strength of the light beam is about the same as an ordinary battery torch bulb and there is no possibility of any harm. As soon as the baby is born the device is taken off.
When you are admitted to the labour ward a research doctor may ask you if you would agree to have this scan on your baby. If you refuse, your decision will have absolutely no effect on the normal medical care you and your baby will receive. If you agree to have a scan please inform your midwife.

Thank you for taking time to read this.

I agree to the study of my baby as described above and explained to me by
Dr J Chipchase.

Signed .....

PUBLICATIONS

Chipchase J. Peebles D Kirkby DR Cope M Rodeck CH. Absolute quantification of oxy- and deoxyhaemoglobin concentration measured by Intensity Modulated Optical Spectrometry in the human fetus during labour. Journal of Obstetrics & Gynaecology 2000 (supplement) Vol 20 :1: 49-50.

Chipchase J. Kirkby D.R. Peebles D. Cope M. Absolute cerebral haemoglobin concentration and oxygen saturation measured with intensity modulated optical spectrometry in the human fetus during Labour. 1999. 3rd International Symposium on Intrapartum Surveillance conference proceedings.

Chipchase J, Kirkby D, Peebles DM, Cope M, Rodeck CH. Cerebral haemoglobin concentration and oxygen saturation measured by intensity modulated optical spectroscopy in the human fetus during labour. J Perinat Medicine 2002;30 (6):502-9.

PRESENTATIONS

Oral

International

Absolute Cerebral Haemoglobin Concentration and Oxygen Saturation measured with Intensity Modulated Optical Spectrometry in the Human Fetus during Labour.

3rd International Symposium of Intrapartum Surveillance June 15-17 1999. Stockholm, Sweden.

National

Future technology in intrapartum fetal monitoring. Fetal Heart Rate Study day for midwives and doctors (100 delegates), organised by Mr P. O'Brien and Mr R Ogle (UCL), held at Institute of Child Health, February 1999.

Inter-departmental

Absolute Cerebral Haemoglobin Concentration and Oxygen Saturation measured with Intensity Modulated Optical Spectrometry in the Human Fetus during Labour.

Research meeting. Dept of O & G. UCL. Nov 1999.

Poster

Chipchase J. Peebles D Kirkby DR Cope M Rodeck CH . Cerebral saturation and Haemoglobin concentration in the human fetus during labour measured by intensity modulated optical spectrometry. British Maternal and Fetal Medicine society 30-31 March 2000. **Prize for best poster in intrapartum category.**

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