Quantitation of cerebral blood volume in human infants by near-infrared spectroscopy

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WYATT, J. S., M. COPE, D. T. DELPY, C. E. RICHARDSON, A. D. EDWARDS, S. WRAY, AND E. O. R. REYNOLDS. *Quantitation of cerebral blood volume in human infants by near-infrared spectroscopy.* J. Appl. Physiol. 68(3): 1086–1091, 1990.—Current methods for measuring cerebral blood volume (CBV) in newborn infants are unsatisfactory. A new method is described in which the effect of a small change (5–10%) in arterial oxygen saturation (Sa_{O_2}) on cerebral oxyhemoglobin [HbO_2] and deoxyhemoglobin [Hb] concentration is observed by near-infrared (NIR) spectroscopy. Previous experiments in which the NIR absorption characteristics of HbO_2 and Hb and the pathlength of NIR light through the brain were defined allowed changes in [HbO_2] and [Hb] to be quantified from the Beer-Lambert law. It is shown here that CBV can then be derived from the expression $CBV = (\Delta[HbO_2] - \Delta[Hb])/(2 \cdot \Delta Sa_{O_2} \cdot H \cdot R)$, where H is the large vessel total hemoglobin concentration and R the cerebral-to-large vessel hematocrit ratio. Observations on 12 newborn infants with normal brains, born at 25–40 wk of gestation and aged 10–240 h, gave a mean value for CBV of 2.22 ± 0.40 (SD) ml/100 g, whereas mean CBV was significantly higher 3.00 ± 1.04 ml/100 g in 10 infants with brain injury born at 24 to 42 wk of gestation and aged 4–168 h ($P < 0.05$).

newborn infant; hemoglobin; arterial oxygen saturation

SEVERAL TECHNIQUES for the determination of cerebral blood volume (CBV) in human subjects have been described. These techniques employ radioiodine-labeled albumin (19), ^{51}Cr-labeled erythrocytes (27), X-ray computed tomography with an intravascular contrast agent (17), gamma emission tomography with ^{99}Tc-labeled erythrocytes (16), and more recently positron and single-photon emission tomography with various radionuclides as intravascular markers (9, 18, 21, 23, 28). All these techniques have the disadvantage of employing ionizing radiation and are only capable of providing intermittent information about CBV. Optical spectroscopy using visible and near-ultraviolet light has been employed to monitor changes in regional CBV in exposed cerebral cortex (12), but this technique is not applicable to the intact head. Near-infrared (NIR) spectroscopy, first described in 1977 (11), enables transillumination of the intact head and offers the possibility of continuous noninvasive quantitation of CBV. Because of the large distance between the sites of light entry and exit and the high degree of scattering in biological tissue, NIR spectroscopy provides a measure of global CBV but is very insensitive to regional fluctuations. This technique has been employed by Jobsis and others to monitor changes in CBV on an arbitrary scale in animals (31), human infants (1, 2), and adults (7, 8), but quantitation of CBV has not been possible. In 1986 we suggested, in a preliminary report, that quantitative determination of CBV by NIR spectroscopy was feasible (34). We describe here a technique for absolute quantitation of CBV in human infants together with the results of observations on 22 infants.

METHODS

Theory. NIR transmission spectroscopy through tissue over distances of up to 8 cm is possible because of the relative transparency of biological tissue to light in the NIR region (750–1,000 nm). The newborn infant's head has a biparietal diameter of 5–9 cm depending on gestational age, and thus transillumination is feasible in many infants. Partial transmission spectroscopy with the optical fibers positioned orthogonally may be performed on those infants in whom linear transillumination is not practicable. Absorption due to the main cerebral chromophores, oxyhemoglobin (HbO_2), and deoxyhemoglobin (Hb) can be quantitated in the following manner.

The Beer-Lambert law describing optical absorption when applied to a homogeneous scattering medium can be expressed as

$$\text{absorption (in OD)} = (a \cdot c \cdot L \cdot B) + G$$

where OD is optical density, a is the absorption coefficient ($mM^{-1} \cdot cm^{-1}$) of the chromophore, c is its concentration (mM), L is the distance between the points where light enters and leaves the medium (cm), B is a pathlength factor, which allows for the increased optical pathlength in a scattering medium, and G is a geometry-dependent factor. If a, L, and B are known and G remains constant, changes in chromophore concentration can be calculated from absorption changes from the formula

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

We have previously described an algorithm for the derivation of concentration changes in HbO_2 and Hb in the brains of newborn infants and experimental animals (32). The absorption coefficients of HbO_2 and Hb were obtained from studies of lysed human blood. The pathlength factor *B* was deduced initially from the NIR absorption by water in transmission spectroscopy of the rat head (32). More recently, time of flight studies in live rats (5) and postmortem infants (33) have allowed direct measurement of the optical pathlength. With the use of advanced optoelectronic techniques it is possible to measure the time taken for an extremely short pulse of near-infrared light to traverse the intact head. From the speed of light and the refractive index of the tissue it is possible to calculate the mean pathlength taken by photons in traversing the head and thus determine the factor *B*. Postmortem measurements in six preterm infants have given a mean value of 4.4 ± 0.3 (SD) (33). For the purpose of this study we have assumed a value for *B* of 4.4. A value for brain density of 1.05 (20) was also assumed, allowing changes in cerebral hemoglobin concentration to be converted from millimoles per liter to millimoles per 100 grams of tissue. Thus changes in oxy- and deoxyhemoglobin concentration ($\Delta[HbO_2]$ and $\Delta[Hb]$) could be derived from absorption changes across the head and alterations in CBV deduced from changes in total hemoglobin concentration ($\Delta[HbO_2] + \Delta[Hb]$).

Absolute quantitation of CBV could then be obtained by observing the effects of a small change in arterial saturation (Sa_{O_2}) on $[HbO_2]$ and $[Hb]$. If the alteration in Sa_{O_2} (ΔSa_{O_2}) is sufficiently small, it can be assumed that there will be no accompanying change in CBV, cerebral blood flow, or cerebral oxygen consumption (see DISCUSSION). Because the extraction of oxygen at each point in the cerebral circulation is absolutely dependent on blood flow and oxygen consumption, it can be seen that the oxygen saturation within each vascular compartment will change by the same amount (ΔSa_{O_2}). (Changes in the amount of oxygen dissolved in plasma may be ignored if the arterial saturation is <95%.) Thus the change in cerebral oxyhemoglobin concentration ($\Delta[HbO_2]$) will be equal to the product of the total cerebral hemoglobin concentration [tHb] and the change in Sa_{O_2} expressed as a fraction

$$\Delta[HbO_2] = [tHb] \cdot \Delta Sa_{O_2}$$

Because it is assumed that CBV does not change during the maneuver, the changes in $[HbO_2]$ and $[Hb]$ must be equal and opposite. Hence

$$\Delta[Hb] = -[tHb] \cdot \Delta Sa_{O_2}$$

Therefore

$$[tHb] = (\Delta[HbO_2] - \Delta[Hb])/(2 \cdot \Delta Sa_{O_2})$$

Mean CBV in milliliters per 100 grams of tissue may be calculated from [tHb] if the large vessel hemoglobin concentration (H) and the cerebral-to-large vessel hematocrit ratio (R) are known (see DISCUSSION)

$$CBV = [tHb]/(H \cdot R)$$

Thus

$$CBV = (\Delta[HbO_2] - \Delta[Hb])/(2 \cdot \Delta Sa_{O_2} \cdot H \cdot R)$$

If $[HbO_2] - [Hb]$ is plotted against Sa_{O_2}, a straight line is obtained with a gradient proportional to CBV.

Infants studied. Twenty-two newborn infants who were admitted to the Neonatal Unit of University College Hospital were studied; 13 were male and 9 were female. Their gestational ages ranged from 24 to 43 (median 36) wk, and birth weights from 730 to 3,900 (median 1,780) g. Their principal diagnoses are listed in the APPENDIX. On the basis of clinical examination and cranial ultrasound scans, 12 infants were thought to have normal brains, and 10 had evidence of cerebral injury (Table 1). Of the 10 infants with brain injury, 4 subsequently died and 5 of the 6 survivors developed cerebral atrophy. None of the infants with normal brains developed cerebral atrophy. NIR spectroscopy was performed at a postnatal age of 4–240 (median 48) h, and recordings were continued for 2–10 (median 4) h. Three infants were breathing spontaneously, and 19 infants were receiving mechanical ventilation.

This study was approved by the University College London Faculty of Clinical Science Committee on the Ethics of Clinical Investigation, and parental consent was obtained before each investigation.

NIR spectroscopy measurements. We have previously described a portable apparatus we have built for NIR transmission spectroscopy of the brain in infants (3). This apparatus was employed for studies in 12 infants, and a commercial prototype of this instrument (Hamamatsu Photonics KK, NIR1000) was employed in the remaining 10 studies. Figure 1 shows a simplified diagram of the apparatus. NIR light from laser diodes was directed through a flexible fiber optic bundle into the head at a site equidistant from the anterior fontanelle and the external auditory meatus. Light emerging from the opposite side of the head was conveyed by an identical bundle to a photomultiplier tube operating in photon-counting mode. The ends of the fibers (optodes) were attached to the scalp by using double-sided adhesive

TABLE 1. *Cerebral blood volume results*

Infant No.	No. of Observations	CBV Mean, ml/100 g	CBV Range, ml/100 g
	Infants with normal brains		
1	1	2.69	
2	1	1.90	
3	3	2.12	1.53–2.85
4	1	2.49	
5	1	2.03	
6	1	2.70	
7	1	1.38	
8	2	2.63	2.27–2.99
9	1	1.88	
10	1	2.40	
11	4	2.52	0.82–3.64
12	4	1.97	1.59–2.39
	Infants with brain injury		
13	2	3.40	2.96–3.85
14	2	2.30	2.06–2.54
15	1	2.54	
16	4	1.74	1.17–2.21
17	3	2.53	2.23–2.89
18	1	4.22	
19	1	2.07	
20	3	4.94	3.84–5.52
21	2	2.42	1.86–2.97
22	3	3.83	3.71–4.01

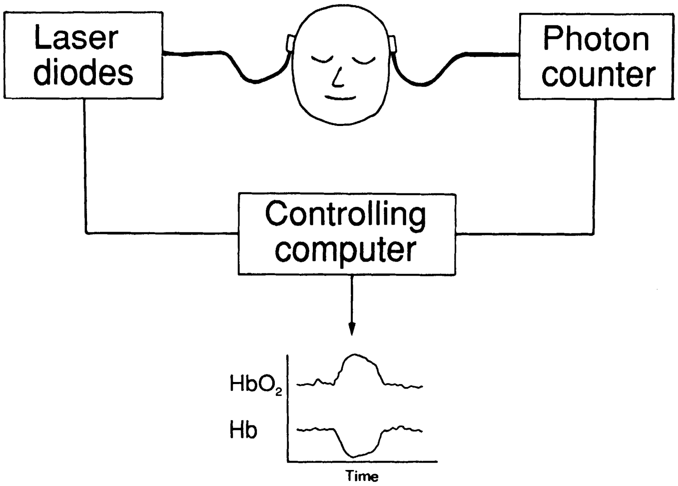

FIG. 1. Block diagram of near-infrared spectroscopy apparatus.

rings, and optical contact was ensured by using standard ultrasound contact gel. In the 10 infants whose biparietal diameters exceeded 8 cm, insufficient signals were obtained with this positioning, and the transmitting optode was therefore attached to the scalp just lateral to the anterior fontanelle, making an angle of 90° with the receiving fiber. The distance between the optodes was measured with mechanical calipers. To prevent interference from background illumination the head was swathed in a light-tight bandage. An optical cut-out device was employed to switch off the laser diodes if the transmitting bundle became detached from the head. The total light power density emitted from the end of the transmitting fiber bundle was ~20 mW/cm^2, and the calculated amount of energy absorbed by brain tissue was more than one order of magnitude below British Standards Institute safety limits (BS4803).

A controlling computer calculated the changes in optical absorption at each wavelength and converted these into changes in $[HbO_2]$ and [Hb] as described above. Readings were obtained at intervals of between 1 and 15 s and were displayed instantaneously at the cotside and recorded on computer disk for subsequent analysis. Arterial oxygen and carbon dioxide tensions were estimated continuously with transcutaneous electrodes (Novametrix model 850) calibrated against arterial blood samples. Sa_{O_2} was recorded by pulse oximeter (Novametrix model 500) or subsequently calculated from the transcutaneous oxygen tension with a standard formula (24). These signals were simultaneously sampled and recorded by the NIR apparatus for later analysis. An arterial blood sample was taken for hemoglobin estimation and the hemoglobin concentration was converted from grams per deciliter to millimoles per liter using a molecular weight of 64,500. A value of 0.69 for the cerebral-to-large vessel hematocrit ratio was assumed (18). Alterations in near-infrared indexes were observed during Sa_{O_2} changes of 5–10% in the range 82–95% induced by a small transient alteration in the inspired oxygen fraction. For each induced alteration in Sa_{O_2} an estimate of CBV was obtained using the formula described above. CBV estimation was performed only if the clinical condition of the infant remained stable during the maneuver and transcutaneous carbon dioxide tension remained constant within 0.5 kPa.

RESULTS

Figure 2 shows representative changes in $[HbO_2 - Hb]$ during a transient alteration in Sa_{O_2}. Figure 3 shows changes in $[HbO_2 - Hb]$ plotted against Sa_{O_2} in the same infant with the regression line and CBV calculated from the slope of this line.

A total of 43 observations was made in the 22 infants. The mean value for CBV was calculated for each infant (Table 1). In infants with normal brains, of gestational age 25–40 (median 29) wk, and birth weight 826–4,400 (median 1,500) g, mean CBV was 2.22 ± 0.40 (SD) ml/100 g. By contrast in the infants with evidence of brain injury, of gestational age 24–42 (median 36) wk, and birth weight 908–4,500 (median 3,100) g, it was 3.00 ± 1.04 (SD) ml/100 g. This difference was statistically significant ($P < 0.05$, Student's t test with 20 df). Among

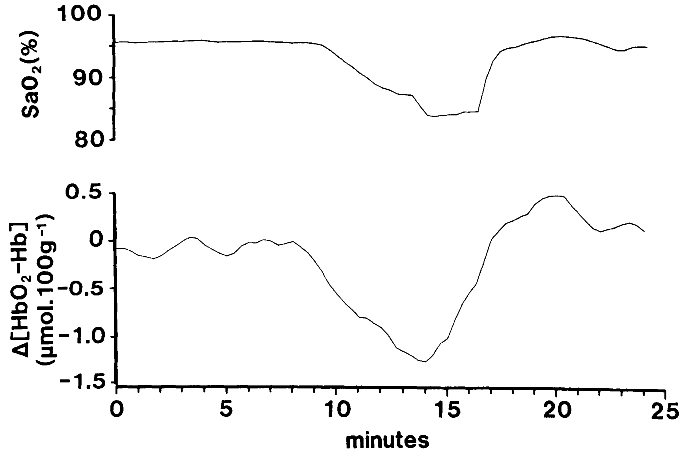

FIG. 2. Observations in a 40-wk gestational term infant studied on the 2nd day of life (*infant 12*). He had meconium aspiration and was receiving mechanical ventilation but had no evidence of cerebral injury. *Top trace*, Sa_{O_2} calculated from transcutaneous P_{O_2}. *Bottom trace*, alterations in cerebral hemoglobin oxygenation determined by NIR spectroscopy. Transient reduction in inspired oxygen concentration was induced at 9 min.

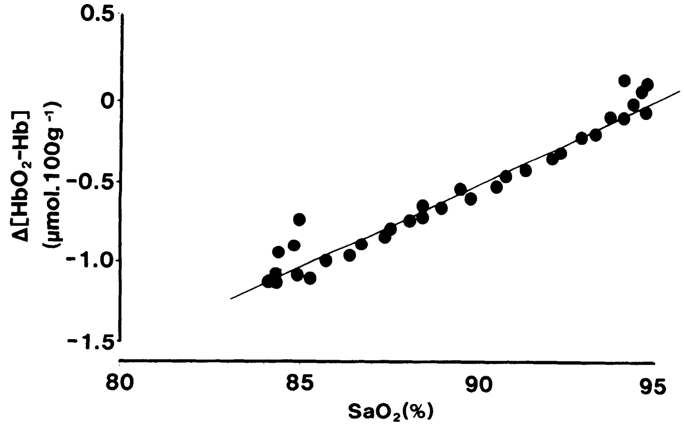

FIG. 3. Alterations in $[HbO_2 - Hb]$ plotted against Sa_{O_2}. Same infant and observations as in Fig. 2. Observations from 9 to 18 min are displayed. Each point represents data averaged over 15-s collection period. Equation of regression line: $y = 0.010x - 9.86$. Calculated cerebral blood volume from this maneuver was 2.39 ml/100 g.

the infants with abnormal brains the highest values for CBV were found in the five with severe birth asphyxia: 3.50 ± 1.22 ml/100 g. Mean arterial carbon dioxide tension was not significantly different between infants with normal brains and those with brain injury.

Formal assessment of reproducibility was not possible as most of the infants studied were clinically unstable, and CBV was therefore fluctuating. However, in six infants (*infants 3, 8, 12, 14, 20,* and *22* in Table 1) whose clinical condition was regarded as reasonably stable, coefficients of variation were obtained ranging from 4 to 21% (mean 12%).

There was no significant difference between results obtained using transmission spectroscopy with linear or orthogonal placing of the optodes. Mean CBV in infants studied with linear geometry was 2.53 ± 0.73 ml/100 g compared with 2.62 ± 1.00 ml/100 g with orthogonal geometry.

DISCUSSION

Validity of the method. This method for measuring CBV incorporates several simplifying assumptions that require consideration. The head is regarded as an optically homogeneous compartment with a constant spatial distribution of hemoglobin and a fixed mean hematocrit. Although regional variations in hemoglobin concentration are well known to occur within brain tissue (9, 23), these will not contribute significant errors to the calculation of total CBV because of the highly scattering nature of brain tissue and the extended path taken by each photon. (For a typical head diameter of 6 cm the mean pathlength is 26.4 cm.)

We have assumed that the hemoglobin saturation of all compartments in the brain is linearly related to Sa_{O_2} within a relatively narrow physiological range. This assumption will be invalidated if global cerebral blood flow or oxygen consumption alters during a small transient change in Sa_{O_2}. Severe hypoxemia is known to be a potent cerebral vasodilator, but studies in animals indicate that a significant rise in global cerebral blood flow does not occur until arterial P_{O_2} falls to ~5.5 kPa (13, 15), equivalent to Sa_{O_2} of ~75%. Numerous studies in animals and human subjects have shown no change in global cerebral oxygen consumption during moderate arterial hypoxemia (13, 25). We do not think, therefore, that the small alterations within the range of Sa_{O_2} employed in this study will have significantly affected global cerebral blood flow or oxygen consumption. If these assumptions are incorrect the relation between hemoglobin oxygenation and Sa_{O_2} during a transient alteration in Sa_{O_2} should not be linear. During this study substantial linearity was observed in the range 82–95% Sa_{O_2}. (We have, however, encountered loss of linearity during other observations when Sa_{O_2} fell transiently below 80% during nursing procedures.) Changes in regional CBV have been observed with minor degrees of hypoxemia (30), but these would not affect our measurements unless there was a gross change in global CBV. In addition NIRS allows continuous observation of global CBV, and we observed no systematic change in CBV during small induced changes of Sa_{O_2}.

Use of the Beer-Lambert relationship is possible only if optical pathlength remains constant during measurements. In the near-infrared region of the spectrum the absorption coefficients of HbO_2 and Hb are much lower than in the visible and ultraviolet regions, and thus within biological tissue optical pathlength is dominated by scattering. In experimental animals the pathlength factor B has been found to be remarkably constant at near infrared wavelengths despite gross changes in oxygenation and perfusion, and before and after death (5). The maximum variation in pathlength observed during these extreme changes of condition was <9%. This observation is confirmed by data obtained from a computer simulation of light transport in brain tissue that supports the relative constancy of optical pathlength with changes in absorption (6). Small fluctuations in oxygenation over the range employed can therefore be assumed to exert a negligible effect.

Spectral distortion in a scattering medium may invalidate quantitative techniques. In the near-infrared region hemoglobin absorption is greatly reduced, and therefore minimal spectral distortion is observed. A linear relationship between hemoglobin measured biochemically and spectroscopically in a scattering medium has been found experimentally over a wide range of hemoglobin concentrations (4, 10).

We have assumed that the relation between optical pathlength and interoptode spacing was the same in infants undergoing transillumination with linear or orthogonal positioning of the optodes. There are currently no experimental data on pathlength with orthogonal positioning, so this assumption cannot yet be tested.

There were unknown and variable proportions of fetal and adult hemoglobin in the blood of the infants studied. This will have had no significant effect on the results, however, as the absorption coefficients of fetal and adult hemoglobin are virtually identical in the NIR region (W. G. Zijlstra, personal communication). The relative lowering of hematocrit in the cerebral circulation is thought to be due to erythrocyte migration toward the center of small vessels, resulting in increased erythrocyte flow rates (18). Although small variations in the large vessel-to-cerebral hematocrit ratio are known to occur with vasodilatation secondary to changing arterial P_{CO_2} (23), we calculate that this will have contributed an error of <3%.

CBV in newborn infants. So far as we are aware, no values for CBV in infants have previously been published. The mean value obtained in infants with normal brains, 2.22 ± 0.4 (SD) ml/100 g, was lower than those found in human adults with the use of other techniques. For example, Sakai and colleagues (23), using single-photon emission tomography, found mean CBV to be 4.81 ± 0.37 ml/100 g.

Underestimation of CBV in the infants could have occurred if the optical pathlength factor B had been considerably shorter than assumed. We have no reason to believe that this was the case. The presence of tissue such as bone, scalp, or cerebrospinal fluid in the light path may cause an apparent underestimate of CBV. The contribution of skull bone to the optical pathlength has been shown to be <10% of the rat head (5). Skin and subcutaneous tissue in the light path represented a thickness of <5 mm in an interoptode spacing of 5–8 cm and therefore can be expected to have contributed an uncertainty of <10%. Cerebrospinal fluid causes virtually no optical scattering, and its contribution to the total optical pathlength was therefore assumed to be negligible.

Regional CBV is thought to be lower in white than gray cerebral matter (17), and the relatively low values for mean CBV we have obtained in infants may reflect a relative preponderance of white matter compared with adult brain.

CBV was significantly elevated in the infants with brain injury. The injury was usually hypoxic-ischemic in nature, although two infants had periventricular hemorrhages. In these latter infants the presence of extravascular blood or blood clots will not have contributed to the calculated CBV. This is because the extravasated blood cannot have participated significantly in oxygen exchange and would therefore have been effectively "invisible" during the transient alterations of Sa_{O_2}. To our knowledge there are no comparable studies of CBV in infants after brain injury. In addition there are very few reliable data on cerebral blood flow in the human newborn after brain injury, primarily because of methodological difficulties. In experimental neonatal animals cerebral blood flow is generally increased after perinatal asphyxia (29). Extracellular fluid concentrations of potassium and adenosine in the brain are also known to be markedly elevated following cerebral hypoxia in animals (14, 25). The elevation of CBV we found after brain injury, especially birth asphyxia, suggested cerebral vasodilatation possibly due to the presence of vasoactive agents released in brain tissue after hypoxic-ischemic injury (26).

CONCLUSIONS

We conclude that NIR spectroscopy can be used for the noninvasive measurement of CBV in newborn infants, that values appear lower than those in adults investigated by other techniques, and that CBV is elevated after hypoxic-ischemic brain injury. Quantitative transmission spectroscopy with linear or orthogonal placement of the optodes, as used in these studies, is only feasible in infants at present. In the near future, when the optical pathlength with other optode geometries has been defined, noninvasive quantitation of CBV in older children and adults should become possible.

APPENDIX

Details of infants studied

Infant No.	Gestational Age, wk	Birth Weight, g	Age, days	Principal Diagnosis	US	Vent	OA	Mean Pa_{CO_2}, kPa
				Infants with normal brains				
1	25	826	9	Hyaline membrane disease	N	MV	180	5.5
2	27	1,030	0	Hyaline membrane disease	N	MV	180	7.8
3	27	890	1	Pulmonary hypoplasia	N	MV	180	7.6
4	27	1,410	1	Hyaline membrane disease	N	MV	180	5.1
5	28	1,500	3	Hyaline membrane disease	N	MV	180	5.7
6	29	1,859	2	Hyaline membrane disease	N	MV	180	5.4
7	29	1,500	1	Hyaline membrane disease	N	MV	180	6.7
8	29	856	1	Hyaline membrane disease	N	MV	180	5.0
9	36	2,552	1	Hyaline membrane disease	N	MV	90	6.0
10	38	3,132	0	Hyaline membrane disease	N	Spont	90	4.9
11	39	2,700	3	Meconium aspiration	N	MV	90	4.8
12	40	4,400	2	Meconium aspiration	N	MV	90	5.1
				Infants with brain injury				
13	24	908	0	Intraparenchymal hemorrhage	IPH	MV	180	6.8
14	26	936	7	Periventricular leucomalacia	PVL	MV	180	4.4
15	27	1,330	1	Intraventricular hemorrhage	IVH	MV	180	7.1
16	36	1,780	2	Seizures	N	MV	90	5.5
17	36	3,400	3	Postnatal asphyxial episode	PE	MV	90	5.6
18	36	2,800	0	Birth asphyxia	PE	MV	180	4.9
19	40	3,740	2	Birth asphyxia	PE	Spont	90	4.7
20	41	4,500	1	Birth asphyxia	PE	MV	90	4.6
21	41	3,700	2	Birth asphyxia	PE	Spont	90	3.3
22	42	3,800	1	Birth asphyxia	PE	MV	90	4.5

IPH, intraparenchymal hemorrhage; IVH, intraventricular hemorrhage; MV, mechanical ventilation; N, normal; Pa_{CO_2}, mean arterial carbon dioxide tension during study; PE, diffuse parenchymal echodensities; OA, interoptode angle; PVL, periventricular leukomalacia; Spont, spontaneous respirations; US, cranial ultrasound appearance; Vent, ventilation.

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REFERENCES

1. Brazy, J. E., and D. V. Lewis. Changes in cerebral blood volume and cytochrome aa_3 during hypertensive peaks in preterm infants.

J. Pediatr. 108: 983–987, 1986.

2. Brazy, J. E., D. V. Lewis, M. H. Mitnick, and F. E. Jobsis. Noninvasive monitoring of cerebral oxygenation in preterm infants: preliminary observations. *Pediatrics* 75: 217–225, 1985.
3. Cope, M., and D. T. Delpy. A system for long term measurement of cerebral blood and tissue oxygenation in newborn infants by near-infrared transillumination. *Med. Biol. Eng. Comput.* 26: 289–294, 1988.
4. Cope, M., D. T. Delpy, E. O. R. Reynolds, S. Wray, J. S. Wyatt, and P. van der Zee. Methods of quantitating cerebral near infrared spectroscopy data. *Adv. Exp. Med. Biol.* 222: 183–189, 1988.
5. Delpy, D. T., S. R. Arridge, M. Cope, D. Edwards, E. O. R. Reynolds, C. E. Richardson, S. Wray, J. S. Wyatt, and P. van der Zee. Quantitation of pathlength in optical spectroscopy. *Adv. Exp. Med. Biol.* 248: 41–46, 1989.
6. Delpy, D. T., M. Cope, P. van der Zee, S. Arridge, S. Wray, and J. S. Wyatt. Estimation of optical pathlength through tissue by direct time of flight measurement. *Phys. Med. Biol.* 33: 1433–1442, 1988.
7. Ferrari, M., E. Zanette, G. Sideri, I. Gianni, C. Fieschi, and A. Carpi. Effects of carotid compression, as assessed by near infrared spectroscopy, upon cerebral blood volume and haemoglobin oxygen saturation. *J. R. Soc. Med.* 80: 83–87, 1987.
8. Fox, E., F. F. Jobsis, and M. H. Mitnick. Monitoring cerebral oxygen sufficiency in anaesthesia and surgery. *Adv. Exp. Med. Biol.* 191: 849–854, 1985.
9. Grubb, R. L., M. E. Raichle, C. S. Higgins, and J. O. Eichling. Measurement of regional cerebral blood volume by emission tomography. *Ann. Neurol.* 4: 322–328, 1978.
10. Hazeki, O., and M. Tamura. Near infrared quadruple wavelength spectrophotometry of the rat head. *Adv. Exp. Med. Biol.* 248: 63–69, 1989.
11. Jobsis, F. F. Noninvasive infrared monitoring of cerebral and myocardial sufficiency and circulatory parameters. *Science Wash. DC* 198: 1264–1267, 1977.
12. Jobsis, F. F., J. H. Keizer, J. C. LaManna, and M. Rosenthal. Reflectance spectroscopy of cytochrome aa_3 in vivo. *J. Appl. Physiol.* 43: 858–872, 1977.
13. Johannssen, H., and B. K. Siesjo. Cerebral blood flow and oxygen consumption in the rat in hypoxic hypoxia. *Acta Physiol. Scand.* 93: 515–525, 1975.
14. Kirshner, H. S., W. F. Blank, and R. E. Myers. Brain extracellular potassium activity during hypoxia in the cat. *Neurology* 25: 1001–1005, 1975.
15. Kogure, K., P. Scheinberg, O. M. Reinmuth, M. Fujishima, and R. Busto. Mechanisms of cerebral vasodilatation in hypoxia. *J. Appl. Physiol.* 29: 223–229, 1970.
16. Kuhl, D. E., M. Reivich, A. Alavi, I. Nyari, and M. M. Staum. Local cerebral blood volume determined by three-dimensional reconstruction of radionuclide scan data. *Circ. Res.* 36: 610–619, 1975.
17. Ladurner, G., E. Zilkha, D. Iliff, G. H. du Boulay, and J. Marshall. Measurement of regional cerebral blood volume by computerised axial tomography. *J. Neurol. Neurosurg. Psychiatry* 39: 152–158, 1976.
18. Lammertsma, A. A., D. J. Brooks, R. P. Beaney, D. R. Turton, M. J. Kensett, J. D. Heather, J. Marshall, and T. Jones. In vivo measurement of regional cerebral haematocrit using positron emission tomography. *J. Cereb. Blood Flow Metab.* 4: 317–322, 1984.
19. Marx, P. Determination of cerebral blood volume and flow-index with RISA. *Acta Neurochir.* 16: 166–167, 1967.
20. Nelson, S. R., M. L. Mantz, and J. A. Maxwell. Use of specific gravity in the measurement of cerebral edema. *J. Appl. Physiol.* 30: 268–271, 1971.
21. Phelps, M. E., S. C. Huang, E. J. Hoffman, and D. E. Kuhl. Validation of tomographic measurement of cerebral blood volume with ^{11}C-labeled carboxyhaemoglobin. *J. Nucl. Med.* 20: 328–334, 1979.
22. Phillis, J. W., G. Preston, and R. E. DeLong. Effects of anoxia on cerebral blood flow in the rat brain: evidence for a role of adenosine in autoregulation. *J. Cereb. Blood Flow Metab.* 4: 586, 1984.
23. Sakai, F., K. Nakazawa, Y. Tazaki, I. Katsumi, H. Hino, H. Igarashi, and T. Kanda. Regional cerebral blood volume and hematocrit measured in normal human volunteers by single-photon emission computed tomography. *J. Cereb. Blood Flow Metab.* 5: 207–213, 1985.
24. Severinghaus, J. W. Simple, accurate equations for human blood oxygen dissociation computations. *J. Appl. Physiol.* 46: 599–602, 1979.
25. Siesjo, B. K. *Brain Energy Metabolism.* New York: Wiley, 1978, p. 423.
26. Siesjo, B. K. Cerebral circulation and metabolism. *J. Neurosurg.* 60: 883–908, 1984.
27. Sklar, R. H., E. F. Burke, and T. W. Langfitt. Cerebral blood volume: values obtained with ^{51}Cr-labeled red blood cells and RISA. *J. Appl. Physiol.* 24: 79–82, 1968.
28. Ter-Pogossian, M. M., and P. Herscovitch. Radioactive oxygen-15 in the study of cerebral blood flow, blood volume, and oxygen metabolism. *Semin. Nucl. Med.* 15: 377–394, 1985.
29. Volpe, J. J. *Neurology of the Newborn* (2nd ed.). Philadelphia, PA: Saunders, 1987, p. 184.
30. Weinbrecht, P. T., L. Johnson, I. S. Longmuir, and J. A. Knopp. Influence of Pa_{O_2} on cerebral macro- and microcirculation as observed by light reflection. *Adv. Exp. Med. Biol.* 200: 131–136, 1986.
31. Wiernsperger, N., A. L. Sylvia, and F. F. Jobsis. Incomplete transient ischemia: a non-destructive evaluation of in vivo cerebral metabolism and hemodynamics in rat brain. *Stroke* 12: 864–868, 1981.
32. Wray, S., M. Cope, D. T. Delpy, J. S. Wyatt, and E. O. R. Reynolds. 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, 1988.
33. Wyatt, J. S., M. Cope, D. T. Delpy, P. van der Zee, S. Arridge, A. D. Edwards, and E. O. R. Reynolds. Measurement of optical pathlength for cerebral near infrared spectroscopy in newborn infants. *Dev. Neurosci.* In press.
34. Wyatt, J. S., M. Cope, D. T. Delpy, S. Wray, and E. O. R. Reynolds. Quantification of cerebral oxygenation and haemodynamics in sick newborn infants by near infrared spectrophotometry. *Lancet* 2: 1063–1066, 1986.