PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Measurements of tissue viability in transplantation

M. S. Thorniley, S. Simpkin, E. Balogun, K. Khaw, C. Shurey, K. Burton and C. J. Green

Phil. Trans. R. Soc. Lond. B 1997 **352**, 685-696
doi: 10.1098/rstb.1997.0050

Measurements of tissue viability in transplantation

M. S. THORNILEY[1], S. SIMPKIN[1], E. BALOGUN[1], K. KHAW[2], C. SHUREY[1], K. BURTON[1] AND C. J. GREEN[1]

[1] *Department of Surgical Research, Northwick Park Institute for Medical Research, Watford Road, Harrow, Middlesex HA1 3UJ, UK*
[2] *Department of Anaesthetics, Mount Vernon Hospital, Northwood HA6 2RN, UK*

SUMMARY

Near-infrared spectroscopy has primarily been used in monitoring changes in cerebral haemoglobin oxygenation and haemodynamics. However its use as a method for the assessment of tissue viability following transplantation has recently been explored experimentally in our laboratory.

The ability to measure changes in oxygenation and perfusion during harvesting and following transplantation of organs or transfer of free and pedicled flaps is potentially important in reconstructive surgery. We have found that near-infrared spectroscopy is extremely useful in detecting vaso-occlusive events and can accurately and reliably distinguish between arterial, venous or total occlusions. Venous congestion indicated by raised levels of deoxygenated haemoglobin with a concomitant increase in blood volume and the presence and magnitude of reactive hyperaemia are both easily recognizable features by near-infrared spectroscopy. We have shown that near-infrared spectroscopy measurements of venous congestion in kidneys (and other tissues) following prolonged storage correlate with medullary vascular congestion confirmed by angiographical and histological analysis of intrarenal perfusion.

Clinically we have shown that flap perfusion can be improved by altering fluid replacement regimes and the addition of ionotropes. Cerebral near-infrared spectroscopy measurements in a liver transplant model showed statistically significant differences within minutes after the anhepatic phase in cerebral perfusion and oxygenation, between animals transplanted with ischaemically damaged livers compared to those isografted with minimally stored livers. Similarly we have found that near-infrared spectroscopy can be used as a monitor to assess the adequacy of fluid or blood replacement in haemorrhagic and hypovolaemic models. We believe that near-infrared spectroscopy provides a sensitive and reliable postoperative method for the assessment of tissue viability following the transfer of free and pedicled flaps and organs.

1. INTRODUCTION

Damaging periods of ischaemia followed by reperfusion with fully oxygenated blood may occur in many clinical conditions and involve a cascade of biochemical and physiological changes which can, in extreme cases, lead to tissue death. These can be considered together under the term ischaemia–reperfusion (IR) injury. The identification of key biochemical changes which correlate with subsequent dysfunction of tissues upon reperfusion at the molecular, ultrastructural and physiological level should allow the development of novel pharmacological strategies to increase tolerance to ischaemic insult in the clinical setting.

The long-term objectives of this research into IR damage can be summarized as (i) the improvement of tissue and solid organ preservation techniques in order to allow longer periods of storage and immediate primary function after transplantation; (ii) the identification of biochemical markers which will predict tissue function before transplantation, and (iii) more satisfactory management of chronic IR-related diseases and the development of strategies for treating patients with early evidence of IR injury to prevent subsequent problems.

Our own studies have focused on the role of IR in tissue storage and reperfusion (transplantation) so much of this paper is concentrated in this area. We believe that many of the experimental techniques and models we describe serve to illuminate IR damage in its widest context. In clinical organ transplantation, there is an urgent need to improve current preservation techniques and to increase the safe storage period tolerated by kidneys (at present about 30 h), livers (15 h), lungs and hearts (4 h), pancreas (6 h) and small bowel (4 h) so that immediate (primary) and long-term life-supporting function are obtained after transplantation whilst allowing time for the organization of clinical facilities, immunological matching of donor and proposed recipient, and potentially specific immunological manipulation of the graft and/or the patient. In addition the increasing sophistication of reconstructive surgery may entail subjecting tissues to long periods of both warm ischaemia (WI) and cold ischaemia (CI). Hence in transplant and reconstructive surgery, protection against IR injury is of paramount importance.

(*a*) In vitro *assessment of tissue viability*

During ischaemia there is rapid inhibition of mitochondrial electron transfer, a net breakdown of adenine nucleotides, accumulation of reduced pyridine nucleotides (Calman *et al.* 1973), loss of homeostasis

involving a fall in intracellular pH (Fuller *et al*. 1988, 1990), mitochondrial calcium loading, cellular swelling and a fall in ATP levels (Hardy *et al*. 1991). In irreversibly injured cells, respiratory control is lost and is accompanied by futile oxidation of cytochromes *a* and a_3 and NADH (Taegtmeyer *et al*. 1985), the latter was attributed originally to substrate deficiency (Chance & Williams 1955) but more recent studies, indicate that this is more likely to be an enzymological defect resulting in an inability to metabolize NADH-linked substrates (Taegtmeyer *et al*. 1985; Hardy *et al*. 1991).

Over the last decade *in vitro* studies of the respiratory chain (RC) complexes have been made in several tissues including cardiac and renal tissue, subjected to ischaemic–reperfusion injury (McMillan-Wood *et al*. 1973; Jennings & Ganote 1976; Pelikan *et al*. 1987; Veitch *et al*. 1992). The results indicated that differences in the sensitivity of these complexes to ischaemic damage were dependent upon the duration of ischaemic episode and the presence of oxygen. It was found that complexes I and IV are the major defective sites during ischaemia and reperfusion. It was shown that damage at complex I occurred during ischaemia and this was exacerbated by reoxygenation; other complexes were apparently unaffected by ischaemia alone but their activity was significantly reduced by reperfusion. It is probably not coincidental that complex I is also a major sources of oxygen-derived free-radical (ODFR) production during normal electron transfer in mitochondria *in vitro* (Cadenas *et al*. 1977; Turrens *et al*. 1982).

In the transplant situation it has been shown that mitochondrial phosphorylative activity is a prerequisite for recovery to restore cellular energy charge (Inomoto *et al*. 1994). Return of function may therefore relate to the preservation of inner mitochondrial membrane integrity, and the structure and activities of the RC complexes I to IV (Taegtmeyer *et al*. 1985; Hardy *et al*. 1991). In essence, the integrity of oxidative metabolic pathways and the capacity to resynthesize ATP rather than the immediate post-ischaemic ATP levels appears to determine the return of function (Taegetmeyer *et al*. 1985). Some workers have reported that finding reduced ATP levels in human livers prior to implantation was not useful in predicting outcome following transplantation but grafts with low *total adenine* nucleotides showed poor function after transplantation (Kamiike *et al*. 1988). Others have shown that a high ATP level does correlate with outcome following liver transplantation (Lanir *et al*. 1988), but that although low ATP content and energy charge indicates a more severe degree of ischaemic damage this is not necessarily irreversible resulting in a non-viable organ. Viability appears to be determined by the ability of the liver to regenerate ATP as measured by high-performance liquid chromatography (HPLC) analysis and by ^{32}P nuclear magnetic resonance (NMR) (Marubayashi *et al*. 1980; Kono *et al*. 1981; Lanir *et al*. 1987; Bowers *et al*. 1992; Okamura *et al*. 1992). Similar observations have been reported in the regenerating liver of rabbits after partial hepatectomy where it was shown that mitochondrial phosphorylative activity plays an important role in initiating liver regeneration (Inomoto *et al*. 1994).

(*b*) In vivo *measurements of respiratory function in transplantation*

The studies discussed so far whilst essential to an understanding of IR injury, all involve techniques which require invasive biopsy, tissue homogenization or cellular fractionation; in addition to being time-consuming, complicated and in the case of NMR, expensive. For a method to be of predictive value in assessing organ viability either before, or immediately after transplantation, it is necessary to use and further develop non-invasive methods for measuring not only oxygen delivery and cellular oxygen utilization but mitochondrial dysfunction. These could provide an early indication of subsequent IR damage in the whole organ.

IR is also expressed at a physiological level as microcirculatory disturbances and interactions between vascular and parenchymal components. These can be studied in intact organs by non-invasive methods such as surface fluorimetry (SF) and near-infrared spectroscopy (NIRS) and the application of these two techniques.

(*c*) *Surface fluorescence*

Two reactions of the respiratory chain are unaltered whether they are measured *in vitro* or *in vivo*: (i) oxidation of all member complexes on exhaustion of substrates; and (ii) their reduction during hypoxia. When Chance and his co-workers (Chance *et al*. 1979; Mayevsky 1983; Mayevsky *et al*. 1984) showed that direct *in vivo* measurements of fluorescence could be made from intracellular pyridine nucleotides in brain and kidney, the possible importance of these measurements as indicators of intracellular hypoxia was emphasized. It was shown that fluorescent signals from cytosolic NADH are negligible compared to those from the mitochondrial pool owing to quenching of fluorescence by cytosolic glyceraldehyde 3-phosphate dehydrogenase (O'Conner 1977). The oxidized flavoprotein signal has only a weak intrinsic fluorescence at physiological temperatures but the level can be enhanced at low temperatures enabling measurement in freeze-trapped tissues (Chance *et al*. 1979). In 1993 the oxidized flavoprotein: NADH ratio was measured in freeze-trapped biopsy samples from human paediatric livers and it was shown that, post transplant, the ratio remained lower than that observed in control livers and this was interpreted as indicative of ischaemia (Tokunaka *et al*. 1993). When non-invasive pyridine nucleotide fluorescence measurements were used alone to determine changes in mitochondrial and cytosolic redox status in perfused blood-free rat livers (Okamura *et al*. 1992), they were found to correlate closely with energy charge and mitochondrial phosphorylative activity (Ozaki *et al*. 1989) as determined by *in vitro* measurements.

The fact that SF measurements of mitochondrial NADH closely correlated with other indices of energy metabolism including the activities of the individual respiratory complexes (Tokunaga *et al*. 1987) suggest

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

that they could be used as a marker of severity of ischaemic damage prior to transplantation and hence a predictor of subsequent success.

(*d*) *Near-infrared spectroscopy*

Near-infrared spectroscopy can be used to make continuous *in vivo* measurements of changes in haemoglobin and tissue oxygenation, the latter indicated by the redox state of cytochrome oxidase, the terminal enzyme of the respiratory chain (Jöbsis 1977). Hence dynamic measurements of oxygen supply, utilization and perfusion can be obtained (Irwin *et al*. 1995; Thorniley *et al*. 1994*a*, *b*, 1995*a*, *c*, 1996). Applications of NIRS are predominately for cerebral monitoring in particular in perinatology (Wyatt *et al*. 1989, 1990; Livera *et al*. 1990, 1991*a*, *b*, 1992), although other measurements have been made during cardiac surgery (du Pleiss *et al*. 1995; Levy *et al*. 1995) and hepatic transplantation (Thorniley *et al*. 1995*a*).

An unfortunate consequence of many major transplant procedures is resultant loss of viability of the transplanted tissue (Marubayashi *et al*. 1980; Shaw *et al*. 1986). This can be due to several reasons including inadequate harvesting technique, poor storage conditions, and surgical problems with failed anastomosis resulting in increased ischaemic time and exacerbated reperfusion injury (Thurman *et al*. 1988; Russell *et al*. 1989). The ability to measure changes in oxygenation and perfusion during harvesting and following transplantation or transfer of free and pedicled flaps is potentially important in reconstructive surgery (Thorniley *et al*. 1995*c*). Rapid detection of a critical change in haemoglobin oxygen saturation could enable earlier and more successful intervention. For example it would be beneficial if the onset and magnitude of the damaging effect of venous ischaemia could be detected and hence reduced (Harrison *et al*. 1983). It has been shown (Irwin *et al*. 1994*b*) in a rabbit hind limb model that NIRS can reliably detect and distinguish between various types of vascular compromise including arterial, venous or total obstruction.

In addition to the failed graft, systemic problems can frequently arise since most surgical procedures are accompanied by haemorrhage necessitating replacement with either fluid or blood. Despite apparently adequate replacement, tissue oxygen delivery may still be poor, resulting in cellular hypoxia and consequent metabolic dysfunction, which can ultimately lead to multiorgan failure (Grant & Nimmo 1995). In the case of livers, even the most well-controlled hepatic transplantation procedures can lead to hypovolaemia and, if graft function is impaired, venous congestion and hence circulatory disturbances arise which exacerbate the situation and can lead to shock (Marzi *et al*. 1991; Takei *et al*. 1991; Grant & Nimmo 1995). A major complication after hepatic transplantation is disturbed cerebral oxygenation and haemodynamics including raised intracranial pressure (Shaw *et al*. 1986; Potter *et al*. 1989; Steltzer *et al*. 1993). This may arise from the effect of a failed graft on the circulation or as a result of inadequate fluid replacement (Marzi *et al*. 1991; Takei *et al*. 1991). As far as we are aware there have been no reported studies examining the effect of hepatic transplantation on cerebral oxygenation measured by NIRS.

The use of NIRS for determining graft function after liver transplantation has been described by Tashiro and co-workers (1993). Even though their measurements were arbitrary, they reported that NIRS measurements of the liver showed a difference in level of deoxygenated haemoglobin post portal reperfusion when comparing animals that had been transplanted with livers for a short and a long storage period (Tashiro *et al*. 1993). In longer-preserved grafts there was a higher initial level of deoxyhaemoglobin, which decreased with time, than that measured in livers stored for a shorter period; this was suggested to be due to hepatic congestion (Tashiro *et al*. 1993; Ohdan *et al*. 1995). This has also been reported for kidney transplants with quantitative NIRS measurements and the results were found to be in agreement with histological findings (Thorniley *et al*. 1994*a*; Lane *et al*. 1996*a*, *b*).

The importance of NIRS in cardiology has only recently become apparent. Myocardial ischaemia followed by reperfusion is associated with a host of distinctive pathophysiologic derangements, the most important of which are reperfusion arrhythmias, transient mechanical dysfunction or 'myocardial stunning', and cell death (Jeroudi *et al*. 1994). Timely coronary reperfusion as treatment for acute myocardial infarction reduces myocardial infarct size, improves left ventricular function and survival (Jeroudi *et al*. 1994). Theoretically, if this 'reperfusion injury' could be treated and eliminated, the outcome for patients with myocardial infarction might further improve (Jeroudi *et al*. 1994). Currently there are no non-invasive methods which can be used to assess myocardial oxygenation. NIRS facilitates measurements deeper into the myocardium than other optical methods (such as NADH fluorometry and visible spectroscopy which penetrate only the epicardial cell layers) and can indicate perfusion quality at greater depths than with methods such as laser Doppler (Thorniley *et al*. 1994*b*).

The use of NIRS in the detection of myocardial ischaemia has until recently been limited to semi-quantitative measurements in dog hearts (Parsons *et al*. 1990). However, the authors recently demonstrated that it is possible to measure changes in myocardial oxygenation in a porcine model in response to LAD occlusion and moreover that the kinetics, magnitude and rate of change of oxy- and deoxyhaemoglobin in the reperfusion phase can be indicative of the severity of the ischaemic period (Thorniley *et al*. 1996*a*).

In this paper our overall objective was to determine if the non-invasive methods, NIRS and SF, can be used to assess organ viability pre- and during transplantation. In order to achieve this we have used several models of storage and transplantation including liver, kidney and muscle to compare NIRS and SF measurements with other physiological indices of organ function (including mitochondrial function) and histological changes including electron microscopy analysis, in addition to survival rates. The models enabled different preservation solutions and pharmacological agents to be assessed. A specific objective of these

studies was to determine the kinetics of haemoglobin oxygenation during *in situ* reperfusion of stored transplanted organs and tissues to provide a framework against which the timing of metabolic injury could be considered. For simplicity we will limit the results to hepatic and cerebral NIRS measurements during liver transplantation.

Our hope is to demonstrate that it might be possible in future to predict organ function and viability by non-invasive measurements of disturbances in oxygen supply and cellular oxygen utilization in the clinical transplant situation.

2. MATERIALS AND METHODS

All animal procedures were carried out according to the Animals (Scientific Procedures) Act, 1986.

(*a*) *NIRS measurements and theory*

NIR transmission spectroscopy through tissues up to several centimetres is possible because of the relative transparency of biological tissues to NIR light (700–1000 nm). Reflectance spectroscopy, in which the transmitting and receiving probes are placed adjacent can be used to enable the zone of interrogation to be more closely defined. In the heart this enables measurements of myocardial oxygenation and haemodynamics to be made. The methodology has been described in detail (Thorniley *et al*. 1994*a*; Irwin *et al*. 1995; Lane *et al*. 1996).

NIRS is based on the Beer–Lambert law which relates optical absorption to the concentration of light-absorbing chromophores present in a measured volume of tissue. NIRS is dependent upon the light absorption properties of the biochemical components, haemoglobin, myoglobin and cytochrome aa_3. Absorption due to oxygenated haemoglobin (O_2Hb) and deoxygenated haemoglobin (HHb) can be quantitated using a modified form of the Beer–Lambert law. The Beer–Lambert law strictly applies only to a non-scattering medium but the equation can be modified for measurements in scattering media such as biological tissue.

$$\text{Absorbance} = aclB + G$$

in which a is the molar absorption coefficient (M^{-1} cm^{-1}) of the chromophore of interest (light-absorbing compound) and its concentration c (M), B is the path length factor which compensates for the increased path length due to scattering, l is the path length and G is a geometry-dependent factor. Hence providing a, l and B are known, the change in concentration can be obtained from change in absorbance.

This instrument uses an algorithm based on published absorption spectra which have been obtained using isolated chromophores in non-scattering media. The HHb and O_2Hb absorption spectra were obtained using haemolysed human blood (Wray *et al*. 1988). The cytochrome difference absorption spectrum was obtained using purified cytochrome extracted from mitochondria (Brunori *et al*. 1981). The algorithm also incorporates wavelength-dependent differential path length factor data derived from 'time-of-flight' studies (Wyatt *et al*. 1990; Essenpreis *et al*. 1993).

Table 1. *Multiplying factors for the CRITIKON* cerebral redox research monitor model 2001*

(The units are mM cm absorption^{-1}. The calculated concentration change is expressed in μM multiplied by the pathlength in cm.)

wavelength/ nm	776.5	819	871.4	908.7
HHb	1.6436	−1.2510	−0.7075	0.6515
O_2Hb	−0.9384	−0.6945	0.5721	1.7344
Caa_3	−0.1398	0.9945	0.1664	−0.9715

Table 2. *Multiplying factors for the NIRO-500 monitor (Hamamatsu)*

(The units are mM cm absorption^{-1}. The calculated concentration change is expressed in μM multiplied by the pathlength in cm.)

wavelength/ nm	772	830	842	909
HHb	1.2209	−0.8437	−0.7215	0.691
O_2Hb	−0.6945	−0.4851	−0.1382	1.9579
Caa_3	−0.0871	0.7550	0.5309	−1.1466

Although the optical path length (B) has been determined for the cerebellum by time-of-flight measurements of an ultrashort pulse of light across the head, there have been no estimates of B in kidneys or livers. As an estimate of B was not made in the present study only, relative changes in chromophore concentration (in μM cm) were measured across the organ or tissue under investigation. However, in the case of kidneys and livers, since we have compared two groups subjected to the same treatment, and the optical path length (and differential path length factor) have been shown to be constant within the NIR region despite gross changes in oxygenation and perfusion before and after death (Cope & Delpy 1988), the relative changes in chromophore concentration determined in this study are directly comparable between groups.

Changes in the concentrations of O_2Hb, HHb and cytochrome aa_3 (Caa_3) were calculated using multiplying factors (tables 1 and 2). The instrument noise on the cytochrome and haemoglobin traces, measured with a sensor on a standard absorbing block and with the path length factor set to 1.0, is less than 0.05 and 0.5 μM, respectively. A path length factor of 1 was used throughout.

A test on the reliability of these algorithms and on the independence of the Caa_3 response compared to O_2Hb has been described in detail (Cope *et al*. 1991; Thorniley *et al*. 1994*a*).

(*b*) *NIRS parameters and physiological significance*

Summation of the changes in the concentrations of O_2Hb and HHb provides a measure of changes in the total tissue haemoglobin (tHb), which reflect changes in tissue blood volume, hence giving an indication of perfusion and blood flow (Wickramasinghe *et al*. 1992;

Thorniley *et al.* 1994*b*; Irwin *et al.* 1994*a*, 1995; Thorniley *et al.* 1996*c*).

A further term, HbD or the oxygenation index can be derived as $[HbD] = [O_2Hb] - [HHb]$. This gives an indication of the net haemoglobin oxygenation status irrespective of any blood volume changes. This term is of great use following tissue transplantation in assessing the onset or recovery from venous congestion.

(*c*) *NIRS terminology*

The HHb and O_2Hb abbreviations are recommended in the National Committee for Clinical and Laboratory Standards.

(*d*) *Statistical analysis*

The mean changes in NIRS parameters at the end of the experimental period were determined and compared to baseline measurements. Data are presented as mean $\pm$ s.e.m. Statistical significance between groups was tested by unpaired Student's *t*-test using two-way analysis of variance and were considered significant when $p < 0.05$.

3. MODEL OF HEPATIC TRANSPLANTATION

(*a*) *Objectives and model*

Our objectives were first to determine if NIRS can be used to measure the changes in cerebral haemoglobin oxygenation and haemodynamics during rat hepatic isografting; and, second to determine if NIRS is sufficiently sensitive to detect the differences in cerebral oxygenation between animals isografted either with minimally-stored or with longer-preserved grafts. Cerebral measurements were made on two groups of rats in which the organs had been isografted either after flush with hypertonic citrate solution and minimal storage (25 min) at 1–2 °C (control, group 1) or after 24 h at 1–2 °C (group 2). From our previous work in this experimental model, we would expect 100% survival in the recipients of minimally stored livers, but only approximately 10% of the rats that receive 24 h stored livers survive with good hepatic function (Thorniley *et al.* 1995*b*). A further objective was to perform NIRS measurements on the liver post transplantation to enable comparison with cerebral measurements.

(*b*) *Surgical procedure*

Male Lewis rats (200–300 g) were used throughout. General surgical procedures were followed (Lee *et al.* 1973; Kamada & Calne 1979, 1983; Chaland *et al.* 1990). Surgical anaesthesia was maintained using enflurane (1–2%) with oxygen and air delivered via a face mask at 0.5 l min^{-1}. Arterial oxygen saturation was maintained at > 95% throughout the procedures (Ohmeda-Biox 3470). The temperature was controlled using a heating pad and monitored throughout using a rectal probe. Continuous F_iO_2 and intermittent blood PCO_2 and PO_2 determinations were made throughout. A Zeiss OPMI-6 operating microscope was used for all microsurgical procedures. Prior to surgery, donor rats were given 1 mg vitamin K i.v. and recipient rats 1 mg vitamin K i.v., 1 ml Haemaccel® i.v. (via the lingual vein) and 5 ml of 5% glucose subcutaneously.

(i) *Donor operation*

The procedure for removing the liver was described previously. The liver was stored in hypertonic citrate solution at 1–2 °C. The total time for the donor operation was 45 min, with a graft cold ischaemic time of 25 min (group 1, control), or 24 h (group 2, stored).

(ii) *Recipient operation*

For simplification the recipient procedures have been divided for description into five events and the effect of each of these events on the NIRS measurements are shown in figures 1 and 2 and in table 3.

(iii) *Preanhepatic phase*

Event 1

The rat was placed in a supine position and a midline incision made. All peritoneal attachments to the liver were removed. The phrenic vein was ligated and divided. The suprahepatic venacava (SVC) was freed from the underlying oesophagus. The BD (bile duct) was dissected free and divided at the liver level just distal to its bifurcation. The HA (hepatic artery) and gastroduodenal arteries were freed from the PV (portal vein) and the HA ligated and divided. The PV was freed from the surrounding tissue up to the origin of the pyloric vein. A small length of aorta (0.5 cm) was dissected free just distal to the mesenteric artery. The infrahepatic venacavae (IVC) was freed proximal to the right renal vein and the right adrenal vein was cauterized. The donor liver was placed in a petri dish containing 10 ml of 4 °C hypertonic citrate solution. The PV was perfused with 2 ml of 4.5% w/v human albumin solution at room temperature and the HA with 1 ml of the solution.

(iv) *Anhepatic phase*

Event 2

The recipient PV was clamped and the vein divided at its bifurcation with the liver hilum. The IVC was clamped and divided at the hepatic junction. The SVC was clamped with a rubber-shod mosquito clamp and was then divided at the hepatic junction. The recipient liver was removed.

Event 3

The donor liver was placed in the recipient in the orthotopic site. The SVC was anastomosed using continuous 8/0 monofilament nylon (Ethilon) sutures. The anastomosis was enveloped with Surgicel absorbable haemostatic gauze. The PV was anastomosed using a 10/0 monofilament nylon (Ethilon) continuous suture.

(v) *Postanhepatic with reperfusion*

Event 4A

Portal flow was then re-established and the mosquito clamp removed from the SVC. The IVC was anastomosed end-to-end; the clamp was removed and the IVC flow re-established.

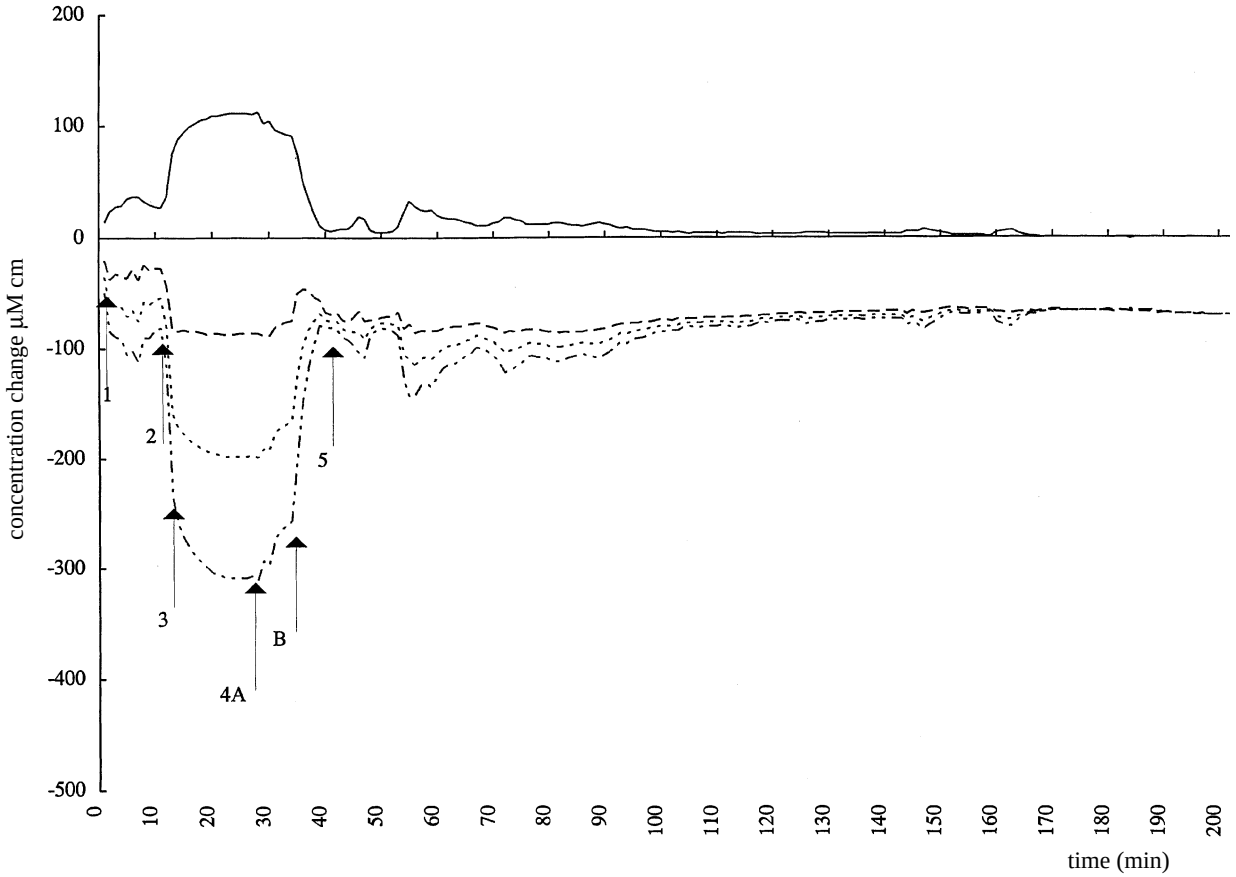

Figure 1. The effect of transplantation of a 25 min stored liver on cerebral NIRS measurements: — HHb, --- O_2Hb, –– tHb, –·––·– HbD. This is an example and not averaged data. As described in the methods section, the values are the changes in concentration in µM cm due to the given procedure from the initial baseline. The arrow numbers refer to the beginning of the new events. Event 1, preanhepatic phase: laparotomy—all vessels freed from attachments. Before clamping measurements taken in all the animals and the mean was determined. Event 2, anhepatic phase: the recipient portal vein, descending venacavae, suprahepatic and hepatic artery were clamped. The recipient liver was removed. Event 3, continuance of anhepatic phase: the donor liver was placed orthotopically. The SVC and PV were anastomosed but remained clamped. Event 4, postanhepatic phase with reperfusion. (A) release of portal and suprahepatic clamps. The IVC was anastomosed and the IVC flow re-established. (B) The aorta was clamped then anastomosed. Event 5, neohepatic phase: aortic clamp removed and arterial flow re-established. All flow re-established.

Event 4B

The donor artery was irrigated with heparinized saline and the recipient aorta clamped with a J-shaped Sugita clamp. An end-to-side aorta-to-aorta anastomosis was performed with a continuous 10/0 monofilament nylon suture.

(vi) *Neohepatic phase*

Event 5

The clamp was removed and arterial flow re-established. The mean times to completion of the SVC and PV and IVC anastomoses were approximately 9, 17 and 25 min, respectively. The arterial anastomosis took a further 10 min.

This therefore entailed NIRS measurements over 19, 27, 35 and 45 min (allowing for approximately 10 min baseline measurements prelaparotomy).

(*c*) *Experimental protocol*

In this study dual NIRS monitoring was performed both on the liver and on the brain. A CRITIKON™ NIRS instrument was used with a neonatal sensor with a 3.5 cm separation between the emitter and detector. This enabled transmission measurements in which the emitter sensor was on the back of the head and the detector placed approximately between the eyes. Hence the area of interrogation was skull plus brain and skin. Following a 10 min period of stable baseline the abdomen was opened and the surgical procedures were performed as described. NIRS measurements were made for 10 min prelaparotomy (baseline), during the harvesting of the recipient animal's liver and throughout the transplantation procedure.

An Hammmatsu (NIRO-500) instrument with small specially designed probes was used to enable transmission measurements of the rat liver to be made immediately before and after release of clamps following anastomosis.

4. RESULTS

(*a*) *The application of NIRS in the assessment of cerebral oxygenation in hepatic transplantation*

The effects of hepatic transplantation on the cerebral haemoglobin oxygenation and blood volume level can

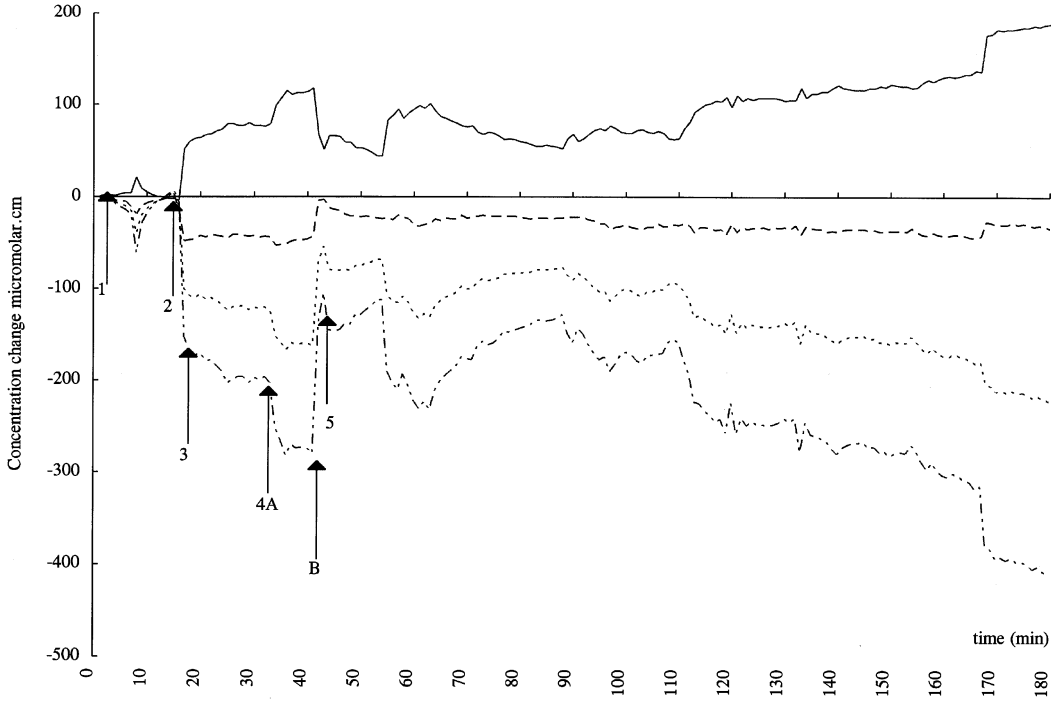

Figure 2. The effect of transplantation of a 24 h stored liver on cerebral NIRS measurements: — HHb, --- O_2Hb, –– tHb, –·–·– HbD. This is an example and not averaged data and is to the same scale as figure 1 to ease comparison. Events as for figure 2.

Table 3. *The resultant changes in concentration of the parameters at the beginning of the defined procedure*

(The p values refer to the levels of significance between the two groups at the defined stage in the transplantation procedures. The 60 min and 120 min events refer to the elapsed time period after laparotomy. 120 min post laparotomy refers to approximately 15 min post event 5.)

event	group	HHb (μM cm)	O_2Hb (μM cm)	tHb (μM cm)	HbD (μM cm)
event 2: effect of	1	13.43 ± 6.42	−30.85 ± 9.68	−17.14 ± 7.37	−44.28 ± 14.68
preanhepatic phase	2	0.8 ± 2.59	−27.6 ± 5.42	−26.6 ± 4.67	−29.2 ± 6.46
event 3: effect of anhepatic	1	81.71 ± 6.82	−150.7 ± 10.03	−68.7 ± 9.16	−232.43 ± 14.48
phase	2	86.6 ± 5.72	−163.8 ± 20.86	−77.4 ± 18.7	−224.0 ± 38.9
event 4: continuance of	1	95.14 ± 8.82	−183.43 ± 11.84	−87.71 ± 12.9	−278.5 ± 16.32
anhepatic phase	2	116.8 ± 3.61	−217.4 ± 21.59	−104.4 ± 20.02	−334.0 ± 22.9
event 5: post anhepatic	1	10.71 ± 9.8	−78.71 ± 20.87	−67.7 ± 17.7	−89.42 ± 27.3
effect of restoration	2	56.6 ± 10.2$^{p<0.03}$	−131.4 ± 23.2	−73.8 ± 20.3	−188.4 ± 29.7$^{p<0.04}$
of blood flow					
neohepatic effect—all flow	1	1.86 ± 10.75	−97.7 ± 22.05	−95.85 ± 20.5	−99.57 ± 28.05
re-established	2	77.2 ± 13.3$^{p<0.001}$	−176.4 ± 18.97$^{p<0.03}$	−99.0 ± 19.6	−253.6 ± 26.3$^{p<0.03}$
120 min post laparatomy	1	0.57 ± 6.73	−71.4 ± 23.9	−70.42 ± 22.0	−72.0 ± 27.4
	2	108 ± 27.7$^{p<0.001}$	−183.4 ± 30.3$^{p<0.015}$	−106.6 ± 21.3	−259.6 ± 48.2$^{p<0.005}$

be seen in figures 1 and 2. During the recipient stages of the transplantation procedures, the total haemoglobin, tHb (cerebral blood volume) decreased and the fall in the O_2Hb level was greater than the rise in the HHb level. Livers that had been stored for a minimal time (25 min) and transplanted resulted in the cerebral HHb level returning to baseline (figure 1). However, isografting of livers which had been stored for 24 h showed a continued divergence of the HHb and O_2Hb levels (figure 2).

From the onset of the anhepatic phase, the mean changes in NIRS parameters were significantly different from baseline. During this phase, it appeared that the changes affecting the cerebral haemodynamics in the two groups were similar and the difference insignificant. There was no significant difference between the fall in cerebral blood volume between the two groups at any stage post anastomosis over the time period monitored.

However there was a significant difference between the cerebral haemoglobin oxygenation index measured in the two groups of animals in the early postanhepatic phase (event 4) when the liver was supplied by blood solely from the portal vein (within 5 min). The oxygenation index had partially recovered in group 1, −89.42 ± 27.3 μM cm compared to −188.4 ± 29.7 μM cm in group 2, the longer-preserved grafts ($p < 0.04$). Similarly the cerebral deoxyhaemoglobin concentration was greater in group 2, 56.6 ± 10.2 μM cm compared to 10.71 ± 9.8 μM cm in group 1 ($p < 0.03$).

In the longer-preserved liver group the differential effect of portal reperfusion followed by arterial

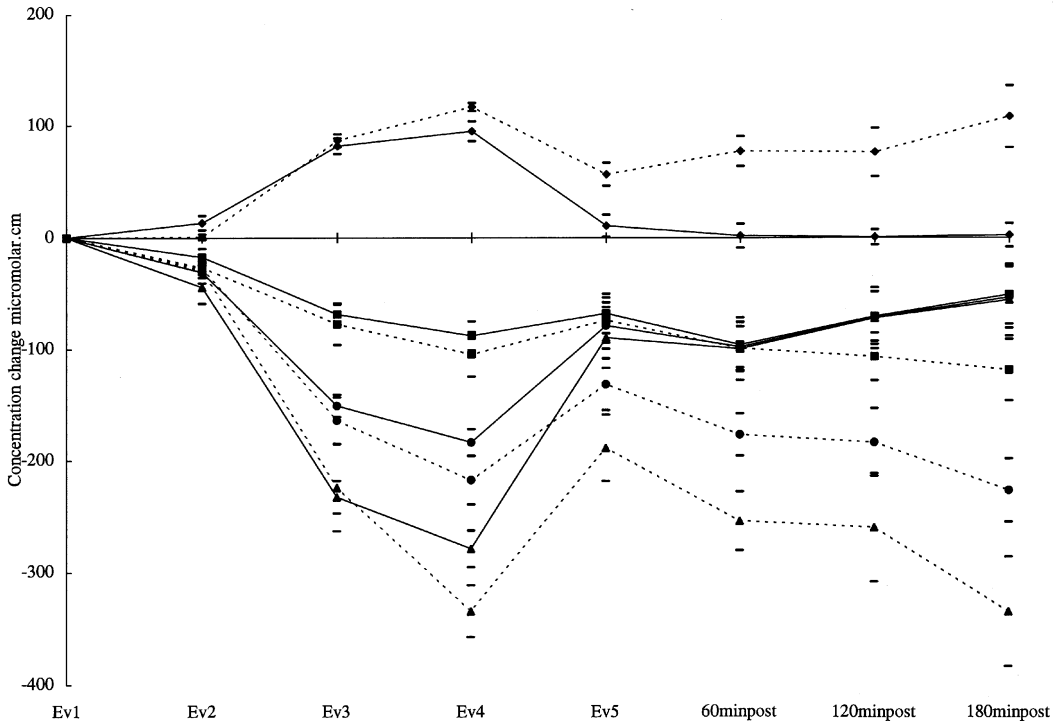

Figure 3. The effect of transplantation of a 25 min and 24 h stored liver on cerebral NIRS measurements: ▲ HbD, ● O_2Hb, ■ HHb, ◆ tHb. This is averaged data and shows the mean ± s.e.m. The solid line refers to group 1 and the dotted group to group 2.

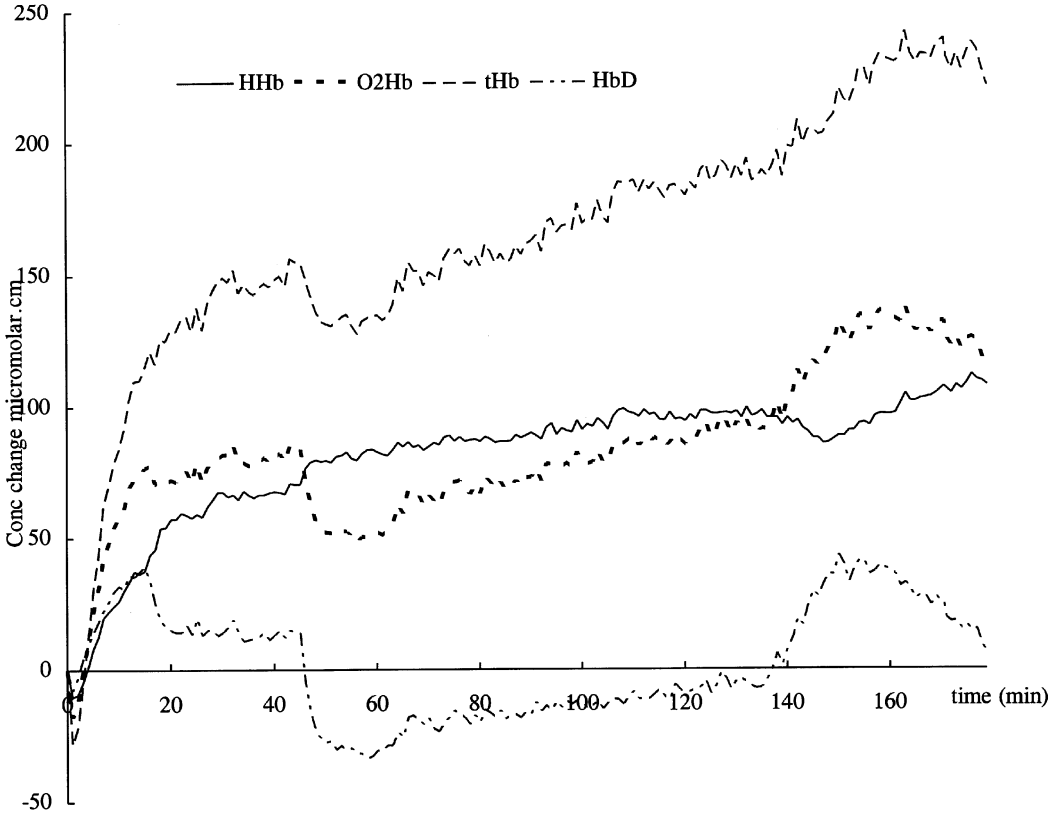

Figure 4. The changes in hepatic NIRS parameters in a 25 min stored transplanted liver: — HHb, --- O_2Hb, –– tHb, –·–·– HbD. This is an example and not averaged data.

clamping compared to the minimally stored livers was evident (figures 1 and 3); there was an initial fall by approximately 50% improvement in the net haemoglobin oxygenation index, HbD; but this improvement was only temporary. From then on, event 5, the neohepatic phase when the aortic clamp was released and the liver was supplied by blood from both the hepatic artery and portal vein, the differences between

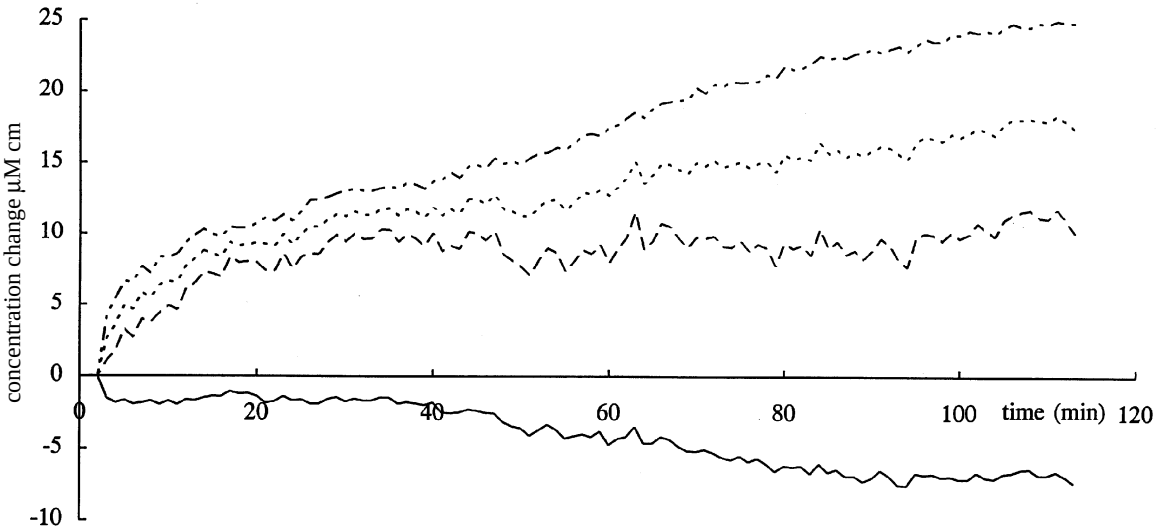

Figure 5. The changes in hepatic NIRS parameters in a 24 h stored transplanted liver: — HHb, --- O_2Hb, –– tHb, –·–·– HbD. This is an example and not averaged data.

the groups for each of the NIRS parameters became even more significant (table 3), with increasing divergence in the O_2Hb, HHb and the HbD. The recovery of the cerebral NIRS parameters, which was observed in animals transplanted with minimally stored livers, correlated with 100% survival whereas isografting of group 2 livers with the continued divergence of the HHb and O_2Hb signals correlated with poor viability following transplantation. Figure 3 shows the effect of transplantation of a 25 min and 24 h stored liver on cerebral NIRS measurements (this is averaged data and shows the mean ± s.e.m.).

(*b*) *NIRS measurements of the isografted liver*

Figure 4 shows the changes in hepatic NIRS measurements in a minimally stored transplanted liver. A rapid initial increase in O_2Hb and HHb was observed which then decreased over the monitoring period (3 h); hence HbD remained approximately zero. However in livers that had been stored for 24 h, figure 5, there was an increasing divergence in O_2Hb and HHb resulting in HbD progressively increasing which is indicative of little if any oxygen utilization.

5. DISCUSSION

During the anhepatic stages there may be little or no perfusion pressure to the mesenteric circulation and if this is prolonged significant ischaemia–reperfusion damage may occur to the intestinal mucosa leading to impairment of the barrier function and allowing intraluminal toxins access to the circulation (Eason & Potter 1990). This can then exacerbate the already compromised patient. This period is usually associated with much haemodynamic imbalance with the cardiac output reduced, as a result of reduction of venous return by about 50–60% (Steltzer *et al.* 1993). Raised intracranial pressure is a feature of hepatic encephalopathy (Potter *et al.* 1989; Carton *et al.* 1994). It has been observed that in a study of six patients undergoing orthotopic liver transplantation the intracranial pressure (ICP) recordings were at the lowest values in the anhepatic phase and raised significantly with reperfusion and in the first postoperative day (Potter *et al.* 1989). The aetiology of the rise in ICP following reperfusion may be related to abrupt inclusion of the ischaemic splanchnic and caval circuits into the circulation (Potter *et al.* 1989).

HbD can be considered to be an indicator of oxygen delivery and is affected by not only perfusion pressure but also by haemoglobin concentration. Since the total haemoglobin concentration, analogous to cerebral blood volume was not different between the groups then the reduced cerebral HbD concentration in the longer-preserved grafts is suggestive of either a greater ratio of HHb:O_2Hb in the blood reaching the brain or the brain has had to increase its metabolic demand. Caa_3 studies show a significantly greater level of reduction in the brains of animals transplanted with longer-preserved livers; this suggests that there is either insufficient oxygen being delivered or there is damage to the respiratory chain and the oxygen cannot be utilized (Schaefer & Biber 1993; Thorniley *et al.* 1994*a*, 1995*b*).

The significant difference in HbD within the first few minutes might facilitate the addition of drugs (e.g. ionotropes) to improve delivery since it has been reported clinically that improved delivery can frequently be associated with improved oxygen consumption (Shoemaker *et al.* 1993; Carton *et al.* 1994). Impaired oxygen transport is frequently the fundamental pathophysiological abnormality in many critical illnesses. In critical illness oxygen supply may not meet the demand and this can lead to functional and structural disturbances of organs and tissues (Shoemaker *et al.* 1993; Grant & Nimmo 1995). Reperfusion of a functioning liver is accompanied with an increase in oxygen consumption (Carton *et al.* 1994). This is in agreement with our results in that there is no measurable difference in the change in concentration between O_2Hb and HHb (HbD at zero) in livers which are functioning. However livers which have been subjected to maximal ischaemic damage

show no measurable oxygen utilization ($[O_2Hb] \gg [HHb]$). *In vitro* studies of mitochondrial activities show damage to complex I and IV and uncoupling of oxidative phosphorylation (Sammut *et al.* 1995). Primary graft non-function is seen within 4–11% of all hepatic transplantation cases; in these cases early re-transplantation is the only therapeutic option but these have poor prognosis (Carton *et al.* 1994). Hence the ability to monitor the organ rapidly can only be of benefit. This suggests that decreased cerebral haemoglobin oxygenation relative to utilization is a major problem in these hepatic transplantation procedures. The lack of recovery of the cerebral NIRS parameters in animals receiving 24 h stored livers can only be speculated. Possibilities are hepatic venous congestion resulting in systemic flow or cerebral oedema resulting in poor cerebral blood flow and perfusion.

The results show that disturbances in the cerebral circulation are evident within 2–5 min of the initial reperfusion of the liver, which is also commonly seen clinically, and indicates that NIRS could be used in the transplant situation enabling early intervention in the event of poor reflow. These findings indicate that the changes in NIRS parameters can be used to monitor changes in the perfusion to the brain which in turn has prognostic implications on survival. The authors are unaware of any other published information on NIRS monitoring of cerebral oxygenation during hepatic transplantation. Future studies will attempt to determine if changes in NIRS parameters associated with graft failure can be prevented to improve prognosis. These results are in agreement with earlier studies in which surface fluorometric measurements of NADH were performed during the hepatic transplantation procedure (Thorniley *et al.* 1995*b*). We observed a significant difference in the reduction state of NADH immediately post revascularization between the two groups; in the 24 h stored livers an abnormal maximal level of NADH oxidation (100%) was measured suggestive of mitochondrial uncoupling, substrate deficiency, complex 1 damage or a combination of these; and hence, less effective production of ATP (Taegtmeyer *et al.* 1985; Pelikan *et al.* 1987; McMillin-Wood *et al.* 1973). Complete NADH oxidation can also be attributed to either substrate deficiency or respiratory chain damage, the latter now considered to be a defect of complex 1 (Hardy *et al.* 1991; Veitch *et al.* 1992) and inability to catalyse the production of NADH. Thurman *et al.* (1988) also concluded that reperfusion of stored livers causes an oxygen-dependent alteration in hepatic microcirculation leading to necrosis. From our studies we believe that SF measurements could be useful in the clinical transplant situation in which damage may have occurred during storage and the first few minutes of reperfusion. Further studies are necessary to elucidate the mechanisms of change in hepatic redox state but we believe that non-invasive measurements of NADH could provide a valuable indication of hepatic metabolic integrity and function. Both NIRS and SF measurements can yield information regarding graft viability and the effect on the cerebral circulation.

In this paper we have attempted to describe the use of NIRS in the transplant situation. Although we have demonstrated that NIRS yields reliable, sensitive and reproducible results in our models it is essential that further investigations are carried out to consider the limitations of the technique for clinical use.

Many major transplantation techniques require hypotensive anaesthesia and there is a critical balance between the degree to which the blood pressure has to be lowered to facilitate raising and transplantation of the graft and the return to normotensive conditions to ensure the graft is not underperfused. In the recovery phase the circulatory state of the graft can be compromised by haemorrhage. Tissue under perfusion or shock following severe haemorrhage results in local hypoxia and metabolic dysfunction and if uncorrected can be a trigger for sepsis syndrome.

Apart from the obvious use of NIRS in measuring changes in haemoglobin oxygenation and haemodynamics its use in assessing mitochondrial dysfunction has become more apparent; in particular since in the last few years the respiratory chain complexes have been found to be sensitive to ischaemia and reperfusion. Hence it is an enormous benefit not only to make predictions about oxygen supply but oxygen utilization as well. NIRS is extremely easy to use, yielding reproducible continuous results and would be of great use in the intensive care unit in investigating the aforementioned problems. This could be of enormous benefit in assessing the balance between supply and utilization of oxygen in tissues especially in pathophysiological states such as sepsis or circulatory compromise. NIRS can be useful intra and postoperatively or in the intensive care unit as a tool in appraising the benefits of fluid or blood replacement.

We acknowledge the support of Johnson & Johnson Medical, UK, and M.S.T. thanks Dr Keith Dalziel, F.R.S., her D.Phil. supervisor, and her family, and in particular her mother, Phyllis Mather, for continuous support.

REFERENCES

Bowers, J. L., Lanir, A., Metz, K. R. *et al.* 1992 ^{23}Na- and ^{31}P-NMR studies of perfused mouse liver during nitrogen hypoxia. *Am. J. Physiol.* **262**, G636–G644.

Brunori, M., Antonini, E. & Wilson, M. T. 1981 In *Metal ions in biological systems XXIII* (ed. H. Siegel), pp. 187–228. New York: Marcel Dekker.

Cadenas, E., Boveris, A., Ragan, C. I. & Stoppani, O. K. 1977 Production of superoxide radicals and H_2O_2 by NADH ubiquinone reductase and ubiquinone cyt-*c* reductase from beef heart Mitochondria. *Arch. Biochem. Biophys.* **180**, 248–257.

Calman, K. C., Quinn, R. O. & Bell, P. R. 1973 In *Organ preservation* (ed. D. E. Pegg), pp. 225–240. London: Churchill Press.

Carton, E. G., Plevak, D. J., Kranner, P. W., Rettke, S. R., Geiger, H. J. & Coursin, D. B. 1994 Perioperative care of the liver transplant patient. *Anesth. Analg.* **78**, 382–399.

Chaland, P., Braillon, A. & Gaudin, C. 1990 Orthotopic liver transplantation with hepatic artery anastomosis. *Transplantation* **49**, 675–678.

Chance, B., Schoener, B., Oshino, R., Itshak, F. & Nakase, Y. 1979 Oxidation–reduction ratio studies of mitochondria in freeze-trapped samples. *J. Biol. Chem.* **254**, 4764–4771.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Chance, B. & Williams, G. R. 1955 The respiratory chain and oxidative phosphorylation. *J. Biol. Chem.* **217**, 383–393.

Cope, M. & Delpy, D. T. 1988 A system for long-term measurement of cerebral blood and tissue oxygenation on newborn infants by near infra-red transillumination. *Med. Biol. Eng. Comput.* **26**, 289–294.

Cope, M., Van der Zee, P., Essenpreis, M., Arridge, S. R. & Delpy, D. T. 1991 Data analysis methods for near infra-red spectroscopy of tissue: problems in determining the relative cytochrome aa_3 concentration. *Proc. SPIE* **1431**, 251–261.

du Pleiss, A. J., Newburger, J., Jonas, R. A. *et al.* 1995 Cerebral oxygen supply and utilisation during infant cardiac surgery. *Ann. Neurol.* **37**, 488–497.

Eason, J. & Potter, D. 1990 Anaesthesia for liver transplantation (2). In *Anaesthesia review* (ed. L. Kaufman), pp. 147–160. Edinburgh: Churchill Livingstone.

Edwards, A. D., Brown, G. C., Cope, M. *et al.* 1991 Quantification of concentration changes in neonatal human cerebral oxidised cytochrome oxidase. *J. Appl. Physiol.* **71**, 1907–1913.

Essenpries, M., Cope, M., Elwell, C. E., Arridge, S. R., Van der Zee, P. & Delpy, D. T. 1993 In *Optical imaging of brain function and metabolism* (ed. U. Dirnagle), pp. 9–20. New York: Plenum Press.

Frank, C. H., Barlow, C. H. & Chance, B. 1976 Oxygen delivery in perfused rat kidney: NADH fluorescence and renal functional state. *Am. J. Physiol.* **231**, 1082–1089.

Fuller, B. J., Busza, A. L. & Proctor, E. 1990 Possible resuscitation of liver function by hypothermic reperfusion *in vitro* after prolonged (24-hour) cold preservation – a ^{31}P NMR study. *Transplantation* **50**, 511–535.

Fuller, B. J., Gower, J. D. & Green, C. J. 1988 Free radical damage and organ preservation, fact or fiction? *Cryobiology* **25**, 377–393.

Grant, I. S. & Nimmo, G. R. 1995 Oxygen transport. *Care of the Critically Ill* **11**, 67–70.

Hardy, L., Clark, J. B., Darley-Usmar, V. M., Smith, D. R. & Stone, D. 1991 Reoxygenation-dependent decrease in mitochondrial NADH: CoQ reductase (Complex I) activity in the hypoxic/reoxygenated rat heart. *Biochem. J.* **274**, 133–137.

Harrison, D. H., Girling, M. & Mott, G. 1983 Methods of assessing the viability of free flap transfer during the post-operative period. *Clin. Plast. Surg.* **10**, 21–36.

Inomoto, T., Tanaka, A., Mori, S. *et al.* 1994 Changes in the distribution of the control of the mitochondrial oxidative phosphorylation in regenerating rabbit liver. *J. Biochem.* **80**, 875–881.

Irwin, M. S., Thorniley, M. S., Dore, C. & Green, C. J. 1995 Near infra-red spectroscopy: a non-invasive monitor of perfusion and oxygenation within the microcirculation. *Br. J. Plas. Surg.* **48**, 14–22.

Irwin, M. S., Thorniley, M. S. & Green, C. J. 1994*a* Application of near infra-red spectroscopy for monitoring oxygenation within the microcirculation of free flaps. *Microsurgery* **15**, 213.

Irwin, M. S., Thorniley, M. S. & Green, C. J. 1994*b* A non-invasive method for distinguishing between arterial, venous and total vascular occlusion. *Clin. Sci.* **87** (Suppl. 31), 26–27.

Jennings, R. B. & Ganote, C. E. 1976 Mitochondrial structure and function in acute myocardial ischemic injury. *Circ. Res.* **38**, 80–89.

Jeroudi, M. O., Hartley, C. J. & Bolli, R. 1994 Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. *Am. J. Cardiol.* **73**, 2B–6B.

Jöbsis, F. F. 1977 Non-invasive infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* **198**, 1264–1268.

Kamada, N. & Calne, R. Y. 1979 Orthotopic liver transplantation in the rat. *Transplantation* **28**, 47–50.

Kamada, N. & Calne, R. Y. 1983 A surgical experience with five hundred and thirty liver transplants in the rat. *Surgery* **93**, 64–69.

Kamiike, W., Burdelski, M., Steinhoff, G., Ringe, B., Lauchart, W. & Pichlmayr, R. 1988 Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. *Transplantation* **45**, 138–143.

Kono, Y., Ozawa, K., Tanaka, J., Ukikusa, M., Takeda, H. & Tobe, T. 1981 Significance of mitochondrial enhancement in restoring hepatic energy charge after revascularization of isolated ischaemic liver. *Transplantation* **33**, 150–155.

Lane, N., Thorniley, M. S., Fuller, B. & Green, C. J. 1996*a* The effect of mannitol and polyethylene glycol on the haemodynamic and diuretic actions of frusemide. *Transplantation* **62**, 575–582.

Lane, N., Thorniley, M. S., Manek, S., Fuller, B. & Green, C. J. 1996*b* Secondary ischaemia and tissue damage in transplanted stored kidneys. *Transplantation* **61**, 689–696.

Lanir, A., Clouse, M. E. & Lee, R. G. L. 1987 Liver preservation for transplant: Evaluation of hepatic energy metabolism by ^{31}P NMR. *Transplantation* **43**, 786–790.

Lanir, A., Jenkins, R. L., Caldwell, C., Lee, R. G. L., Khettry, U. & Clouse, M. E. 1988 Hepatic transplantation survival: correlation with adenine nucleotide level in donor liver. *Hepatology* **8**, 471–475.

Lee, S. A., Charters, C., Chandler, J. G. & Orloff, M. J. 1973 A technique for orthotopic liver transplantation in the rat. *Transplantation* **16**, 664–669.

Levy, W. J., Levine, S. & Chance, B. 1995 Near infra-red measurement of cerebral oxygenation: Correlation with electroencephalographic ischaemia during ventricular fibrillation. *Anaesthesiology* **83**, 738–746.

Livera, N. L., Spencer, S. A., Thorniley, M. S., Wickramasinghe, S. A. & Rolfe, P. 1991*a* Effects of hypoxaemia and bradycardia on neonatal cerebral haemodynamics. *Arch. Dis. Childh.* **66**, 376–380.

Livera, N. L., Spencer, S. A., Wickramasinghe, Y. A. B. D., Thorniley, M. S. & Rolfe, P. 1990 Cerebral haemodynamic changes in the newborn detected using near infra-red spectroscopy. *Early Human Development* **22**, 173.

Livera, N.L., Spencer, S.A., Wickramasinghe, Y. A. B. D., Thorniley, M. S. & Rolfe, P. 1991*b* Comparison of blood volume changes detected using near infra-red spectroscopy and venous occlusion plethysmography. *Early Human Development* **25**, 53.

Livera, N. L., Spencer, S. A., Wickramasinghe, Y. A. B. D., Thorniley, M. S. & Rolfe, P. 1992 Cyclical fluctuations in neonatal cerebral blood volume measured by near infra-red spectroscopy. *Arch. Dis. Childh.* **67**, 62–63.

McMillin-Wood, J., Sordahl, L. A., Lewis, R. M. & Schwartz, A. 1973 Effect of chronic myocardial ischaemia on the activity of carnitine palmitylcoenzyme A transferase of isolated canine heart mitochondria. *Circ. Res.* **32**, 340–347.

Marubayashi, S., Takenaka, M., Dohi, K., Ezaki, H. & Kawasaki, T. 1980 Adenine nucleotide metabolism during hepatic ischemia and subsequent blood reflow periods and its relation to organ viability. *Transplantation* **30**, 294–296.

Marzi, I., Knee, J., Menger, M. D., Harbauer, G. & Buhren, V. 1991 Hepatic microcirculatory disturbances due to portal vein clamping in the orthotopic rat liver transplantation model. *Transplantation* **52**, 432.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Mayevesky, A. 1984 Brain NADH redox state monitored *in vivo* by fibre optic surface fluorometry. *Brain Res. Reviews* **7**, 49–68.

Mayevsky, A., Zarchin, N., Kaplan, H., Harveri, I. J., Haselgroove, H. & Chance B. 1983 Brain metabolic responses to ischaemia in the Mongolian gerbil: *in vivo* and freeze trapped redox scanning. *Brain Res.* **276**, 95–97.

O'Conner, M. J. 1977 Origin of labile NADH tissue fluorescence. In *Oxygen and physiological function* (ed. F. F. Jöbsis), pp. 90–99. Dallas: Professional Information Library.

Ohdan, H., Fukuda, Y., Suzuki, S., Amemiya, H. & Dohi, K. 1995 Simultaneous evaluation of nitric oxide synthesis and tissue oxygenation in rat liver allograft rejection using near infra-red spectroscopy. *Transplantation* **56**, 530–535.

Okamura, R., Tanaka, A., Uyama, S. & Ozawa, K. 1992 Low-temperature fluorometric technique for evaluating the viability of rat liver grafts after simple cold storage. *Transplant Int.* **5**, 165–169.

Ozaki, N., Tokunaga, Y., Ikai, I. *et al.* 1989 Pyridine nucleotide fluorometry in preserved porcine liver with fluorocarbon emulsion. *Transplantation* **48**, 198–201.

Parsons, W. J., Rembert, J. C., Bauman, R. P., Greenfield, J. C. & Piantadosi, C. A. 1990 Dynamic mechanisms of cardiac oxygenation during brief ischaemia and reperfusion. *Am. J. Physiol.* **259**, 1477–1485.

Pelikan, P. C. D., Niemann, J. T., Xia, G., Jagels, G. & Criley, J. M. 1987 Enhancement of mitochondrial oxidative phosphorylation capability by hypoperfusion in isolated perfused rat heart. *Circ. Res.* **61**, 880–888.

Potter, D., Peacher, T. & Eason, J. 1989 Intracranial pressure monitoring during orthotropic liver transplantation for acute liver failure. *Transplant. Proc.* **21**, 3528.

Russell, R. C., Roth, A. C., Kucan, J. O. & Zook, E. G. 1989 Reperfusion injury and oxygen free radicals. *J. Reconstr. Microsurg.* **5**, 79.

Sammut, I. A., Thorniley, M. S., Simpkin, S., Bates, T. E. & Green, C. J. 1995 Changes in hepatic mitochondrial metabolic parameters following transplantation. *Biochem. Soc. Trans.* **23**, 527S.

Schaefer, C. F. & Biber, B. 1993 Effects of endotoxaemia on the redox level of brain cytochrome *aa*3 in rats. *Circ. Shock* **40**, 1–8.

Shaw B. W., Gordon R. D., Iwatsuki S. & Starzl T. E. 1986 Hepatic retransplantation. *Transplant Proc.* **17**, 264.

Shoemaker, W. C., Montgomery, E. S., Kaplan, E. & Elwyn, D. H. 1993 Physiological patterns in surviving and non-surviving patients. *Arch. Surg.* **106**, 630–636.

Sinclair, J. S., Thorniley, M. S. & Green, C. J. 1994 A new tubed myocutaneous porcine flap for investigation of metabolic events following burn injury. *Microsurg.* **15**, 213–214.

Steltzer, H., Hiesmayr, M., Tuchy, G. & Zimpfer, M. 1993 Perioperative liver graft function: the role of oxygen transport and utilisation. *Anesth. Analg.* **76**, 574–579.

Taegtmeyer, H., Roberts, A. F. C. & Raine, A. E. G. 1985 Energy metabolism in reperfused heart muscle: metabolic correlates to return of function. *J. Am. Coll. Cardiol.* **6**, 864–870.

Takei Y., Marzi I., Gao W., Gores G. J., Lemasters J. J. & Thurman R. G. 1991 Leucocyte adhesion and cell death following orthotopic liver transplantation in the rat. *Transplantation* **51**, 959–965.

Tashiro, H., Suski, H. & Kanashiro, M. 1993 A new method for determining graft function after liver transplantation using near infra-red spectroscopy. *Transplantation* **56**, 1261–1263.

Thorniley, M. S., Lahiri, A., Glenville, B. *et al.* 1996*a* Non-invasive measurement of cardiac oxygenation and haemodynamics during transient episodes of coronary artery occlusion and reperfusion. *Clin. Sci.* **91**, 51–58.

Thorniley, M. S., Lane, N. J. & Green, C. J. 1994*a* Non-invasive measurement of respiratory chain dysfunction following hypothermic renal storage and transplantation. *Kidney Int.* **45**, 1489–1496.

Thorniley, M. S., Lane, N., Simpkin, S. Fuller, B., Jenabzadeh, M. Z. & Green, C. J. 1996*b* Monitoring of mitochondrial NADH levels by surface fluorimetry as an indication of ischaemia during hepatic and renal transplantation. In *Oxygen transport to tissue XVII* (eds C. Ince, J. Kesecioglu, L. Telci & K. Apkir), pp. 431–444. New York: Plenum Press.

Thorniley, M. S., Irwin, M. S. & Green, C. J. 1994*b* Measurement of skeletal muscle blood flow using near infra-red spectroscopy and validation by comparison with laser Doppler flowmetry. *Clin. Sci.* **87** (Suppl. 31), 26–27.

Thorniley, M. S., Sammut, I. A., Simpkin, S. & Green, C. J. 1995*a* An investigation into the effect of hepatic transplantation on cerebral oxygenation and haemodynamics. *Biochem. Soc. Trans.* **23**, 525S.

Thorniley, M. S., Simpkin, S., Fuller, B., Jenabzadeh, M. Z. & Green, C. J. 1995*b* Monitoring of surface mitochondrial NADH levels as an indication of ischaemia during liver isograft transplantation. *Hepatology* **21**, 1602–1609.

Thorniley, M. S., Sinclair, S. J. & Green, C. J. 1995*c* The use of a critikon cerebral redox research monitor model 2001 for assessing flap viability during reconstructive surgery *Clin. Sci.* **89**, 32P.

Thurman, R. G., Marzi, I., Seitz, G., Thies, J., Lemasters, J. J. & Zimmerman, F. 1988 Hepatic reperfusion injury following orthotopic liver transplantation in the rat. *Transplantation* **46**, 502–506.

Tokunaga Y., Ozaki N., Wakashiro, S. *et al.* 1987 Fluorometric study for the non-invasive determination of cellular viability in perfused rat liver. *Transplantation* **44**, 701–706.

Tokunaka, A., Tanaka, A., Kitai, T., Yamaoka, Y., Tanaka, K. & Ozawa, K. 1993 Delayed oxidation of intramitochondrial pyridine nucleotide oxido–reduction state as compared with tissue oxygenation in human liver transplantation (abstract). *Sixth Congr. Euro. Soc. for Organ Transplantation*, **129**.

Turrens, J. F., Freeman, B. A. & Crapo, J. D. 1982 Hyperoxia increases H_2O_2 release by lung mitochondria and microsomes. *Arch. Biochem. Biophys.* **217**, 411–421.

Veitch, K., Homobroeckx, A., Caucheteux, D., Pouleur, H. & Hue, L. 1992 Global ischaemia induces a biphasic response of the mitochondrial respiratory chain. *Biochem. J.* **281**, 709–715.

Wickramasinghe, Y. A. B. D., Livera, L. N., Spencer, S. A., Rolfe, P. & Thorniley, M. S. 1992 Plethysmographic validation of near infrared spectroscopic monitoring of cerebral blood volume. *Arch. Dis. Childh.* **67**, 407–411.

Wray, S., Cope, M., Delpy, D. T., Wyatt, J. S. & Reynolds, E. O. R. 1988 Characterization of the near infrared absorption spectra of cytochrome aa_3 and haemoglobin for the non-invasive monitoring of cerebral oxygenation. *Biochim. Biophys. Acta* **933**, 184–192.

Wyatt, J. S., Cope, M., Delpy, D. T. *et al.* 1990 Measurement of optical path length for cerebral near-infrared spectroscopy in newborn infants. *Devl Neurosci.* **12**, 140–144.

Wyatt, J. S., Edwards, A. D., Azzopardi, D. & Reynolds, E. O. R. 1989 Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic–ischaemic brain injury. *Arch. Dis. Childh.* **64**, 953–963.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES