INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

DOPPLER EVALUATION OF BRAINSTEM FLOW

IN ASPHYXIATED NEONATES WITH

DELAYED AUDITORY BRAINSTEM EVOKED RESPONSES

BY BRIJ J. KAPADIA

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2000

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

1/6/2000
Date

Chair of Examining Committee
Dr. Bernard Karmel, N.Y.S. Institute for Basic Research

1/25/00
Date

Executive Officer
Dr. Richard L. Chappell

Dr. Ekkehart Trenkner, College of Staten Island

Dr. Theodore Lidsky, N.Y.S. Institute for Basic Research

Dr. Andrew Wieraszko, College of Staten Island

Dr. Orlando Manfredi, New York Medical College

Supervising Committee

The City University of New York

Abstract

DOPPLER EVALUATION OF BRAINSTEM FLOW IN ASPHYXIATED NEONATES WITH DELAYED AUDITORY BRAINSTEM EVOKED RESPONSES

by

Brij J. Kapadia

Adviser: Professor Bernard Z. Karmel, Ph.D.

Background

In animals, it has been demonstrated that asphyxia leads to transient ischemia and reperfusion of the brain. As a consequence of reperfusion, there is free-radical mediated neural damage, and progressive hypoperfusion of the brain lasting for approximately one week after the event. Studies using positron emission tomography have corroborated these findings in human neonates. The sonographic/Doppler findings during the phase of hypoperfusion have thus far not been characterized. Also, delayed auditory brainstem evoked responses (ABRs) have been documented in asphyxiated neonates in the first few days of life. The association of altered brainstem flow with delays in ABR component latencies has thus far not been studied.

Methods

ABRs were recorded and pulsatility indices in the basilar artery and branches of the middle cerebral artery were measured using the Doppler technique in 40 neonates admitted to the neonatal intensive care unit (NICU). A ratio

of brainstem to cortical impedance was computed. Asphyxia was defined on the basis of arterial blood gases. Neurological outcome at discharge was based on evaluation by an independent pediatric neurologist.

Results

We found that asphyxiated neonates had higher basilar to middle cerebral artery impedance ratios. Higher ratios were associated with delayed ABR wave III component latencies as well as poor neurological outcome during hospital stay. In asphyxiated neonates, high impedance and low-flow states in the basilar territory were significantly associated with higher ratios.

Conclusions

Asphyxia causes delayed low-flow states in the basilar territory, that are associated with delayed ABR component latencies and impaired neurological outcomes.

PREFACE, FOREWARD, AND ACKNOWLEDGEMENTS

The most thrilling years of my education have been spent in Graduate School. When I started in the fall of 1992, I had no idea that my perspectives would be so widened, and my thought processes so enriched, by the interaction with neuroscientists I encountered during graduate study.

Though I had the ability to experiment with molecular genetics and biology, I chose a clinical topic for my dissertation and sought to make the best use of easily available methodologies to address an important medical issue. I finished all course-work and had collected most of the data for my dissertation by 1996, but continued to write the actual thesis during residency.

I would like to dedicate this thesis to my wife, Poonam, who endured every moment I spent away from her working on the dissertation, and to my parents, Mina and Jairaj, and my brothers, Yash and Kashyap. All deserve special mention, first, for having me believe that no task is impossible, and second, for kindling in me the flame of endeavor.

I would like to acknowledge the support of many people that have stood by me during these arduous years. First, I would like to extend my sincere regards and gratitude to Drs. Bernard Karmel and Orlando Manfredi, who played more

vital roles than mentors in making this entire project possible. Next, I would like to thank Dr. David Soifer for guiding me in the initial stages, Dr. Anantham Harin for encouraging this study in the NICU, Dr. Judith Gardner for allowing me to steal Dr. Karmel away from his work, and Drs. Theodore Lidsky and Darlynne Devenny for providing inspiration along the way. I would also like to thank Drs. Ekkehart Trenkner and Andrej Wieraszko for reading the thesis and providing constructive suggestions, Daniel Merton at Thomas Jefferson University Hospital for helping me gain competency in cerebral Doppler sonography, and Drs. Freedland and Flory for enhancing my computer and statistical skills.

Table of Contents

LIST OF TABLES

DOPPLER EVALUATION OF BRAINSTEM FLOW IN ASPHYXIATED NEONATES WITH DELAYED AUDITORY BRAINSTEM EVOKED RESPONSES

I. INTRODUCTION

The auditory brainstem evoked response (ABR) waveform is the brain electrical activity recorded from the scalp produced in the first 8-12 msec following auditory stimulation and consists of 5-7 positive components time-locked to the stimulus (Jewett, Romano, & Williston, 1970; Picton, Hillyard, Kraug, & Galambos, 1974). These minute electrical deflections measuring 0.2-1.0 μV represent non-propagated, volume-conducted events reflecting the sequential activation of brainstem auditory nuclei and pathways (Jewett, 1970; Jewett, & Williston, 1971).

A shortening of peak latencies has been observed from birth to adulthood, with a decrease in conduction latencies occurring from the formation of the auditory system at about 24-28 weeks gestation to about six weeks post term (Salamy, McKean, & Buda, 1975; Starr, Amlie, Martin, & Sanders, 1977). Prior studies in our laboratory have demonstrated an association between prolonged ABR component latencies (CL's) and brain abnormalities (such as ventricular dilatation and intracranial hemorrhage) detected by cranial sonography (Karmel, Gardner, Zapulla, Magnano, & Brown, 1988). Other studies have demonstrated an association between delayed ABR

CL's and consequences of perinatal and neonatal insults in infants 1-2.5 years of age (Sohmer, & Student, 1978; Sohmer Gafni, Tannenbaum, at al., 1979).

Although related to central nervous system (CNS) compromise, the exact etiology of transiently prolonged ABR CL's has not been elucidated. We hypothesized that delays in ABR CL's were associated with reduction in blood flow to the brain. We used Doppler sonography to study the association between Doppler-derived pulsatility indices and brainstem electrophysiology. By measuring Doppler blood flow velocity in the basilar artery (BA) and terminal middle cerebral artery (tMCA) and using the pulsatility index as a measure of distal impedance, we obtained an estimate of brainstem and cortical flow, respectively.

Neurophysiological studies (Jewett, 1970; Jewett, Romano, & Williston, 1970), lesioning experiments (Buchwald, & Huang 1975; Achor, & Starr, 1980a,b), and human neuropathological results (Starr, & Hamilton, 1976; Stockard, & Rossiter, 1977; House, & Brackmann, 1979) suggest that generators of ABR waves III and V are located in the brainstem. While wave III is generated in the superior olivary nucleus located at the ponto-medullary junction, wave V is generated in the inferior colliculus located in the midbrain.

Using 2-[14C] deoxyglucose, which gets trapped in

functionally active neurons, it has been demonstrated by autoradiography that regional brain metabolic requirements depend on the level of functional activity of neural systems (Kennedy et al., 1975; Plum, Gjedde, & Samson, 1976). Further, local cerebral blood flow (CBF) has been shown to adjust to the level of energy generation and metabolic activity in neural circuits (Edvinsson, MacKenzie, & McCulloch, 1993). As the auditory system is the most metabolically active component of the neonatal CNS, brainstem auditory nuclei have the highest perfusion when compared to other parts of the brain (Edvinsson, MacKenzie, & McCulloch, 1993).

Thus, we created a ratio of BA (brainstem) to tMCA (cortical) impedance, and hypothesized that delays in ABR CL's might be associated with a preferential reduction of flow in the basilar territory.

Perinatal asphyxia causes an initial precipitous drop in CBF, which is followed by luxury perfusion when perfusion pressure is restored. The restoration of blood flow and oxygen is critical to survival of the brain as a whole, but in ischemic tissues results in free-radical mediated membrane damage, delayed hypoperfusion, and progression of neural injury for a few days after the ischemic event (Rosenberg, 1986; Krause, White, Aust, Nayini, & Kumar, 1988). Decreased flow to the brainstem may be one of the

mechanisms leading to delayed ABR conduction latencies in asphyxiated neonates.

We hypothesized that asphyxiated neonates would have a higher ratio of BA to tMCA impedance as compared to non-asphyxiated neonates. In asphyxiated neonates, we hypothesized that the higher ratio of brainstem to cortical impedance would be related to higher basilar impedance and, further, in such neonates higher basilar impedance would be associated with delayed ABR CL's.

Perinatal asphyxia is the leading cause of neurological and intellectual impairment in the pediatric population (Brann, 1985). Of asphyxiated neonates that did not expire, 25 per cent or more exhibited permanent brain damage (Ellenberg, & Nelson, 1988; Ergander, Eriksson, & Zetterström, 1983).

The significance of our studies is that Doppler sonography and ABR testing are non-invasive and can be performed in the neonatal intensive care unit (NICU) relatively quickly. When used in combination, these tests may become significant clinical tools to assess flow-electrophysiology relationships in high-risk neonates. If there is an association between reduced brainstem flow and delayed brainstem impulse conduction, steps can be taken to maintain flow at an optimal level. As changes in flow may predate electrophysiological dysfunction there may be a

window of opportunity for therapeutic intervention. For example, aggressive reperfusion may need to be delayed until excitotoxicity has been treated, or free-radical scavengers have had time to prepare the nervous system to receive restored oxygen supply. This approach may help in reducing long-term deleterious effects on the developing nervous system and reduce morbidity associated with perinatal asphyxia, thereby improving long-term outcome.

II. BACKGROUND AND SIGNIFICANCE

IIA. Cerebral Circulation

The brain has a high metabolic rate, and despite comprising only 2% of total body weight, requires 17% of cardiac output and 20% of total oxygen consumed by the body. Yet, the brain has very little circulatory reserve, and scant oxygen or glucose stores, making it vitally dependent on adequate perfusion (Stephens, 1969).

Regional brain metabolic activity and nutritive requirement is regulated by the level of functional activity (Kennedy et al., 1975; Plum, Gjedde, & Samson, 1976), and local cerebral metabolic rate of glucose has been correlated with regional cerebral blood flow (Younkin, Delivoria-Papadopoulos, Reivich, Jaggi, & Obrist, 1988). Thus, local CBF adjusts to the energy level and activity in neural circuits (Edvinsson, MacKenzie, & McCulloch, 1993), and measuring the flow characteristics in supplying vessels may

provide an estimate of the metabolic and functional activity of these areas.

In human newborns higher metabolic rates of glucose were noted in subcortical areas, midbrain, brainstem, and cerebellum as compared to the cerebral cortex (Chugani, Phelps, & Mazziotta, 1986). The auditory system was noted to be the most metabolically active component of the neonatal CNS (Kennedy, Sakurada, Shinohara, Jehle, & Sokoloff, 1978), and compared to other parts of the brain, brainstem auditory nuclei had the highest perfusion (Edvinsson, MacKenzie, & McCulloch, 1993). Histopathological studies have revealed the vulnerability of the neonate's brainstem to anoxia (Friede, 1972; Grunnet, Curless, Bray, & Jung, 1974). These features make the auditory areas in the brainstem vulnerable to fluctuations in flow as occur in asphyxia.

IIAi. Anatomy of Cerebral Circulation

The MCA arises from the internal carotid artery, and at the sylvian fissure gives off deep perforating branches, also known as the lateral lenticulostriate arteries, that supply the lentiform nucleus, internal capsule and caudate nucleus. The MCA also gives off insular and opercular branches that ramify over the hemispheric surface to supply a large proportion of white matter and cortex (Carpenter, 1978).

Brainstem perfusion is accomplished by the posterior

inferior cerebellar artery, a branch of the vertebral artery that supplies the posterolateral medulla, and by branches of the BA (Carpenter, 1978). The BA arises from the union of the vertebral arteries at the caudal border of the pons, and averages 1.5-4 mm in width, and 3 cm in length in adults (Smoker, Price, Keyes, Corbett, & Gentry, 1986). The first major branch of the BA, the anterior inferior cerebellar artery, courses in the cerebellopontine angle cistern toward the internal auditory canal, and supplies the inferolateral pons, cerebellum, and nuclei of the abducens, facial, and vestibulocochlear nerves (Martin, Grant, Peace, Theiss, & Rhoton, 1980). Short and long segment circumflex perforating branches arise along the entire length of the BA, mainly from its cephalic portion (Shrontz et al., 1986; Marinkovic, & Gibo, 1993) and supply the ventral pons and rostral brainstem (Barkhof, & Valk, 1988). This pattern of perfusion may account for a preferential effect on ABR wave III CL when flow is altered in the BA.

The tegmentum of the midbrain contains the inferior colliculus, a relay nucleus in the auditory pathway and the generator of the ABR wave V CL. Though the inferior colliculus is supplied by the posterior cerebral arteries, which are terminal branches of the BA, additional blood is also supplied by collateral flow from the circle of Willis, as well as from the superior cerebellar arteries.

IIAii. Physiological Changes in CBF during Development

Changes in CBF velocity during the transition from the fetal to the neonatal stage following delivery have been documented in animals and in humans. Pertinent human literature is briefly reviewed below.

Fetal blood flow is mainly localized around the brainstem. After birth, BA velocity has been shown to reach a steady state earlier than the major cerebral arteries, suggesting that areas of the brainstem supplied by the BA might have a protected circulation (Hayashi, Ichiyama, Uchida, Tashiro, & Tanaka, 1992).

After a transient decrease lasting 4-6 hours following birth (Sonesson, Winberg, & Lundell, 1987; Winberg, Sonesson, & Lundell, 1990; Hayashi, Ichiyama, Uchida, Tashiro, & Tanaka, 1992), a two to three fold increase of end-diastolic velocity in the MCA has been documented in term and preterm neonates over the next 18 to 20 hours (Hayashi, Ichiyama, Uchida, Tashiro, & Tanaka, 1992; Low, Froese, Galbraith, Smith, & Karchmar, 1993). Thereafter, the peak systolic and end-diastolic velocities increased linearly during the first few weeks of life (Bode, 1988). A slower increase over the next five years occurred till the velocities attained 70% of adult values. The increase in diastolic flow exceeded the systolic flow, such that the systolic to diastolic flow ratio decreased over the first

year, indicating a steady decrease in cerebrovascular resistance and pulsatility. This reduction in resistance may be partially accounted for by a doubling of cerebral capillary density over the first year of life (Diemer, & Henn, 1964).

There appeared to be an optimal range of flow velocities in the intracranial arteries such that either too high or too low CBF velocities were detrimental to the developing nervous system. Thus, infants having CBF velocity > 3 SD above the mean or CBF velocity < 2 SD below the mean were found to have severe neurological impairment (Levene et al., 1989).

Many studies have attempted to document a normal range and there is great variation, chiefly because of different equipment, different modes of analysis and reporting, and the measurements of velocities in different cerebral arteries.

IIAiii. Measurement of Flow Using Doppler Sonography

The application of Poiseuille's law to flow in a vessel is defined in Appendix 1. Briefly, doubling the radius of a tube decreases the resistance to one sixteenth of the original value. Doppler shift is proportional to flow speed, and increases with radius (or diameter) squared. Poiseuille's law applies for flow speed calculated for the entire vessel, and states that flow speed increases with

larger diameters. Thus, vasodilatation in the cerebral circulation delivers more flow and is associated with higher velocities in the major cerebral vessels.

Vessels less than 1 mm in diameter contribute most to arterial reflections; with arterioles playing the most significant role (O'Rourke, 1967). Changes in the caliber of arterioles are responsible for the pulsatile form of the distal pressure wave. Thus, the shape of the velocity pulse measured upstream actually assesses relative impedance of the arteriolar bed downstream (Burns, 1995a). If there is vasoconstriction in the distal arteriolar bed, the reflection coefficient rises, and a more pulsatile velocity is noted upstream. Thus, by measuring flow characteristics in major upstream vessels, such as the BA and tMCA, we can measure the influence of smaller vessels that control perfusion at the tissue level but are too small to be interrogated directly by Doppler (Evans, Barrie, Asher, Bentley, & Bell, 1980).

The pulsatility index (PI), is defined as the difference between the maximum and minimum Doppler shift frequencies, divided by the mean or time average of the maximum Doppler shift frequency over the cardiac cycle. Using the PI to gauge perfusion has a major advantage over using velocity measures, as the index is a ratio of Doppler shift frequencies, and hence is independent of Doppler angle

(Burns, 1995b).

Proximal arterial tracings in high distal impedance circuits (distal vasoconstriction) show flow reversal (negative Doppler shift below the baseline) in early diastole and lack of flow in late diastole resulting in a high PI. In circuits with low distal impedance (distal vasodilatation) there is high end-diastolic flow velocity throughout diastole resulting in a low PI (Burns, 1995a; Pryds, Greisen, Lou, Friis-Hansen, 1990).

Increased CBF velocities measured by the Doppler technique were associated with increased volemic CBF measured by the xenon clearance technique (Pryds, Greisen, Lou, Friis-Hansen, 1990). In newborn piglets (Hansen, Stonestreet, Rosenkrantz, & Oh, 1983) and in newborn dogs (Batton, Hellman, & Hernandez, 1983) there is an excellent correlation between CBF velocity (area under waveform) and volemic CBF measured by radioactive microspheres, or by tissue autoradiographic techniques. The use of range-gated Doppler sonography has been validated in newborn lambs (Sonesson, & Herin, 1988). In the human newborn, CBF velocity was correlated with total CBF measured by Xe-133 clearance technique (Greisen et al., 1984), and with regional CBF in anterior cerebral artery distribution measured by positron emission tomography (PET) (Perlman, Herscovitch, & Corriveau, 1985).

IIAiv. Autoregulation

Autoregulation is the process by which a tissue or end organ regulates its perfusion. With specific reference to the CNS, it is the process by which a constant cerebral blood flow is maintained over a wide range of perfusion pressures (Lassen, & Christensen, 1976; Johnson, 1986; Paulson, Strandgaard, & Edvinsson, 1990).

Local perfusion is related neither to the anatomic organization of cerebral vasculature nor to regional differences of innervation of blood vessels. Because local CBF adjusts to the level of energy generation, the level of activity in neuronal circuits determines local cerebral blood flow (Edvinsson, MacKenzie, & McCulloch, 1993). Probably for this reason blood flow to the central auditory pathways is always high (Edvinsson, MacKenzie, & McCulloch, 1993).

In the adult human autoregulation is operative over a range of mean blood pressures varying from 60 to 150 mmHg (Paulson, Strandgaard, & Edvinsson, 1990), and responds to changes in perfusion pressure within 3 to 15 seconds (Florence, & Seylaz, 1992). Three major mechanisms play a role in the process of autoregulation, and these are described below. In the brain there is evidence that major arteries are under myogenic control (Hall, 1982), while pial and smaller arterioles imbedded in the tissue are under

metabolic control (Kontos et al., 1978).

IIAiv(a). Effect of metabolic factors

The metabolic hypothesis suggests that the local concentrations of vasoconstrictor or vasodilator metabolites play a role in determining vascular caliber.

(i). Carbon dioxide (CO_2). CO_2 has a potent effect on CBF, which doubles after an increase in arterial CO_2 tension ($PaCO_2$) from 40 to 80 mmHg in healthy adults (Harper, & Glass, 1965; Hauge, Thoresen, & Walloe, 1980; Markwalder, Grolimund, Seiler, Roth, & Aaslid, 1984). The smallest pial vessels exhibited the highest degree of dilatation in response to changes in $PaCO_2$ (Auer, & Johannson, 1980; Wei, Kontos, & Patterson, 1980). Busija, Heistad, & Marcus (1981) observed an increase in pial vascular diameter from 388 µm to 450 µm using an operating microscope during hypercapnia. In contrast to pial (surface) vessels, the intracerebral (parenchymal) vessels showed relatively little change in response to changes in $PaCO_2$ (Mchedlishvili, 1986).

The vasodilatory response to CO_2 is exaggerated in infants when compared to adults. This is supported by work on preterm infants using radioactive xenon (Greisen, & Trojaborg, 1987). Much of the change is due to alteration in blood pressure consequent to variation in $PaCO_2$ (Fenton, Woods, Evans, & Levene, 1992). In 19 ventilated preterm babies a rise of 1 kPa in $PaCO_2$ produced a median rise in

anterior cerebral artery velocity of 44% per kPa increase in $PaCO_2$ (Levene, Shortland, Gibson, & Evans, 1988).

In preterm infants with periventricular hemorrhage and in term infants with birth asphyxia a state of vasoparalysis was demonstrated in response to changes in CO_2 levels, during which there was a pressure-passive circulation (Pryds, Greisen, Lou, & Friis-Hansen, 1989; 1990). A reduction in PI was also shown in 11 healthy full term asphyxiated babies who rebreathed for 5 minutes (Archer, Evans, Paton, & Levene, 1986). This effect was mainly due to a rise in diastolic velocity, indicating cerebral vasodilatation. These findings were confirmed in preterm infants (van Bel, van de Bor, Baan, & Ruys, 1988), with an additive effect of associated hypoxia.

(ii). Oxygen. In the brain, blood flow is highly dependent on tissue oxygen consumption (Eklof, Lassen, Nilsson, & Siesjö, 1973). Granger, Goodman, & Granger (1976) suggested that terminal arterioles are more sensitive than larger arterioles to reduced tissue oxygen tension. Enhanced autoregulation might be attributed to lowered tissue oxygen tension following consumption by metabolically active tissues (Granger, Goodman, & Granger, 1976). Any drop in arterial pressure and flow would lower the tissue oxygen tension and lead to relaxation of larger arterioles in order to restore blood flow. The effect of oxygen on CBFV was ten

times smaller than that of carbon dioxide (Menke et al., 1993).

(iii). Prostaglandins. Constant production of vasodilatory prostaglandin from the vessel wall also plays a role in autoregulation (Busse, Forstermann, Matsuda, & Pohl, 1984). In cases of reduced flow, there might be reduced washout of prostaglandin and thus its continued presence in greater quantities may enhance autoregulation. Ischemia induces the production of PGE and F_2, that act as potent vasodilators and serve to restore blood flow to deprived tissues. However, $PGF_2\alpha$ is a potent vasoconstrictor, and plays an important role in producing a phase of delayed hypoperfusion.

(iv). Hydrogen ions. Increased neural activity results in an increase in perivascular hydrogen ion concentration and a decrease in local pH. Anaerobic glycolysis during hypoxia or ischemia also results in local acidosis. A fall in local pH mediates arteriolar dilatation. A rise in local tissue CO_2 causes an increase in the hydrogen ion and also results in arteriolar dilatation (Kontos, Raper, & Patterson, 1977; Kontos, Wei, Raper, & Patterson, 1977; Faraci, & Heistad, 1990).

(v). Adenosine. Adenosine has a potent cerebral vasodilator effect and increases cerebral blood flow during hypoxia (Wahl & Kuschinsky, 1976; Laudignon, Farri, Beharry,

Rex, & Aranda, 1990). During normoxia, newborn and adult brain produced adenosine levels within the normal vasodilatory range. During severe hypoxia, adenosine levels peaked at 2-4 minutes in CSF (Laudignon, Farri, Beharry, & Aranda, 1991).

In hypoxia-induced hypotension, an increase in extracellular adenosine stimulated A_2 receptors causing cerebral vessel dilatation necessary for a compensatory increase in CBF and maintenance of CBF autoregulation (Winn, Welsh, Rubio, & Berne, 1988). Adenosine also had a neuroprotective effect through stimulation of A_1 receptors on the neural cells (Fredholm, & Hedqvist, 1980), and has been shown to counteract excitotoxicity (Benveniste, Drejer, Schousboe, & Diemer, 1984).

Low levels of extracellular adenosine may lead to insufficient cerebral perfusion and deficient autoregulation, a major problem during the neonatal period (Winn, Welsh, Rubio, & Berne, 1988).

IIAiv(b). Myogenic influence on autoregulation

According to the myogenic hypothesis, arterioles respond to intravascular pressure, with pressure elevation causing constriction, and fall in pressure causing vasodilatation (Johnson, 1980). If arterial pressure to the regulated organ is reduced, the metabolic mechanism apparently overcomes the myogenic response (Borgstrom,

Grande, & Mellander, 1984). External calcium concentration plays a role in membrane depolarization, and the addition of calcium channel blockers like nifedepine or verapamil abolish autoregulation.

IIAiv(c). Neurogenic influence on autoregulation

When systemic blood pressure is reduced there is an increase in sympathetic outflow, which selectively constricts large arteries (Brough, Cowley, & Guyton, 1975; Cowley, 1982). Large artery constriction causes a transient fall in flow. However, the brain is unusual in that arterioles lack sympathetic innervation, and the flow reduction quickly returns to normal due to autoregulatory correction downstream. Autoregulation counters hypotension by producing vasodilatation in the arterioles, causing these resistance vessels to be dilated to the point to restore constant blood flow at normal arterial pressure.

In neonates sympathetic vasoconstriction alters the autoregulatory range (Lassen, 1978), so that the lower limit of autoregulation is reached at a higher than normal arterial pressure, and the upper limit is also elevated. Thus, sympathetic innervation and autoregulation act together to protect the capillary network against the disruptive effects of elevated capillary pressure. When arterial pressure is above the autoregulatory range, the rise in capillary pressure can lead to disruption of the

blood brain barrier (Lassen, 1978).

IIAv. Impaired Autoregulation

Autoregulation is abolished or impaired in brain injury (Enevoldsen, & Jensen, 1977). Ischemia provoked generalized vasomotor paralysis with true loss of autoregulation, mainly because of acidosis. Arterial hypoxia to less than 60% saturation abolished cerebral blood flow autoregulation in man (Haggendal, & Johansson, 1965).

The initial events in perinatal asphyxia consist of a precipitous drop in blood flow and oxygen delivery to the brain. In an animal model of hypoxic ischemia Rosenberg (1986) altered inspired gas concentrations and respiratory rates in artificially ventilated neonatal lambs and noted bradycardia and hypotension below the limits of autoregulation within the last 10 to 15 minutes of the asphyxial insult. In their model of perinatal asphyxia, ischemia was the initial event, accompanied by hypoxemia and hypercarbia.

The events in post-asphyxial recovery have been extensively studied using various techniques. Either physiologic compensation, as described below, or therapeutic intervention serves to restore perfusion pressure.

IIAv(a). Redistribution of Cardiac Output

Following an asphyxial event there was a prompt redistribution of cardiac output to the brain, heart and

adrenal glands (Behrman, Lees, & Peterson, 1970; Rudolph, 1984; Ashwal, Majcher, Vain, & Longo, 1980; Friedman, & Kirkpatrick, 1977; Davies, & Tweed, 1984). A two-fold increase in the proportion of cardiac output going to the brain was documented in term fetuses. An intact sympathoadrenal system, intact oxygen chemoreceptors, and an adequate circulatory volume were essential for this reflex to be effective. Hypertension, noted immediately after asphyxia, was also particularly important in delivering blood to the brain (Lou et al., 1979; Johnson, Palahnuik, Tweed, Jones, & Wade, 1979).

IIAv(b). Luxury Perfusion

Hypoxic tissues require increased oxygen delivery, which is accomplished by increasing cerebral blood flow. In animals and primates with perinatal asphyxia, an increased flow of 50% to 500% has been documented (Cavazzuti, & Duffy, 1982; Purves, & James, 1969; Behrman, Lees, & Peterson, 1970; Ashwal, Majcher, Vain, & Longo, 1980), most marked in the brainstem and least marked in the cerebral white matter (Ashwal, Dale, & Longo, 1984; Johnson, Palahnuik, Tweed, Jones, & Wade, 1979; Ashwal, Majcher, & Longo, 1981; Lou, Tweed, & Davies, 1985; McPhee, Kotagal, & Kleinman, 1985). Hypoxemia and hypercapnia with resultant rise in perivascular hydrogen ion (Johannsson, & Siesjö, 1975; Lassen, 1968), and elevations of extracellular potassium,

adenosine, and prostaglandins, each have been implicated in post-asphyxial vasodilatation (Rosenberg, 1986). Endogenous opioids also played a role (Lassen, 1968; Lou, Tweed, & Davis, 1989) by suppressing the rate of oxygen consumption in the telencephalic brain (cortex), permitting a greater fraction of blood flow to be diverted to the brainstem.

In term asphyxiated neonates Doppler studies documented increased flow velocities (with reduced resistance) lasting 6 to 130 hours after the insult (Archer, Evans, Paton, & Levene, 1986; Archer, Levene, & Evans, 1986; Levene et al., 1989; van Bel, Hirasing, & Grimberg, 1984; Ramaekers, & Casaer, 1990). In these neonates highest flow velocities were associated with isoelectric EEGs. Asphyxiated term neonates with post-asphyxial encephalopathy studied by the Doppler technique showed increased CBF velocities in the anterior cerebral artery with a decreased PI (representing cerebral hyperemia with vasodilatation) at a median age of 26 hours (Levene et al., 1989). In the same study no infant with CBF velocity exceeding three standard deviations above the mean survived without severe neurological impairment.

A PI below 0.55 predicted adverse outcome after birth asphyxia (Archer, Levene, & Evans, 1986), confirmed in three other cases (Yoshida-Shuto, Yashura, & Kobayashi, 1992). A high velocity also predicted poor outcome (Levene et al., 1989), and seen after 24 hours may represent luxury

perfusion. van Bel, van de Bor, Stijnen, Baan, & Ruys, (1987) found a high velocity and reduced PI in 17 infants between 32 and 48 hours of life. Increased CBF in asphyxiated infants with poor outcome has also been shown by the nitrous oxide technique (Frewen et al., 1991).

IIAv(c). Pressure-passive Circulation

Asphyxia and resulting acidosis produced by partial occlusion of the umbilical vessels in term fetal sheep suppressed autoregulation so that eventually the cerebral circulation became pressure-passive (Lou et al., 1979). Consequently, raising the blood pressure to 60-70 mmHg produced marked hyperemia of the brain, and lowering the blood pressure to 30 mmHg lowered flow in large cortical areas to near-zero levels. Similar failure of autoregulation has also been demonstrated in a xenon-133 study of cerebral blood flow in asphyxiated term and preterm neonates (Lou, Lassen, & Friis-Hansen, 1979).

Infants with the poorest outcome had the highest CBF in the first day of life (Pryds, Greisen, Lou, & Friis-Hansen, 1990). There was loss of reactivity of cerebral blood flow to arterial blood pressure and arterial carbon dioxide in infants with the poorest outcome, loss of reactivity to blood pressure but retained reactivity to carbon dioxide in infants with intermediate outcome, and retained reactivity to both in infants with normal outcome.

Thus, a state of vasoparalysis (maximal vasodilatation) and hyperemia may exist in the postasphyxial human neonate that may be correlated with the degree of brain injury and the severity of the asphyxial insult. However, the areas with maximal reperfusion and reoxygenation also become vulnerable to the effects of free-radical-induced injury. In animal models the hyperemic phase is followed within a few hours by delayed hypoperfusion of the brain.

IIAv(d). Progression to delayed hypoperfusion and brain injury

Increasing experimental evidence points to a second and delayed phase of brain hypoperfusion that may ultimately determine progression of neural injury. In the study by Levene et al. (1989), following the initial phase of hyperemia, 20% of asphyxiated term infants with poor outcome had very low CBF velocity. Similarly, a high PI (indicating distal vasoconstriction and low-flow states) correlated with an adverse outcome in a group of 60 preterm infants (van Bel, van de Bor, Baan, & Ruys, 1988).

Subsequent to the initial phase of hyperemia, there was a phase of delayed cerebral hypoperfusion with a 30% reduction in cerebral oxygen consumption (Rosenberg, Murdaugh, & White, 1989; Rosenberg, Parks, Murdaugh, & Parker, 1989). PET studies done in the first week of life in term infants that survived the initial asphyxial event

showed a relative reduction in CBF in parasagittal cortices, symmetrical and more prominent posteriorly. The extent of decrease in CBF correlated with the severity of clinical manifestations that included proximal limb weakness (Volpe, Herscovitch, Perlman, Kreusser, & Raichle, 1985).

Myocardial depression resulting from hypoxia and acidosis following the asphyxial event also resulted in diminished cardiac output and hypotension (Vannucci, & Plum, 1975; Young, Petroff, Aquila, Cheung, & Gore, 1992; Behrman, Lees, & Peterson, 1970). As a result there was a marked reduction of cerebral blood flow, making the parasagittal cortex especially vulnerable in term fetal monkeys (Reivich, Brann, & Shapiro, 1972) and near-term fetal sheep (Williams, Gunn, Synek, & Gluckman, 1990).

Asphyxia followed by reventilation in neonatal pigs revealed that free-radicals were produced not during the asphyxial event, but during the reperfusion and hyperemia phase following reventilation (Pourcyrous, Leffler, Bada, Korones, & Busija, 1993). Free-radicals then mediated both delayed hypoperfusion and reduced cerebral oxygen consumption as both these events could be prevented by prior administration of free-radical scavengers, superoxide dismutase and catalase. Free-radical-mediated mitochondrial injury as well as calcium-activated cascades probably contributed to the reduction in cerebral oxygen consumption

(Rosenberg, Parks, Murdaugh, & Parker, 1989).

IIB. Auditory Brainstem Evoked Responses (ABRs)

As described earlier, auditory stimulation produces 5-7 positive waves within the first 8-12 msec following stimulation that represent non-propagated volume-conducted far-field potentials (Jewett, Romano, & Williston, 1970; Jewett, & Williston, 1971), reflecting the sequential activation of auditory brainstem nuclei and pathways (Jewett, Romano, & Williston, 1970; Picton, Hillyard, Kraug, & Galambos, 1974).

Estimates of inter- and intra-subject variability for ABR waveforms in neonates have shown the response to be not only consistent across subjects, but also quite reliable from average to average within a given subject (Salamy, & McKean, 1976). Similar findings have been reported in adults (Jewett & Romano, 1972; Amadeo, & Shagass, 1973; Picton, Hillyard, Kraug, & Galambos, 1974). The ABR is unaffected by changes in states of consciousness as occur in different stages of sleep (Amadeo, & Shagass, 1973), or during selective attention (Picton, Hillyard, Kraug, & Galambos, 1974). Reproducible and undistorted ABR waveforms have been obtained even during deep coma induced by barbiturate overdose (Starr, & Achor, 1975).

IIBi. Auditory Pathway

The cochlear portion of the labyrinth serves as the

auditory transducer. Energy transmitted by fluid pressures produces traveling waves in the basilar membrane that move from the base of the cochlea to the apex (Békésy, 1960). Displacement of the basilar membrane in response to acoustic stimuli causes bending of hairs of the hair cells in contact with the tectorial membrane.

The cochlear nerve originates from bipolar cells located in the spiral ganglion. The central processes of these neurons make their first synapses in the cochlear nuclei located on the lateral surface of the inferior cerebellar peduncle at the ponto-medullary junction. The auditory pathways then ascend in the brainstem, make synapses in the superior olivary nuclei in the tegmentum of the pons, as well as in the inferior colliculus in the midbrain, before terminating in the metathalamus and superior temporal gyrus in the cortex.

Neurophysiological studies (Jewett, Romano, & Williston, 1970), lesioning experiments (Buchwald, & Huang 1975; Achor, & Starr, 1980a,b), and correlation with human neuropathology (Starr, & Hamilton, 1976; Stockard, & Rossiter, 1977; House, & Brackmann, 1979) suggest that the voltage deflections making up the ABR waveform are generated from spatially separate structures along the auditory pathway.

Auditory stimulation in cats produced waves I, II, III,

& IV in electrodes implanted at or near the VIIIth nerve, the cochlear nucleus, the superior olivary complex, and the inferior colliculus, respectively. Each wave showed an increase in amplitude, a polarity inversion, or both, as the recording electrode was successively passed in the vicinity of the components of the auditory tract (Jewett, Romano, & Williston, 1970; Jewett, & Williston, 1971; Jewett, & Romano, 1972).

Further, bilateral destruction of the superior olivary nuclei caused wave III to disappear (Jewett, 1970). Buchwald and Huang (1975) precisely identified the generator of wave IV to be in the lateral lemniscus and preolivary complex, and wave V was found to be dependent on the integrity of the rostral inferior colliculus. Despite this specificity in the cat it is believed that in the human waves IV, V, VI, and VII reflect the algebraic summation of activity originating from multiple local generators.

IIBii. Developmental Changes in the ABR

ABR inter-peak component latencies (IPL's) are incrementally prolonged across waves I-V early in development. As a function of maturation, the IPL's shorten differentially to reach adult values.

The decrease in ABR latencies was attributed to myelination or to an increase in the number of synapses. Prolonged latencies in the quaking mouse (an autosomal

recessive mutant with disturbances in myelin metabolism) (Sidman, Dickie, & Appel, 1964), delays in component latencies produced by triethylene-induced reduction of myelin density (Amochaev, Johnson, Salamy, & Shah, 1979), and demyelination-induced prolongation of ABR component latencies in multiple sclerosis (Robinson, & Rudge, 1977), support the theory that normal myelination of the auditory tract is essential for normal conduction of auditory impulses.

Further, a gradual increase in cerebroside levels in the inferior colliculus over days 15-26 of post-natal age in rat pups was associated with shortening of the ABR II-V interval (Shah, Bhargava, Johnson, & McKean, 1978). This suggests that ongoing myelogenesis is a major contributor to the shortening of ABR latencies in early post-natal life.

IIBiii. Delayed ABR Component Latencies

Latencies get prolonged and amplitudes are decreased with an increase in stimulus rate or a decrease in intensity. Since the fastest click rate used (as fast as 200/sec, one click every 5 msec) is much slower than axonal refractory periods, axonal transmission velocity cannot be changed. Thus, prolonged latencies have been attributed to stimuli taking longer to drive neurons to synaptic threshold, and decreased amplitudes have been attributed to post-synaptic activation of a smaller population of neurons

(Buchwald, 1985).

ABR wave amplitudes and latencies vary independently with disease processes (Buchwald, 1985). Decrease in conduction velocity due to axonal demyelination may lead to prolonged latencies in multiple sclerosis, without any change in amplitude (Buchwald, 1985). Cooling the pontine auditory path prolongs latencies after wave III without producing changes in wave amplitude. Prolonging stimulus rise time prolongs ABR wave latencies without accompanying effects on amplitude. Changing the excitability of muscarinic or nicotinic receptors also alters ABR wave amplitudes (Buchwald, 1985).

Prolonged ABR component latencies have been associated with abnormalities detected on cranial sonography (Karmel, Gardner, Zapulla, Magnano, & Brown, 1988), and have been used to follow clinical recovery after shunt surgery in cases of hydrocephalus. ABRs have also been used for the assessment of hearing loss (Sohmer, & Gafni, 1979), and to discriminate VIIIth cranial nerve pathology (Zappulla, Karmel, & Greenblatt, 1981). ABR studies have been done in children with sleep apnea and sudden infant death syndrome (Orlowski, Nodar, & Lonsdale, 1979), perinatal asphyxia (Kilney, Connelly, & Robertson, 1980), kernicterus (Kaga, Kitazumi, & Kodama, 1979), intracranial hemorrhage (Marshall, Reichert, Kerley, & Davis, 1980), and

developmental disorders (Sohmer, Gafni, Tannenbaum, et al., 1979).

Shucard, Shucard, & Thomas (1988) suggested that infants with impaired or delayed auditory functioning (as reflected by the ABR) may exhibit a delay in cortical responsiveness to complex auditory stimuli such as human speech (as reflected in the cortical auditory evoked response). Such infants may then manifest a subsequent delay in receptive or expressive language development.

Thus, delayed ABR CL's in asphyxiated neonates may represent transient periods of neurological dysfunction, with potential long-term consequences related to developmental delays. Altered CBF velocities in the BA may be associated with delayed ABR CL's, especially during the phase of delayed hypoperfusion following an asphyxial event.

IIC. Neonatal Asphyxia and Hypoxic Ischemic Encephalopathy (HIE)

Perinatal asphyxia and birth injuries are the main factors leading to neurological and intellectual impairment in the pediatric population (Brann, 1985; Volpe, 1990). An estimated 750,000 children with cerebral palsy and 850,000 children with mental retardation were documented in 1985 in the United States alone (Freeman, 1985). In a study by MacDonald, Mulligan, Allen, & Taylor (1980) 38,405 consecutive deliveries were studied, and asphyxia was found

to occur in 1.16 per cent of infants. In a study of 17,196 infants by Peters et al. (1984) the incidence of mild to moderate asphyxia was 18 per cent, and of severe asphyxia, 4 per cent. Severe asphyxia was associated with an increased mortality, and an increased incidence of cerebral palsy in survivors.

Besides cerebral palsy, a higher incidence of less severe neurological impairments such as learning disabilities, academic failure, and behavioral problems has been reported as well, ranging between 25 to 50 per cent in children born with very low birth weight (Volpe, 1990). Data analyzed from the National Collaborative Perinatal Project revealed that of the survivors reaching their 7th birthday, 21 per cent of cases of poor neurological outcome could be attributed to asphyxia-related variables (Nelson, 1988). The incidence of neurological impairments at age 7 in surviving infants with Apgar scores at birth of 0 to 3 at 5 minutes was 1%, at 15 minutes was 9%, and at 20 minutes was 57% (Nelson, & Ellenberg, 1986). The actual extent of neurological problems may have been underestimated, as 69 per cent of infants with Apgar scores less than 3 at 10 minutes or later, died before reaching one year of age (Nelson, & Ellenberg, 1981).

Neonatal encephalopathy may present clinically with seizures, altered consciousness, and altered tone. Varied

etiologies have been associated with HIE, including birth asphyxia, metabolic errors, infection, and congenital malformations. Clinical correlates of HIE include birth depression (Apgar scores < 6 at 5 minutes), delayed spontaneous respiration, acidosis (cord umbilical artery blood pH < 7.0 or base deficit > -10mmol/l), fetal distress with sustained bradycardia (heart rate < 100 beats per minute), and thick meconium staining of liquor. Signs of multisystem failure, which may appear shortly after an asphyxial event include refractory convulsions occurring in 24-48 hours, renal failure, and poor myocardial function resulting in hypotension.

Electroencephalography (EEG) is the best early electrophysiological predictor currently available, and a continuous low voltage EEG, flat EEG, or EEG with burst-suppression within the first 6 hours, predicted adverse outcome in 43 of 47 infants (Hellström-Westas, Rosénn, & Svenningsen, 1995). Cranial sonography identifies cerebral edema, the earliest imaging manifestation of HIE, and has been used to document brain injury. A summary of normal and abnormal non-Doppler sonography as done in the clinical setting is provided for the interested reader in Appendices 2 & 3.

IID. Specific Hypotheses

We hypothesize that delays in brainstem neural

conduction will be associated with a preferential reduction of blood flow to the brainstem. Using Doppler sonography to measure blood flow velocity in the basilar artery that supplies the brainstem, we can derive pulsatility indices to estimate perfusion. Thus, we hypothesize that high impedance in the basilar artery, suggestive of low-flow states in the brainstem, will be associated with delayed ABR conduction latencies. Further, by computing a ratio of brainstem to cortical impedance, we hypothesize that preferential reduction of flow to the brainstem will result in a higher impedance ratio, and we hypothesize that higher ratios will be related to delays in ABR conduction latencies.

In asphyxiated neonates we hypothesize that there will be a phase of delayed hypoperfusion detectable by Doppler sonography, and in such neonates we hypothesize that delayed brainstem neural conduction will be associated with a higher basilar impedance, and a higher brainstem to cortical impedance ratio.

III. METHODS

IIIA. Measurement of Pulsatility Indices in Cerebral Vessels

IIIAi. The Doppler Technique

Cranial sonography was performed using an ATL Ultramark 9 HDI ultrasound unit and a phased array 3-5 MHz scan head. The Doppler technique was used to measure flow

characteristics in the cerebral vessels. The technique is briefly reviewed here.

IIIAi(a). The Doppler Equation

Doppler signals are dependent on 1, velocity of RBCs; 2, angle of insonation; 3, frequency of transmitted ultrasound; and 4, velocity of sound in the medium. The latter two factors are constant from one test to another (Bode, & Eden, 1989; Raju, 1991; Newell, & Aaslid, 1992).

When an ultrasound beam of a transmitted frequency (ft) encounters a moving target the reflected frequency (fr) is changed by an amount (fd), and the following equation applies: (where v = velocity of moving object, θ = angle of insonation, and c = velocity of sound, 1540 cm/s)

$$fd = (ft - fr) = 2v\cos\theta/c$$

The cosine of a smaller angle approximates to 1. Hence it is important to insonate a target with the beam pointing directly at it, like looking down the barrel of a gun. The effect of angle is minimal (< 4%) when the angle of insonation is 0-15 degrees, 18% at 35 degrees, and 50% at 60 degrees (Raju, 1991). Thus, angle of insonation was kept as low as possible, and was below 20 degrees in all cases studied.

IIIAi(b). The Doppler Signal

The best transducer position was obtained by visually inspecting the spectral tracing and by estimating optimal

loudness of the sound. As neonatal vessels are small, the range-gate was uniformly set at 2 mm in all cases.

IIIAi(c). Spectral Analysis

Fourier transform spectral analysis is the best method to analyze frequency components, and was automatically performed by the sonography machine. The spectral analyzer takes in 5 msec of complex signal, resolves the frequencies within it, and displays the results as an array of pixels (spots on a TV-type screen). The length of each pixel represents 5 msec of data and the height is usually one-hundredth of the total frequency range. The gray scale assigned to each pixel is a measure of the power of the frequency component of that pixel. The best estimate of the mean velocity of flow over the entire cardiac cycle is obtained by tracing the peak velocity envelope, integrating the frequencies over time, calculating the mean velocity from the Doppler equation, and then halving it (Evans, 1985).

IIIAi(d). Pulsatility Index

The pulsatility index of Gosling (Gosling, King, Newman, & Woodcock, 1969) was the earliest index used to describe these waveforms. The frequency components making up the periodic time-velocity waveform of fmax are analyzed. High pulsatility waveforms have greater amplitude of high-frequency components. If the graph is normalized so that the

amplitude A_0 of the zero frequency component (steady flow) is unity, the pulsatility index is then the area under the curve. PI is higher for pulsatile waveforms, and lower for dampened waveforms (Burns, 1995a).

This index is relatively insensitive to heart rate, but is difficult to calculate. Hence, it has been replaced by various indices calculated directly from the Doppler tracing in the time domain. These indices are ratios of Doppler shift frequencies, hence are independent of Doppler angle (Burns, 1995b).

The pulsatility index (PI) calculated by the Doppler machine is defined as the difference between the maximum and minimum Doppler shift frequencies, divided by the mean or time average of the maximum Doppler shift frequency over the cardiac cycle. The mean used in this ratio is identical to A_0. These indices are independent of the Doppler angle hence pulsatility can be assessed even in vessels too small or tortuous to be imaged.

These indices can also be directly measured off the spectral tracing. However, for all measurements reflecting the shape or spectral content of the Doppler waveform, it is recommended to take an average value after calculating the index for each of several cardiac cycles (five heartbeats in adults to account for beat-to-beat variability). Automated real-time calculation of these indices averaged over 5 beats

saves time and reduces operator variability as long as signal-to-noise-ratio is favorable (Rickey, & Fenster, 1996).

The resistive index (RI) of Pourcelot (1976) (RI = [PSV-EDV]/PSV, where PSV = peak systolic velocity, and EDV = end-diastolic velocity) is another index often used in literature. Just as for the PI, changes in probe angle affect values for PSV and EDV similarly, thus RI is not affected by probe angle and can be used to compare serial determinations of CBF velocities.

RI is a fair indicator of cerebrovascular resistance. However, the best predictor of cerebrovascular resistance is change in EDV. Changes in PSV also affect RI, but these changes would reflect pump forces such as blood pressure and cardiac output (Raju, 1991).

IIIAi(e). Transducers

As the velocity of ultrasound in biological tissue is relatively constant, higher frequency transducers are required to obtain ultrasound of smaller wavelength. Transducers with a high frequency are also capable of resolving small parts. Thus, for our study a phased array 3-5 MHz transducer was chosen. A good quality 5 MHz transducer is capable of resolving about 1 or 2 mm lateral resolution. Temporal resolution is limited to 40 msec with a frame rate of 25 per second.

The maximum Doppler shift that can be detected is half the pulse repetition frequency, also known as the Nyquist frequency. The corresponding maximum velocity obtained by using this value in the Doppler equation is termed the Nyquist limit. The Nyquist limit varies with the depth setting. When deep structures are examined only slow velocities can be sampled as enough time must be given for the echoing pulses to return to the transducer. If the Nyquist limit is exceeded the phenomenon of aliasing takes place, where the highest velocities are represented in the reverse channel.

High-amplitude low-velocity signals are generated by movement of the vessel wall rather than by blood within it, and constitute interference, which is filtered out from the returning signal by the wall-thump filter. Wall-thump filters are usually set between 100 and 500 Hz. For our study the wall filter was set between 100 to 200, high enough to eliminate the audible thumping of the vessel walls.

IIIAi(f). Bioeffects and Safety

The American Institute for Ultrasound in Medicine (AIUM, 1988) has set maximum acceptable levels for peak output at 100 mW/cm^2. Acoustic power is defined as the flow of energy across the ultrasound field per unit of time, and is measured in watts. The intensity refers to the power per

unit area in the field, usually square centimeters. For continuous wave ultrasound the intensity measured at the peak is termed intensity spatial peak (Isp).

An average value calculated for the intensity at a specified distance averaged across the field is termed the intensity spatial average (Isa). Pulsed ultrasound is intermittent so that energy values are briefly high, and the equivalent measurement is of the intensity spatial peak temporal average (Ispta). The AIUM (1988) considers that 'there have been no biological effects for unfocused ultrasound below 100 mW/cm^2, and even at higher intensities when the product of intensity and exposure time is less than 50 $joules/cm^2$ (1 J/s = 1 Watt)'.

Two known bioeffect mechanisms have been associated with the use of ultrasound. The thermal bioeffect refers to a rise in temperature of tissues when exposed to acoustic energy. The effect depends on the amount of energy, the area of exposure, and the thermal characteristics of the tissue. The ISPTAd represents the per second, time-averaged, acoustic intensity measured at the derated (in situ) spatial peak (focal point) location and averaged over one of the following: (a) the repetition for single line modes (M-mode, Doppler), or (b) the scan repetition interval for scanning modes. The ISPTAd is related to the thermal bioeffect mechanism, and is the primary acoustic parameter regulated

by the Food and Drug Administration (FDA). It is presented on the on-screen display in increments of 1 mW/cm^2. The FDA limit for intended use in neonatal heads is 94 mW/cm^2.

The cavitational bioeffect refers to the effect of ultrasound energy on bubbles of gas- or vapor-pockets in tissue. The mechanical index (MI) is related to the cavitational bioeffect mechanism and is presented on the on-screen display in 0.1 increments.

The reference manual of the manufacturer of the ultrasound machine used in our study (Ultramark 9 HDI Reference Manual, 1992) states that "in all cases the ISPTAd and the MI output preset values are less than the FDA limits for the application". Thus, when in the "Neonatal Head" examination type preset listed under the "Radiology" mode, the system has an output limit of 94 mW/cm^2.

The phased array probe, P5-3, has a maximum attainable acoustic intensity ISPTAd = 66 mW/cm^2 and MI = 0.8 at 9 cm depth in 2-D imaging mode at 5 MHz scanning frequency, and ISPTAd = 650 mW/cm^2 at 6.9 cm depth and MI = 1.5 at 6.3 cm depth in the pulsed Doppler imaging mode at 3 MHz scanning frequency.

Before starting the scan, the unit was set to the Neonatal Head preset mode, and power output was further reduced to 1.0 mW/cm^2 SPTAd in gray scale imaging mode, and 74-88 mW/cm^2 SPTAd in Doppler mode. These levels are below

the upper limit set by the FDA for intended use in neonatal heads. Also the pulse repetition frequency was reduced from the preset value of 2500 for neonatal heads to 1250-1500, which further reduced the number of Doppler pulses sent out per second, and also improved the sensitivity for detection of slow flow.

The actual ISPTAd and MI values attained during scanning of neonatal heads under the modified settings on the ATL Ultramark 9 HDI machine are listed below:

	ISPTAd (mW/cm^2)	MI
2-D mode (gray-scale imaging)	01.1	0.3
Imaging in the Color mode	12.0	0.4
Pulsed Doppler insonation	74.0 to 88.0	0.3

IIIAii. Testing Protocol

40 neonates admitted to the NICU at St. Vincent's Medical Center between the period of January 1995 to May 1996 were studied. The Institutional Review Board approved all protocols at St. Vincent's Medical Center. Informed consent was obtained from the parent prior to the test. Ultrasound equipment was available for experimental purposes only when all diagnostic sonograms and emergency scans had been completed for the day. At that time the ultrasound unit was wheeled up to the NICU. All clinically stable new admissions with parental consent were considered for testing. If no new admissions met the criteria, repeat tests

were performed on neonates studied earlier in the week. The operator had no awareness of their clinical status.

Scans were performed at the bedside in the NICU while the neonates were asleep. Lights in the room were dimmed prior to the study, and the neonate was fed and swaddled to minimize movements. If the neonate was not asleep prior to the scan, testing was delayed until the baby was calm. The neonate was kept supine with the head in a neutral position, and this position was maintained during the study.

Using the anterior fontanel as a window, flow measures in the BA were obtained in the sagittal plane. Then scanning via the anterolateral foramina, the terminal branches of the MCAs were insonated and flow measures were obtained. Spectral tracings during five consecutive cardiac cycles were visually assessed until stable and optimal waveforms were obtained. Peaks and troughs were manually identified in the waveform, and an on-line time averaged mean velocity (representing the area under the curve) was computer-generated. The following velocity measures were obtained for each vessel: end-diastolic velocity (EDV), peak-systolic velocity (PSV), and time averaged mean velocity (TAMV). The pulsatility index (PI), defined as PSV-EDV/TAMV, was derived from the above measures.

A typical cranial ultrasound study, including gray-scale imaging and Doppler velocity measurement, took 40

minutes to complete. Approximately 30% of the examination time was spent in setting up the machine at the bedside, initiating the menu system, entering demographic information, and arranging the probe for testing. 30% of the time was spent in the 2-D gray-scale imaging mode, and about 9% of the time in color mode (both operate at the same power output levels). About 5 seconds were spent in the Doppler mode to acquire velocity signals from each vessel insonated. 30% of time was spent in the freeze mode (zero power output) during which time calculations and waveform analyses were performed and screen annotations were made.

IIIB. Measurement of Delayed Neural Conduction by ABRs

All ABRs were recorded at 32 weeks post-conception age in premature infants, and no earlier than 24 hours after birth in infants older than 32 weeks post-conception age. The infant's ear was dried and cleaned at least three times by NICU nurses and was visually inspected to exclude retained debris prior to ABR testing. A resident performed otoscopy if there was suspicion of occlusion of the external auditory canal. There was no occasion when an ABR test had to be postponed because of otitis media or externa in the group of neonates studied.

ABRs were recorded at the bedside in the NICU under clinical protocols. We used 100 μs monaural rarefaction clicks presented at an intensity of 75dB adult NHL and a

rate of 10.1 Hz. One channel of brain electrical activity was recorded from the vertex and referenced to the ipsilateral mastoid with forehead as ground. The auditory stimulus was presented via a Sony MDK-2 earphone that was standardized against TDH-39 pediatric earphones. Frequencies were matched and were linear with respect to the TDH-39 earphones, except in the very low frequency range, which are not typically measured using clicks in ABR recordings.

Responses were bandpass filtered between 100 Hz and 3000 Hz (-12 dB). Peak-to-peak voltage level was used to establish an artifact-rejection algorithm. The data were digitized at 20 kHz for 10 ms and averaged over 1024 artifact-free repetitions to produce an ABR waveform that was stored and displayed using a modified IBM-AT microcomputer. At least three replicable waveforms were obtained and inspected. If waveforms were not replicable, additional waveforms were obtained. The three most replicable waveforms were then averaged to form a composite waveform and CL's were identified by visual inspection. When the identity of CL's was not clear, additional recordings were made at lower sound intensities, e.g., 65 dB NHL. ABR waveforms where CL's and IPL's were not discernible (n=1) were classified as abnormally delayed and were assigned CL values equal to (mean + 2SD) of post-conception age-matched controls for statistical hypothesis testing.

IIIC. Clinical Variables

Values for pH, $PaCO_2$, and PaO_2 from arterial blood gas analyses (ABGs) and corresponding bicarbonate values from blood chemistry analyses done under clinical protocols in the NICU were obtained from the clinical record. The presence or absence of fetal heart rate changes, meconium staining or oligohydramnios, placental infarction or abruptio, Apgar scores < 7, resuscitation with sodium bicarbonate, prolonged intubation (> 7 days), neonatal bradycardia or hypotension, positive cranial imaging, and positive EEGs were obtained from the clinical record. The pediatric service made referrals to an attending pediatric neurologist as clinically indicated. Reports of these clinical neurological evaluations were also obtained from the clinical record.

IIID. Matching of Doppler, ABR and Blood Gas Information

A total of 40 neonates were studied, some more than once. We matched each Doppler test to the closest ABR, ABG, and serum chemistry measure to create Doppler-ABR-ABG data triplets for each case. As PET studies have shown the brain hypoperfusion effect of asphyxia to last 7 days, we included only those cases in which the interval between the Doppler and ABR tests was within the 7-day range. Thus, we excluded those cases in which the interval between ABR and Doppler tests exceeded 5 days when the ABR test followed the Doppler

test, and 2 days when the ABR test preceded the Doppler test. These criteria yielded 41 matched ABG-Doppler-ABR triplets in 34 neonates, with mean interval between ABR and Doppler tests of 1.47 days. Three neonates had incomplete Doppler examinations with missing data for terminal middle cerebral artery pulsatility indices, as this vessel was not interrogated during the initial stages of the study. Data analyses were then carried out on the 38 matched triplets as samples for tests of hypotheses.

IV. RESULTS

IVA. Variables Analyzed

IVAi. Measures of flow.

PI's (measures of downstream impedance and estimates of perfusion) in the brainstem (basilar distribution, BA PI) and cortex (terminal middle cerebral artery distribution, tMCA PI) were entered into the analysis. To obtain an index of differential perfusion of brainstem versus cerebral cortex, a ratio of brainstem to cortical impedance was computed by dividing BA PI by tMCA PI. A higher ratio of brainstem to cortical impedance indicated either high basilar PI (high resistance and low-flow state in brainstem) or low tMCA PI (low resistance and high flow state in cortex).

IVAii. Measures of asphyxia.

Asphyxia was determined by giving a positive score to

any neonate with an ABG documenting pH < 7.15, $PaCO_2$ > 45 mmHg and PaO_2 < 80 mmHg.

IVAiii. ABR measures.

ABR wave III and V CL's were entered into the analysis. As described earlier, ABR wave III is generated by the superior olivary nucleus, located at the ponto-medullary junction, and is supplied by branches of the basilar artery. Wave V CL is generated by the inferior colliculus, located in the midbrain tegmentum, and is supplied by terminal branches of the basilar artery as well as by collateral supply into the circle of Willis from the posterior cerebral arteries.

IVAiv. Clinical variables.

Neurological evaluation by the attending pediatric neurologist was scored according to the following scale: 0, normal or no evaluation; 1, normal tone with positive but non-focal examination; 2, hypotonia; 3, hemiplegia or hemiparesis; 4, clinical diagnosis of hypoxic-ischemic encephalopathy.

Each of the following variables were given a positive score if the diagnosis was established in the clinical record: the presence of fetal heart rate changes, meconium staining or oligohydramnios, placental infarction or abruptio, Apgar scores < 7, resuscitation with sodium bicarbonate, prolonged intubation (> 7 days), neonatal

bradycardia or hypotension, positive cranial imaging, and positive EEGs.

Flow measures, ABR measures, and clinical data including ABG and serum chemistry values were stored in a database (Rbase 4.5). Data were analyzed using Systat 7.0 for Windows. Two sample student's t-tests and univariate or multivariate regression were used as applicable. An alpha level of 0.05 was used to determine significant effects for all statistical tests.

IVB. Descriptive Statistics

Mean birth weight was 2299 g (SD: 995 g), mean gestational age was 35.1 weeks (SD: 4 weeks), mean head circumference was 31 cm (SD: 3.9 cm), and mean interval between Doppler and ABR tests was 0.4 days (SD: 1.8 days).

Neonates with ABGs having pH < 7.15, $PaCO_2$ > 45 mmHg, and PaO_2 < 80 mmHg were classified as having suffered an asphyxial event. Twelve data triplets in eight neonates met these criteria, and the mean interval of an asphyxial event from birth was 0.5 days.

Mean interval between the asphyxial event and the first Doppler study was 2.9 days and mean interval between Doppler test and corresponding ABR study was 0.4 days.

Twenty-two neonates had normal or no neurological evaluation. Four neonates had normal tone with positive but non-focal examination. Two neonates had hypotonia, one

neonate had hemiplegia, and two neonates were classified clinically as having hypoxic-ischemic encephalopathy.

IVC. Data Analysis

IVCi. Association of ABR Wave III and V CL's with Regional PI's

Tables 1 & 2 show results from univariate regression of regional PI's against ABR wave III & V CL's. BA PI was positively related to ABR wave III CL (F = 5.80, p = 0.021), but not to wave V CL (F = 0.19, p > 0.05). tMCA PI was related to neither ABR wave III (F = 0.11, p > 0.05) nor wave V CL (F = 0.28, p > 0.05). The ratio of BA to tMCA PI was positively associated with ABR wave III CL (F = 4.3, p = 0.045), but not with wave V CL (F = 0.52, p > 0.05).

IVCii. Contribution of Impedances in Individual Vessels to Ratio of Brainstem to Cortical Impedance: Differential Flow

Table 3 shows the results from univariate regression of the ratio of BA to tMCA PI against individual PI's. In the entire group BA PI was positively associated with the ratio of brainstem to cortical impedance (F = 9.4, p = 0.004), while tMCA PI was negatively associated with the ratio (F = 25.92, p = 0.001).

IVCiii. Effect of Asphyxia on Ratio of Brainstem to Cortical Impedance

Table 4 shows the results from two-sample t-tests on the ratio of BA to MCA PI, grouped by asphyxia. Asphyxiated

neonates had a significantly higher mean ratio of brainstem to cortical impedance (t = -3.02, p = 0.005).

IVCiv. Effect of Asphyxia on the Association of Regional PI's with Ratio of Brainstem to Cortical Impedance

Table 5 shows the results of univariate regression of BA and tMCA PI's against the ratio of brainstem to cortical impedance. In non-asphyxiated neonates we found that the ratio of brainstem to cortical impedance was negatively associated with impedance in tMCA (F = 22.38, p = 0.001). A developmental fall in cortical resistance may account for this finding (Hayashi, Ichiyama, Uchida, Tashiro, & Tanaka, 1992). In asphyxiated neonates, on the other hand, the ratio was positively associated with BA PI (F = 5.43, p = 0.042). A doubling of the coefficient of basilar PI in asphyxiated neonates, and a non-significant but small increase in the coefficient of tMCA PI indicate a higher impedance in both territories in asphyxiated neonates, more so in the brainstem than in the cortex. Thus, a higher ratio of brainstem to cortical impedance in asphyxiated neonates was associated with higher impedance (low-flow state) in the basilar territory.

IVCv. Effect of Asphyxia on the Association of BA PI with ABR Wave III CL

Our data have shown a higher ratio of brainstem to cortical impedance in asphyxiated neonates, and a higher

basilar PI in such neonates. Our data have also shown a positive association of BA PI with ABR wave III in the whole group. Hence, the next step was to check the association of BA PI with ABR wave III in asphyxiated neonates.

Table 6 shows the results of univariate regression of regional PI's against ABR wave III CL. In non-asphyxiated neonates none of the flow measures are significantly associated with delayed ABR CL's (p's > 0.05). In asphyxiated neonates a positive association between basilar PI and ABR wave III CL (F = 9.22, p = 0.013) suggests that low-flow states in the brainstem following asphyxia may be responsible for delays in conduction along the brainstem components of the auditory tract (specifically wave III, the generator of which is located in the territory supplied by the BA).

IVCvi. Association of BA PI with Birth-to-Test Interval and Demographic Variables

Table 7 shows results from univariate regression of BA PI against various demographic variables. Birth to test interval, representing the time between birth and the Doppler study, was not significantly associated with BA PI (p > 0.05). Similarly, birth weight, estimated gestational age and head circumference in our group of neonates were not associated with BA PI (p's > 0.05).

IVCvii. Clinical Variables Associated with Asphyxia

Table 8 shows results from two-sample t-tests on various clinical variables grouped by asphyxia. Birth weight, head circumference and estimated gestational age were not significantly different in asphyxiated infants when compared with non-asphyxiated infants in our group (p's > 0.05). Infants with asphyxia had a significantly higher ratio of brainstem to cortical impedance (p = 0.005). Asphyxiated neonates also had a significantly higher incidence of prenatal fetal heart rate changes (p = 0.001), meconium staining or oligohydramnios (p = 0.001), placental infarction or abruptio (p = 0.003), low Apgar scores (p = 0.001), resuscitation with sodium bicarbonate (p = 0.001), prolonged intubation (p = 0.044), neonatal bradycardia or hypotension (p = 0.002), positive cranial imaging (p = 0.014), positive EEGs (p = 0.011), and positive neurological examination (p = 0.001).

IVCviii. Association of Clinical Variables with Ratio of Brainstem to Cortical Impedance

Using univariate regression (results shown in Table 9) we established that the ratio of brainstem to cortical impedance was positively associated with asphyxia, accounting for 20% of the variance (univariate) ($F = 9.12$, $p < 0.005$), and was positively associated with neurological examination, also accounting for 20 % variance (univariate) ($F = 9.08$, $p < 0.005$). Birth weight, gestational age, head

circumference, gender, and Apgar scores were not significantly associated with the ratio of impedance in our group of neonates (p's > 0.05). pH, $PaCO_2$, and PaO_2 from ABGs matched temporally to the Doppler study did not account for significant variance in the ratio, indicating that the acid-base status might have recovered after the asphyxial event.

IVCix. Multivariate Association of Clinical Variables with Ratio of Brainstem to Cortical Impedance

We performed multivariate regression of clinical variables against the ratio of BA to tMCA PI. The four variables found to be associated with the ratio ($p < 0.05$) in the univariate model were entered into a multivariate model and forward and backward step regression analysis was performed. The multivariate model revealed that asphyxia, neurological examination, positive cranial sonography and electroencephalography together accounted for 51% of the variance (multivariate) in ratio of BA to tMCA PI ($F = 2.9$, $p = 0.037$). However, the variables making up the model did not achieve significance individually. Stepping the model resulted in exclusion of all variables except asphyxia, which was positively associated with BA to tMCA ratio ($F = 9.2$, $p = 0.005$).

V. DISCUSSION

Our results support our initial hypotheses. We found a

positive association between BA PI and ABR wave III. A significant positive association was noted in the entire group as well as in asphyxiated neonates, but not in non-asphyxiated neonates. As the generators of wave III are located in the territory supplied by the basilar artery, this association suggests that high impedance and low flow states in the basilar territory might be associated with delayed conduction in the brainstem, producing prolonged ABR wave III CL's.

On the other hand, there was no association between BA PI and ABR wave V. The generator of wave V, the inferior colliculus, is situated more rostrally in the brainstem and receives perfusion not only from the basilar artery but also from the posterior part of the circle of Willis. Thus, reduced BA flow may be compensated for by increased flow from the circle of Willis.

Our data suggest that there is a period of delayed hypoperfusion in neonates that may be detected by Doppler ultrasound. A higher mean ratio of brainstem to cortical impedance in asphyxiated versus non-asphyxiated neonates suggests that there is either a higher impedance and low-flow state in the basilar territory, or a lower impedance and high flow state in the territory supplied by the middle cerebral artery.

Our regression results show that in the entire group,

and more specifically, in asphyxiated neonates, BA PI is positively associated with the ratio of brainstem to cortical impedance. This suggests that the higher ratios seen in asphyxiated neonates are likely related to elevations in basilar impedance and low-flow states in the brainstem. In non-asphyxiated neonates BA PI was not significantly associated with the ratio.

Impedance in tMCA was negatively related to the ratio of brainstem to cortical impedance in the entire group as well as in non-asphyxiated neonates, indicating that in these neonates impedances were falling as expected with growth and flow to the cortex was increasing. However, this association lost significance in asphyxiated neonates, with a reduction, albeit small, in the coefficient of tMCA PI, indicating a corresponding reduction in flow to the cortical areas supplied.

The positive association of the ratio of basilar to cortical impedance with ABR wave III suggests that higher ratios and low-flow states in the brainstem are associated with delays in impulse conduction in the neurons comprising the generators of wave III. In asphyxiated neonates but not in non-asphyxiated neonates, higher brainstem to cortical impedance ratios were associated with higher impedance and low-flow states in the basilar territory. Further, the positive association of BA PI with ABR wave III in

asphyxiated neonates but not in non-asphyxiated neonates reaffirms the notion that asphyxia likely results in delayed hypoperfusion in the brainstem, the consequences of which cause delays in the ABR.

VA. Brain injury during ischemia and reperfusion

VAi. Brain Edema

The brain has a high consumption of oxygen and glucose, and depends almost exclusively on oxidative phosphorylation for energy production. During ischemia delivery of oxygen and glucose to the brain is interrupted, and impairment of oxidative phosphorylation enhances vulnerability to excitotoxicity (Beal, 1998).

Brain energy stores are scant, and ATP and phosphocreatinine get depleted within 4 to 5 minutes following bilateral ischemia in gerbils (Nowak, Fried, David Lust, & Passonneau, 1985). This results in a failure of the ATP-dependent membrane-bound Na^+/K^+ ATPase pump, resulting in loss of ionic gradients (Yanagihara & McCall, 1982; Hossman, Sakaki, & Zimmermann 1977). Neurons and glia depolarize (Katsura, Kristian, & Siesjö, 1994), and following activation of voltage-dependant calcium channels, excitatory amino acids such as glutamate and aspartate are released into the extracellular space (Hagberg et al., 1985). Moreover, inactivation of energy-dependent presynaptic reuptake of glutamate occurs and leads to

further accumulation of extracellular glutamate.

Activation of glutamate receptors results in intracellular calcium overload (Park, Nehls, Teasdale, & McCulloch, 1989). There is also rapid intracellular accumulation of sodium and chloride, followed by chloride-favored osmotic entry of water. These events occur in neurons and glia, but predominantly in glial cells, and cause acute cellular swelling, termed cytotoxic edema (Kempski, & Volk, 1994; Young, Rappaport, Chalif, & Flamm, 1987; MacKnight, & Leaf, 1975).

Secondary metabolic events that follow glutamate toxicity may further induce cell swelling in vitro (Tower, 1992), and in vivo (Gordon, Simpson, Statman, & Silverstein, 1991). Secondary brain damage is also associated with vasogenic edema resulting from tissue necrosis and impaired blood-brain barrier permeability (Betz, Keep, Beer, & Ren, 1994; Klatzo, 1994).

Using diffusion weighted MR and measuring apparent diffusion coefficient (ADC) to follow the time course of cytotoxic edema after an acute hypoxic-ischemic event in 7-day old rats, Rumpel, Nedelcu, Aguzzi, & Martin, (1997) have shown a biphasic decrease in ADC, suggesting restricted diffusion in glial cells undergoing cytotoxic edema. An immediate decrease in ADC normalized within three hours after the hypoxic-ischemic event, and was followed by a

delayed decrease in ADC occurring between 8-48 hours after recovery. These findings were associated with increased labeling of GFAP immunoreactive glial cells. The delayed phase was also associated with an increase in T2 signal, a marker of vasogenic edema, and was accompanied by eosinophilic neuronal shrinkage. Maximum T2 signal was obtained at 24-48 hours after the hypoxic-ischemic event and was associated with neuronal necrosis and apoptosis, as measured by the TUNEL method.

Brann & Myers (1975) studied the effects of severe intrauterine partial asphyxia in term fetal monkeys by using halothane anesthesia to lower maternal blood pressure for up to 5 hours. This resulted in fetal asphyxia, hypoxia, hypercapnia, acidosis and hypotension. Following delivery and resuscitation the animals were sacrificed at 1 to 96 hours of life. Diffuse gyral flattening was noted and was confirmed by electron microscopy to represent intracellular edema. Cortical necrosis was also seen to involve primarily parieto-occipital parasagittal cortex. A similar pattern of neuronal injury was noted seen in 90% of term infants surviving severe asphyxia (Volpe, & Pasternak, 1977; Volpe, Herscovitch, Perlman, Kreusser, & Raichle, 1985).

It has been proposed that local edema exceeding 25-30% of the tissue volume was associated with irreversible injury to most neurons (Garcia et al., 1983). While it has been

postulated that edema compresses local capillaries and potentiates the lesion (Symon, Branston, & Chikovani, 1979), it has also been suggested that progressive potassium-induced edema increases intercapillary distance and may thus alter neural function (Auen, Bourke, Barron, San Filippo, & Waldman, 1979), possibly accounting for the delays in ABR CL's we observed in asphyxiated neonates.

Regional glial edema has been implicated in impaired myelination. While the impairment may be transient and reversible with removal of edema, the consequences might exceed temporally the period of glial dysfunction. Perinatal hypoxic ischemic encephalopathy was associated with damage restricted to areas of primary myelination in the cortex and subcortical white matter, as well as in the thalami, basal ganglia, brainstem, and spinal cord, as detected by magnetic resonance imaging (MRI) and PET (Azzarelli, Caldmeyer, Phillips, & DeMyer, 1996). Moreover, delayed or deficient myelination was identified on MRI in eight of thirty-two children with neonatal cerebral injury and developmental delay (Johnson et al., 1987). Thus, delayed ABR CL's in our study may be related to ischemia-induced impairment in myelination, as conduction latencies are directly dependent on intact or adequate myelination of the auditory tract.

Studies in surviving asphyxiated neonates have documented raised intracranial pressure (both by noninvasive

transfontanellar and by invasive subarachnoid transducers) on the second and third days of life (Clancy et al., 1988; Levene, Evans, Forde, & Archer, 1987; Adhikari, Moodley, & Desai, 1990). In a study of 32 term asphyxiated neonates, raised intracranial pressure (> 10 mmHg) occurred in 22% neonates, reaching a maximum at 36 to 72 hours after the insult, and correlated with evidence of early brain necrosis seen on cranial computed tomography (CT) (Lupton, Hill, Roland, Whitfield, & Flodmark, 1988).

Each of these alterations in the physiological milieu may affect CBF velocities and concomitantly or consequently affect nerve conduction latencies, resulting in delayed ABR CL's in asphyxiated neonates.

VAii. Ischemia and Excitotoxicity

The excitotoxic neurotransmitter hypothesis is directed largely at events during ischemia. Selectively vulnerable cell bodies receive afferent projections that release large amounts of glutamate during ischemia-induced depolarization (Siesjö, 1992). In vulnerable neurons glutamate is bound by two ionotropic receptors, activated either by NMDA or by AMPA. NMDA receptor activation opened a calcium channel allowing calcium influx. AMPA receptor activation opened a sodium channel allowing sodium influx. Raised intracellular sodium induced reversal of normal calcium extrusion and resulted in raised intracellular calcium.

Nicholson, Bruggengate, Steinberg, & Stockle (1977) showed that anoxia triggered an intracellular accumulation of calcium. However, it was soon shown in cultured neurons (Rothman, 1984) and in brain slices (Garthwaite, & Garthwaite, 1986) that glutamate and related excitatory amino acids triggered neuronal death. Choi (1987) showed that whereas early cell swelling was usually reversible, cells exposed to glutamate showed a calcium-mediated delayed cell death.

We speculate that delays in ABR CL's that recover prior to discharge from the NICU are electrophysiological markers of early and reversible cellular edema. The reversibility of electrophysiological delay may be accounted for by the fact that glia are predominantly affected by early cytotoxic edema. However, when there is sustained ischemia, or there are repeated undetected insults, or if the first insult is severe enough to induce secondary excitotoxicity, there may be superimposed vasogenic edema and secondary excitotoxicity affecting primarily neurons. This may result in more prolonged delays in ABR CL's, as observed in severely asphyxiated neonates.

High intracellular calcium, if sustained, was excitotoxic and set off a cascade of events. There was activation of phospholipase A_2 (Edgar, Stroszanjder, & Horrocks, 1982) which degraded membrane phospholipids to

yield arachidonic acid (Rehncrona, Westerberg, Akesson, & Siesjö, 1982). There was enhanced production of nitric oxide synthase (Choi, 1988; Dawson, & Snyder, 1994), which activated the calcium-calmodulin-dependant pathway to produce nitric oxide from arginine. Production of lactic acid as a result of anaerobic glycolysis resulted in a fall in intracellular pH (Rehncrona, Rosen, & Siesjö, 1980). High intracellular calcium also caused conversion of xanthine dehydrogenase to the oxidase from (McCord, Roy, & Schaffer, 1985). The breakdown of ATP yielded large amounts of hypoxanthine (Saugstad, & Schrader, 1978), which served as a substrate for xanthine oxidase in the reperfusion phase. Nuclear DNA was damaged by calcium-induced nucleases (Tullis, & Rubin, 1982), forming many single-strand regions.

VAiii. Reperfusion and Free-radical Induced Brain Injury

The free-radical hypothesis is directed largely at events during reperfusion, and has been implicated in the events leading to delayed hypoperfusion and progression of neuronal injury. Three major observations support this theory: (a) morphologic studies have shown that most of the structural damage occurred during reperfusion, (b) there was progressive hypoperfusion of the brain during reperfusion, and (c) prolonged suppression of protein synthesis ensued (Krause, White, Aust, Nayini, & Kumar, 1988).

Reperfusion brought oxygen to the ischemic tissue. The accumulated hypoxanthine from ATP breakdown was metabolized by xanthine oxidase, with the production of superoxide (McCord, 1985; Dykens, Stern, & Trenkner, 1987). Arachidonic acid was also metabolized by cyclo-oxygenase to produce superoxide (Bakhle, 1983). During reperfusion the overproduction of free-radicals overwhelmed antioxidative defense mechanisms such as superoxide dismutase, glutathione peroxidase and catalase, resulting in inactivation and consumption of the antioxidants and an associated failure to replenish the latter in ischemic tissue (Chan, 1996).

Excessive superoxide displaced iron from ferritin stores (Thomas, Morehouse, & Aust, 1985; Krause et al., 1985), which catalyzed oxidative lipid peroxidation (Krause et al., 1985), as well as caused double-strand breaks in DNA (Brawn, & Fridovich, 1981). However, nitric oxide can react with superoxide to yield peroxynitrite, the latter decomposing to yield hydroxyl ions, highly toxic free-radicals (Beckman, Ye, Chen, & Conger, 1996). While all these three free-radicals have potentially destructive properties, both nitric oxide and peroxynitrite predispose to a mitochondrial permeability transition, and could thus trigger a pore opening in partially calcium-loaded mitochondria (Schweizer, & Richter, 1994; Packer, & Murphy, 1995).

Arachidonate metabolism led to production of prostaglandins ($PGF_2\alpha$) (Bakhle, 1983) and leukotrienes (LTD_4) (Tagari, Du Boulay, Aitken, & Boullin, 1983), both of which caused vasoconstriction, and were responsible for delayed hypoperfusion or secondary ischemia. In the presence of excess arachidonic acid, there was preferential production of thromboxane over prostacyclin (Bakhle, 1983), the former being a vasoconstrictor with platelet activating properties (Bunting, Moncada, & Vane, 1983).

Specific populations of brain neurons were exceptionally susceptible to damage and death as a consequence of ischemia and reperfusion. These included the pyramidal neurons in layers 3 and 5 of the cortex and in the CA1 zone of the horn of Ammon in the hippocampus. Evidence suggested that these neurons were selectively vulnerable to free-radical induced damage because they were deficient in glutathione peroxidase (Ushijima, Miyazaki, & Morioka, 1986), and had a high endogenous content of iron (Zaleska, & Floyd, 1985) stored in the ferric form, mostly in ferritin and transferrin (Crichton, 1979). During reperfusion superoxide promoted the reduction of iron in the ferric form and release of ferrous iron. Iron-dependent free-radical reactions then caused extensive peroxidation of the polyunsaturated fatty acids of membrane lipids (Bromont, Marie, & Bralet, 1989; White, Rafols, DeGarcia, Skjaerlund,

& Krause, 1992; White et al., 1988; Babbs et al., 1986; Kogure, Watson, Busto, & Abe, 1982; Watson et al., 1984).

Damage in the form of lipid peroxidation during reperfusion occurred in two phases (Sato, Hashimoto, & Kosaka, 1990). Immediate microvacuolation was noted in the first 15 minutes of reperfusion. Progressive damage then occurred during the following 6 hours, and by 48-72 hours neurons had disintegrated. The physiological effects of such damage ranged from alteration of fluidity and permeability of the membrane (Irvine, 1980), to compromise of membrane receptors and ion channels. At 8 hours of reperfusion, tissue and ionic gradients had equilibrated with extracellular fluid, indicating loss of ionic partitioning despite normal levels of ATP. These reactions were also involved in the genesis of post-ischemic hypoperfusion, which was inhibited by superoxide dismutase and deferoxamine (Cerchiari, Hoel, Safar, & Sclabassi, 1987), or U74006F (Hall, & Yonkers, 1988), a lipid peroxidation chain terminator (Braughler et al., 1987). Further, neural degeneration mediated by superoxide radicals generated by xanthine oxidase could be prevented by the addition to culture media of free-radical scavengers (Dykens, Stern, & Trenkner, 1987).

Capillary perfusion was reduced to 30% of normal in the cortex by 90 minutes of reperfusion following global

ischemic insults lasting between 10 and 20 minutes (Krause, White, Aust, Nayini, & Kumar, 1988). Reperfusion substantially suppressed protein synthesis in vulnerable brain areas, including the cortex (Hossman, & Kleihues, 1973; Cooper, Zalewska, Kawakami, & Hossman, 1977; Dienel, Pulsinelli, & Duffy, 1980), and several proteins produced by the brain were gradually lost over several days (Matsumoto, Yamamoto, Homburger, & Yanagihara, 1987).

Elevated PI's in the basilar territory in our group of asphyxiated neonates may represent delayed hypoperfusion noted in other studies. Though we have no direct biochemical evidence of the cellular events occurring in these neonates we speculate that the mechanisms described thus far may play a major role in affecting the auditory tract as high basilar PI's were associated with prolonged ABR CL's.

There is increasing evidence that post-ischemic inflammatory responses contribute to brain injury (reviewed by Feuerstein, Wang, & Barone, 1998). The effects of hypoxia, calcium-induced second messenger systems, and free-radicals induce the synthesis of various transcription factors such as nuclear factor κB (O'Neill, & Kaltschmidt, 1997), STAT3 (Planas et al., 1996), interferon regulatory factor 1 (Iadecola et al., 1999), and hypoxia inducible factor 1 (Ruscher et al., 1998). These transcription factors lead to the expression of numerous proinflammatory genes.

Thus, ischemic brain cells produce various mediators of inflammation, such as platelet-activating factor, tumor necrosis factor α and interleukin 1β (Rothwell, & Hopkins, 1995). There is induction of various endothelial cell-surface adhesion molecules such as intercellular adhesion molecule 1, P-selectins and E-selectins (Zhang, Chopp, Zhang, Jiang, & Powers, 1998; Haring, Berg, Tsurushita, Tagaya, & del Zoppo, 1996; Lindsberg, Carpen, Paetau, Karjalainen-Lindsberg, & Kaste, 1996). The adhesion molecules then interact with complementary surface receptors in neutrophils, macrophages, and monocytes. These blood-borne inflammatory cells adhere to the endothelial lining, and cross the wall of the vessels to enter the brain parenchyma. They are guided to areas of injured brain by chemokines such as interleukin and monocyte chemoattractant protein 1 (Yamasaki et. al., 1995; Ivacko et. al., 1997). At about five to seven days after the ischemic event, the inflammatory cells have a predominant presence in the viscinity of the infarcted tissue (Iadecola, 1997).

Post-ischemic inflammatory cells may physically obstruct the microvasculature, extending the area of infarction (del Zoppo, Schmid-Schonbein, Mori, Copeland, & Chang, 1991), or may produce toxic mediators that potentiate the degree of ischemia. For example, inducible nitric oxide synthase that generates toxic amounts of nitric oxide, is

produced not only by inflammatory cells, but also by ischemic neurons (Forster, Clark, Ross, & Iadecola, 1999). Nitric oxide produced by inducible nitric oxide synthase may be seen as late as four days after a 5-minute episode of global ischemia, and be involved in the mechanism of brain damage, especially in the hippocampal CA1 neurons that deteriorate very slowly (Caldwell, O'Neill, Earley, & Leonard, 1994). Excess neuronal nitric oxide was associated with rapid adenosine triphosphate reductions and cellular damage from peroxynitrite formation in acutely hypoxic cerebrocortical slices (Litt et al., 1999). In addition, ischemic neurons may express cyclo-oxygenase 2, which heads off a complex biochemical cascade resulting in the conversion of polyunsaturated fatty acids to prostaglandins and thromboxane (Marnett, & Kalgutkar, 1999) that have potent vasoactive properties.

These studies provide evidence for prolonged and delayed effects on neurons that far exceed the duration of the initial ischemic event, and support our hypothesis that the delays in ABR CL's observed in the first week or two after an asphyxial event may reflect cellular effects in the brainstem auditory tract.

VB. Possible therapeutic interventions

Free-radicals overwhelm endogenous scavenging mechanisms and play an important role in ischemia-induced

cell damage. Overproduction of free-radical scavenging enzymes, such as superoxide dismutase, protects against stroke (Yang et. al., 1994). However, as copper-zinc superoxide dismutase has an extremely short half-life (6 minutes) and is unable to cross the blood-brain barrier, the use of unmodified superoxide dismutase in ameliorating ischemic brain injury has met with variable results (Chan et. al., 1993). However, polyethylene gylcol-conjugated superoxide dismutase that has an increased half-life, has successfully reduced infarct volume in rats with focal cerebral ischemia (He, Hsu, Ezrin, & Miller, 1993). Malonate and MPTP, both neurotoxins causing energy depletion, secondary excitotoxicity, and free radical generation, produce neurodegenerative diseases in mice by mechanisms similar to the ones observed in hypoxic ischemic encephalopathy. Recent observations suggest that free radical spin traps provide significant protection against MPTP (Matthews et al., 1999b), and oral supplementation with creatine may buffer against ATP depletion and and MPTP-induced dopamine depletion in rats, thereby exerting a neuroprotective effect (Matthews et al., 1999a). Whether these agents have similar neuroprotective effects in the setting of asphyxia remains to be seen.

There is ample evidence to support the role of glutamate receptor activation in the initiation of ischemic

cell death. Thus, a major thrust of therapeutic intervention was to block receptors activated by glutamate (Lees, 1997) by a variety of methods, each resulting in reduction of the severity of the infarct. Glutamate antagonism may be achieved by the use of noncompetitive blockers of the ion channel associated with the NMDA receptor (e.g. aptiganel), competitive antagonists of the glutamate recognition site (e.g. selfotel), or of tne glycine recognition site (e.g. ACEA 1021, GV150526), or of the polyamine site (e.g. eliprodil) of the NMDA receptor. Drugs that interfere with glutamate release by sodium channel blockade (e.g. lubeluzole) may also be used. At neuroprotective doses selfotel is poorly tolerated, however, aptiganel can achieve therapeutic concentrations, with only a few tolerable side effects such as hypertension, sedation, confusion, or hallucination, and at higher doses, catatonia. Glycine antagonists have fewer side effects, but have poorer penetration (reviewed in Lees, 1997).

While the therapeutic time window for the administration of NMDA-receptor antagonists is only one or two hours after the ischemic event, AMPA-receptor antagonists are effective even when administered after a substantially longer interval (Turski et al., 1998). Activation of group-II and group-III metabotropic receptors may also be neuroprotective (Bond, O'Neill, Hicks, Monn, &

Lodge, 1988). However, there are significant limitations to the use of glutamate receptor antagonists. Besides the fact that glutamate is an important physiological neurotransmitter in the brain, there are serious psychomimetic, cardiovascular and respiratory side effects (Lees, 1997) associated with the use of glutamate receptor antagonists. For example, dizocilpine (MK801), a non-competitive NMDA receptor antagonist has been reported to induce behavioral sensitization (Carlezon, Meddrek, & Wise, 1995), and enhance opiate catalepsy and lethality (Vanderschuren, Schoffelmeer, & De Vries, 1997). Competitive NMDA receptor antagonists have shown similar side effects (Jeziorski, White, & Wolf, 1994).

Our study shows an association between delayed ABR CL's and elevated impedance in the basilar territory that extends for a few days after the asphyxial event. While earlier detection of the effect of the asphyxial event on brainstem neurons may be difficult, the ability to identify this association between flow and electrophysiology during NICU stay using Doppler sonography is useful. Consequently, it may allow the physician to use at least some of the glutamate receptor antagonists that are not constrained by a tight therapeutic window. However, as few animal studies have been done using these medications in a neonatal asphyxia model, clearly more research will be required

before these agents can be used in a clinical setting.

Anoxia from ischemia causes energy depletion and depolarization of neurons and glia. In the epicenter of the ischemic lesion cells may permanently depolarize, however, in the penumbra (where residual perfusion persists), the cells may repolarize. The effect of local increases in glutamate and potassium may cause the cells that partially recover to undergo repetitive depolarizations, also called peri-infarct depolarizations (Hossmann, 1996). These depolarizations have been recorded in rodent stroke models and may continue to occur for six to eight hours after the ischemic event. The number of depolarizations is directly related to infarct size (Mies, Iijima, & Hossmann, 1993). NMDA- and AMPA-receptor antagonists block peri-infarct depolarizations and have been shown to reduce infarct size (Iijima, Mies, & Hossmann, 1992).

Reducing the effects of post-ischemic inflammatory cell activation may provide another avenue for therapeutic intervention. A reduction in ischemic damage has been noted in animals when adhesion molecules or their receptors have been blocked by neutralizing antibodies, or when ischemia has been induced in mice having deletion of intercellular adhesion molecule-1 (Connolly et al., 1996). Also, blockade of mediators of inflammation such as interleukin-1 (Loddick, & Rothwell, 1996) can ameliorate the extent of ischemic

injury.

Neutrophil activation has been shown in the setting of neonatal asphyxia, and these agents may play a role in the prevention of delayed hypoperfusion.

In adults with prior stroke or transient ischemic attacks, antiplatelet agents have significantly reduced stroke recurrence (Diener, 1998). Low-dose acetylsalicylic acid acts by inhibiting thromboxane formation, and when used alone reduced stroke recurrence by 18%. Dipyridamole acts by raising platelet CAMP and CGMP levels and thereby reducing platelet aggregation, and when used alone, reduced stroke recurrence by 16%. However, when used in combination, the two agents had an additive effect, producing a reduction in stroke recurrence of 37%.

However, dipyridamole also acts by blocking the uptake and transport of adenosine into cells, thereby potentiating adenosine-mediated neuroprotective effects (Fitzgerald, 1987). Adenosine reduces the release and post-synaptic effects of glutamate (Rudolphi, & Schubert, 1996). Adenosine induces the synthesis of neurotrophins and acting together with polypeptide growth factors, promotes the regeneration of damaged neurites (Neary, Rathbone, Cattabeni, Abbracchio, & Burnstock, 1996).

How closely the findings seen in adult stroke models match the delayed hypoperfusion seen in asphyxiated neonates

remain to be seen. Perhaps, the fetal sheep models (Williams, Gunn, & Gluckman, 1991; Gunn, Gunn, de Haan, Williams, & Gluckman, 1997) may provide the closest reflection of events occurring in fetal and perinatal asphyxia, and consequently, in neonatal hypoxic ischemic encephalopathy. Especially since neonatal cardiovascular physiology in the presence of a patent ductus arteriosus, as well as cerebrovascular autoregulatory mechanisms, are substantially different from the adult, some of the neuroprotective agents studied in adult stroke models may not be appropriate to use, and may, in fact, be contraindicated for use in neonates. Thus, there is clearly need for further research in this field, and in the area of neuroprotection, to help identify which agents if appropriately modified can be safely used in the neonatal clinical setting.

VC. Conclusions

Normal physiology of cerebral blood flow ensures stable perfusion of the brainstem at all times. While brainstem flow stabilizes earlier than cortical flow in normal development, during asphyxia flow is diverted from cortical areas to the brainstem as a compensatory process (Hayashi, Ichiyama, Uchida, Tashiro, & Tanaka, 1992). However, asphyxia is invariably associated with hypotension and transient ischemia (Behrman, Lees, & Peterson, 1970),

followed by reperfusion. During reperfusion increased flow to ischemic areas may act as a double-edged sword. Besides restoring blood flow and oxygen to ischemic tissue, reperfusion eventually leads to free-radical mediated neurotoxicity and production of vasoconstrictive compounds that mediate secondary ischemia.

Thus, delayed hypoperfusion may be a mechanism by which brainstem dysfunction occurs in asphyxiated neonates. This mechanism may be operational in addition to the hypotension-induced ischemic lesions noted in the watershed areas of major cerebral vessels, seen mainly in the parasagittal cortex.

We have documented low-flow states in the brain in asphyxiated neonates, more significantly in the brainstem, which are associated with prolonged CL's of neurons traversing the brainstem, as well as with subsequent neurological outcome. This suggests that there may be a phase of altered electrophysiology in neonates that do not suffer the full-blown ischemic or hemorrhagic consequences of asphyxia. Further studies will be required to characterize the developmental consequences of such low-flow states.

Clearly these low-flow states cannot be evaluated by routine cranial imaging prior to discharge. Based on our findings, measurement of cerebral blood flow velocities in

asphyxiated neonates by the Doppler technique may provide vital and useful information about the perfusion of the brainstem. Association of low-flow states with prolonged ABR component latencies documents neurological impairment.

In our group of neonates studied, asphyxia was defined on the basis of arterial blood gas values. The clinical variables that were significantly associated with asphyxia in our group are extensively studied and widely accepted markers of fetal and neonatal distress. In our group, asphyxiated neonates had significantly higher incidence of prenatal fetal heart rate changes, meconium staining or oligohydramnios, placental infarction or abruptio, low Apgar scores, resuscitation with sodium bicarbonate, prolonged intubation, neonatal bradycardia or hypotension, positive cranial imaging, positive EEGs, and positive neurological examination.

While baseline fetal heart rate and fetal heart rate accelerations have not been found to be associated with fetal hypoxia and metabolic acidosis (Low, Pancham, & Worthington, 1977), fetal heart rate decelerations are predictive of fetal asphyxia. The probability of fetal asphyxia with metabolic acidosis was less than 50% in the presence of late decelerations however, in the presence of hypoxia late decelerations were noted in 50% neonates (Low, Pancham, & Worthington, 1977).

The presence of meconium in amniotic fluid, and its amount relative to amniotic fluid has been studied extensively. In general, the mere presence of meconium in the amniotic fluid in term infants was not associated with an increased risk of cerebral palsy. However, thick meconium staining of the amniotic fluid detected at labor was shown to have the worst prognosis (Grant, 1989). Low (1997) found moderate to severe meconium in 32% of fetuses with significant metabolic acidosis at delivery, however, there was a high false-positive rate of 95%. An increased risk was seen in those infants with meconium and low 5-minute Apgar scores (Nelson, & Ellenberg, 1986).

A weak association between low 1-minute Apgar scores and later motor and cognitive development has been noted (Broman, Nicholas, & Kennedy, 1975). However, the more important association has been between low 5-minute Apgar scores and severe motor and cognitive defects (Nelson, & Broman, 1977; Ergander, Eriksson, & Zetterström, 1983), as well as with prolonged low Apgar scores and cerebral palsy (Nelson, & Ellenberg, 1979). Though there is a high false positive rate associated with prolonged low Apgar scores, a delay in onset of respiration lasting > 20 minutes was associated with subsequent handicap in 20-25% of neonates (Steiner, & Nelligan, 1975; Scott, 1976).

Asphyxiated neonates in our group had significantly

higher ratios of brainstem to cortical impedance. We also regressed clinical variables against the ratio of brainstem to cortical impedance and found significant positive associations with the presence of asphyxia, positive neurological examination, positive gray-scale cranial sonography, and positive EEGs.

Sonographic characteristics of post-asphyxial neurological injury in preterm neonates comprise germinal matrix hemorrhage (Hambleton, & Wigglesworth, 1976; Larroche, 1979) and periventricular white matter post-infarction hemorrhages. In term neonates, ischemia in the watershed areas of major cerebral arteries (including parasagittal cortex, hippocampus, striatum, and dentate gyrus) has been demonstrated (Adams, Brierley, Connor, & Treip, 1966). Structural correlates of these lesions have been documented in animal models of hypoxic-ischemic encephalopathy.

From our data it is evident that in the post-asphyxial neonate there might exist a spectrum of neuropathological states, ranging from hemorrhage to infarction, with electrophysiological disturbance, delayed nerve conduction, and altered cerebral blood flow occurring between the extremes. Currently available clinical tests cannot characterize the less severe or even transient impairment that may follow asphyxial injury, thus limiting neurological

evaluation of such neonates.

Using Doppler imaging of the cerebral vessels, which adds 5 minutes of scan-time to the presently routine standard cranial sonographic assessment, information about regional cerebral blood flow can be obtained in at-risk neonates. Using ABRs to estimate the efficiency of impulse conduction in the auditory tract, information about the electrophysiological integrity of the CNS can be obtained. Using both techniques to evaluate asphyxiated NICU neonates we may obtain a better understanding of the alterations in physiology in the post-asphyxial state, especially during the phase of delayed hypoperfusion.

While this phase has been studied using PET and SPECT (Volpe, Herscovitch, Perlman, Kreusser, & Raichle, 1985; Shankaran, Kottamasu, & Kuhns, 1993), these tests are expensive and require transport of the neonate to a cyclotron. MR testing in mechanically ventilated neonates is also not feasible. Thus, we have described a relatively simple, non-invasive and inexpensive bedside technique to study an important problem in the neonatal population. By reducing the interval between ABR and CBF testing it might become possible to assess the vascular reserve of the brainstem and provide a means of functional testing. By measuring BA PI prior to ABR testing and during ABR testing an increment in flow might be expected in the territory

served by the BA. An inability to match increased metabolic requirements by increased flow, especially in asphyxiated neonates, may be indicative of a loss of vascular reserve. Power Doppler is an advanced post-processing technique whereby signal amplitude in the returning Doppler signal is encoded to a color scale. This technique is particularly sensitive to slow flow and may provide an index of tissue perfusion. Using digital output from the ultrasound machine it may then be possible to compare flow within individual areas of the brainstem, that may help in further clarifying the flow-ABR relationship.

From this thesis we conclude that delayed brainstem neural conduction, more specifically prolonged ABR wave III CL, is associated with high impedance in the basilar artery and low-flow states in brainstem. Further, the association of higher ratios of brainstem to cortical impedance with delayed wave III CL leads us to conclude that there is a preferential reduction in blood flow to the brainstem when compared to the cortex.

In asphyxiated neonates we conclude that there is a phase of delayed hypoperfusion that is detectable by Doppler sonography. We further conclude that asphyxiated neonates have a higher ratio of brainstem to cortical impedance ratio, and in such neonates there is higher impedance n the basilar territory as compared to the cortex, again

suggesting that there is a preferential reduction of blood flow to the brainstem. In such neonates, we conclude that higher basilar impedance is associated with delayed ABR wave III CL.

Table 1. ASSOCIATION OF ABR WAVE III CL WITH REGIONAL PI's[1]

Univariate regression of regional PI's against ABR wave III CL:

	Coeff	F	Mult-R	p
In the whole group:				
BA PI	0.27	5.80	0.37	0.021
tMCA PI	-0.04	0.11	0.06	0.745
Ratio of BA to tMCA PI	0.17	4.30	0.33	0.045

[1] ABR: auditory brainstem evoked responses; CL: component latency; PI's: pulsatility indices; Coeff: coefficient; Mult-R: multiple R; BA: basilar artery; tMCA: terminal middle cerebral artery

Table 2. ASSOCIATION OF ABR WAVE V CL WITH REGIONAL PI's[2]

Univariate regression of Regional PI's against ABR wave V CL:

	Coeff	F	Mult-R	p
In the whole group:				
BA PI	0.07	0.19	0.07	NS
tMCA PI	-0.08	0.28	0.09	NS
Ratio of BA to tMCA PI	0.08	0.52	0.12	NS

[2] ABR: auditory brainstem evoked responses; CL: component latency; PI's: pulsatility indices; Coeff: coefficient; Mult-R: multiple R; BA: basilar artery; tMCA: terminal middle cerebral artery

Table 3. ASSOCIATION OF REGIONAL PI's WITH RATIO OF BRAINSTEM TO CORTICAL IMPEDANCE[3]

Univariate regression of Ratio of BA to tMCA PI against individual PI's:

	Coeff	F	Mult-R	p
In the whole group:				
BA PI	0.65	9.40	0.46	0.004
tMCA PI	-0.89	25.92	0.65	0.001

[3] PI's: pulsatility indices; BA: basilar artery; tMCA: terminal middle cerebral artery; Coeff: coefficient; Mult-R: multiple R;

Table 4. ASSOCIATION OF ASPHYXIA WITH RATIO OF BRAINSTEM TO CORTICAL IMPEDANCE[4]

Asphyxia was defined as pH < 7.15, $PaCO_2$ > 45 mmHg, PaO_2 < 80 mmHg

Two sample t-tests on Ratio of BA to tMCA PI grouped by Asphyxia: (t = -3.02; p = 0.005)

Group	N	Mean	SD
Non-asphyxiated	26	1.199	0.505
Asphyxiated	12	1.786	0.658

[4] PI's: pulsatility indices; BA: basilar artery; tMCA: terminal middle cerebral artery; SD: standard deviation

Table 5. EFFECT OF ASPHYXIA ON THE ASSOCIATION BETWEEN REGIONAL PI's AND RATIO OF BRAINSTEM TO CORTICAL IMPEDANCE[5]

Univariate regression of Ratio of BA to tMCA PI against individual PI's:

	Coeff	F	Mult-R	p
In the whole group:				
BA PI	0.65	9.40	0.46	0.004
tMCA PI	-0.89	25.92	0.65	0.001
In non-asphyxiated neonates:				
BA PI	0.39	1.62	0.25	0.215
tMCA PI	-0.77	22.39	0.69	0.001
In asphyxiated neonates:				
BA PI	0.65	5.44	0.59	0.042
tMCA PI	-0.74	1.96	0.40	0.192

[5] PI's: pulsatility indices; BA: basilar artery; tMCA: terminal middle cerebral artery; Coeff: coefficient; Mult-R: multiple R;

Table 6. EFFECT OF ASPHYXIA ON THE ASSOCIATION BETWEEN ABR WAVE III CL AND REGIONAL PI's[6]

Univariate regression of Regional PI's against ABR wave III CL:

	Coeff	F	Mult-R	p
In the whole group:				
BA PI	0.27	5.79	0.37	0.021
tMCA PI	-0.04	0.11	0.06	0.745
Ratio of BA to tMCA PI	0.17	4.30	0.33	0.045
In non-asphyxiated neonates:				
BA PI	0.05	0.07	0.05	0.797
tMCA PI	-0.09	0.42	0.13	0.523
Ratio of BA to tMCA PI	0.15	1.58	0.25	0.220
In asphyxiated neonates:				
BA PI	0.38	9.22	0.69	0.013
tMCA PI	0.44	2.97	0.48	0.116
Ratio of BA to tMCA PI	0.12	0.63	0.24	0.448

[6] PI's: pulsatility indices; ABR: auditory brainstem evoked responses; CL: component latency; Coeff: coefficient; Mult-R: multiple R; BA: basilar artery; tMCA: terminal middle cerebral artery

Table 7. ASSOCIATION OF BA PI WITH DEMOGRAPHIC VARIABLES[7]

Univariate regression of BA PI against demographic variables:

	Coeff	F	Mult-R	p
Birth weight	0.00	0.66	0.13	NS
Estimated gestational age	-0.02	1.55	0.20	NS
Head circumference	0.01	0.62	0.13	NS
Birth to test interval	0.00	0.44	0.11	NS

[7] PI: pulsatility index; BA: basilar artery; Coeff: coefficient; Mult-R: multiple R

Table 8. CLINICAL VARIABLES ASSOCIATED WITH ASPHYXIA[8]

Asphyxia was defined as pH<7.15, $PaCO_2$ > 45 mmHg, PaO_2 < 80 mmHg

Two sample t tests on Asphyxia:

Variable	Pooled Var t	df	p
Fetal heart rate changes	-5.077	36	0.001
Meconium staining / oligohydramnios	-2.829	36	0.008
Placental infarction / abruptio	-3.205	36	0.003
Mode of delivery	-3.944	36	0.001
Low Apgar scores	-5.993	36	0.001
Resuscitation with sodium bicarbonate	6.333	36	0.001
Prolonged intubation	-2.429	36	0.020
Neonatal bradycardia / hypotension	-3.302	36	0.002
Positive cranial sonography/CT/MRI	-3.216	36	0.003
Positive electroencephalogram	-2.676	36	0.011
Positive neurological examination	-4.430	36	0.001
Ratio of BA to tMCA PI	**-3.020**	**36**	**0.005**
Estimated gestational age	-1.944	36	NS
Birth weight	-1.011	36	NS
Head circumference	-1.177	34	NS

[8] pCO2: partial pressure of carbon dioxide in arterial blood; pO2: partial pressure of oxygen in arterial blood; df: degrees of freedom; CT: computed tomography; MR: magnetic resonance imaging; BA: basilar artery; tMCA: terminal middle cerebral artery; PI: pulsatility index

Table 9. UNIVARIATE REGRESSION OF CLINICAL VARIABLES AGAINST RATIO OF BRAINSTEM TO CORTICAL IMPEDANCE[9]

Univariate regression of Ratio of BA to tMCA PI against clinical variables:

	Coeff	F	Mult-R	p
Asphyxia	0.59	9.12	0.45	0.005
Neurological examination	0.20	9.08	0.45	0.005
Cranial sonography findings	0.07	4.29	0.33	0.045
Positive EEG	0.57	5.46	0.36	0.025
Birth weight	0.00	0.12	0.06	NS
Estimated gestational age	0.02	0.62	0.13	NS
Head circumference	0.02	0.61	0.13	NS
Length	0.02	0.50	0.12	NS
Gender	0.08	0.15	0.06	NS
1-minute Apgar	-0.07	3.30	0.29	NS
5-minute Apgar	-0.11	2.93	0.28	NS
pH	-0.26	0.02	0.03	NS
$PaCO_2$	0.01	0.39	0.12	NS
PaO_2	-0.00	0.22	0.09	NS

[9] PI: pulsatility index; BA: basilar artery; tMCA: terminal middle cerebral artery; Coeff: coefficient; Mult-R: multiple R; EEG: electroencephalography; pCO2: partial pressure of carbon dioxide in arterial blood; pO2: partial pressure of oxygen in arterial blood

Appendix 1.

Cerebral Circulation: Evaluation by Ultrasound and Doppler

Use of the Doppler Technique in Measuring Cerebral Blood Flow Velocities and Estimating Cerebral Blood Flow

Volume flow in a tube is determined by the pressure difference and by the resistance to flow by Poiseuille's Law (Burns, 1995a,b; Kremkau, 1990a,b,c).

$$\text{Volume flow (ml/s)} = \frac{\text{pressure difference (dyne/cm}^2\text{)}}{\text{flow resistance (g/cm}^4\text{*s)}}$$

$$Q = \frac{\Delta P}{R}$$

The flow resistance depends on the fluid viscosity, tube length and radius as follows:

$$\text{Flow resistance} = \frac{8 * \text{length} * \text{viscosity}}{\pi r^4}$$

$$R = \frac{8Lv}{\pi r^4}$$

Thus, doubling the radius of a tube decreases the resistance to one-sixteenth of the original value. Thus,

$$\text{Volume flow} = \frac{\text{pressure difference} * \pi * r^4}{8Lv}$$

$$Q = \frac{\Delta P \pi r^4}{8Lv}$$

In a tube there is laminar flow, with each successive cylindrical layer sliding on each other, creating a

decreasing profile of flow speeds from the center to vessel wall. Thus, for parabolic flow:

Average flow speed = ½ * maximum flow speed

Turbulent flow occurs at a bifurcation or in the region of a stenosis. The flow pattern becomes random and chaotic with particles flowing in all directions.

$$\text{Reynolds \#} = \frac{\text{average flow speed} * \text{tube diam} * \text{density}}{\text{viscosity}}$$

$$Re = \frac{v(avg)*d*\rho}{v}$$

By the continuity rule, the average flow speed at a stenosis should be greater than the speed proximal or distal to it, as total volume flow must be constant for all the three regions, proximal, at stenosis, and distal to stenosis. This is because fluid is neither created nor destroyed as it passes through the tube or the stenosis.

Volume flow = average speed * tube area

$$Q = v(avg)*A$$

If the stenosis has diameter one half that adjacent to it, the area at the stenosis is one fourth that adjacent to it, and the average flow speed in the stenosis must be quadruple.

Poiseuille's equation converted to average flow speed rather than volume flow is:

$$\text{Average speed} = \frac{\text{pressure difference} * \text{radius}^2}{}$$

$$v(avg) = \frac{\Delta P r^2}{8 * \text{length} * \text{viscosity}}$$

$$v(avg) = \frac{\Delta P r^2}{8Lv}$$

Doppler shift is proportional to flow speed, not volume flow. Thus, flow speed and Doppler shift increases with radius (or diameter) squared. Volume flow depends on radius to the fourth power.

Poiseuille's law states that flow speed is less with smaller diameters, while continuity law states that flow speed is greater with smaller diameters. This applies as the radius in Poiseuille's law applies to the entire vessel, while it applies only to a short segment of the vessel in the continuity law. If the radius of the entire vessel is reduced as in vasoconstriction, flow speed is reduced. If the radius of only a short segment of the vessel is reduced (stenosis, but not complete occlusion) the flow speed in the entire vessel is unaffected, except at the stenosis, where it is increased.

Appendix 2.

Normal Ultrasound and Scanning Planes.

The most anterior coronal section contains mostly frontal lobe, and the anterior limb of the internal capsule may be seen. The anterior cerebral arteries can be seen in the interhemispheric fissure.

Coronal section at the level of the anterior frontal horns of the lateral ventricles

The frontal horns of the lateral ventricles may appear as slit-like structures on either side of midline. Minor asymmetry between the two horns is common, and not pathological. The cavum septum pellucidum lies between the two horns of the lateral ventricles, while the third ventricle appears as a single anechoiec structure under the two horns of the lateral ventricles. The middle cerebral arteries are seen to pulsate in the Sylvian fissure after they bifurcate from the internal carotid artery, and their course in the Sylvian fissure separates the temporal lobe from the insular cortex on each side. The basal ganglia at this level comprise the caudate nucleus immediately inferior to the lateral ventricles, with the denser gray matter nuclei, the putamen and globus pallidus.

Coronal section at the level of the trigones

The glomus of the choroid plexus is seen to fill the cavity of the lateral ventricles. Choroid plexus cysts are a

common finding during pregnancy and have been reported in 3% of newborns. In this plane the white matter around the trigones of the ventricles appears echogenic and is termed the peritrigonal blush (DiPietro, Brody, & Teele, 1986) or halo (Grant, Schellinger, Richardson, Coffey, & Smirniotopoulous, 1983). The blush is thought to represent neurovascular bundles travelling at right angles to the ultrasound probe, is symmetrical, less bright that the choroid and skull base, and has the appearance of fine brush strokes. A flare in this region may represent subtle pre-white matter injury, and persistent flare has been associated with abnormal neurodevelopment. Further posteriorly, the occipital cortex can be imaged and small occipital cysts of leukomalacia can be identified.

Midline sagittal section

The highly echogenic cerebellar vermis in the midline is indented on its anterior surface by the fourth ventricle. The pons lies anterior to this. Inferior to the cerebellum and between the cerebellum and the pons lies the cisterna magna, an anechoiec area. Arising from the superior surface of the cisterna magna and extending upwards is the echogenic quadrigeminal cistern containing the vein of Galen. Pulsations of the basilar artery can be seen and recorded through this cistern.

Angled parasagittal section

The entire c-shaped extent of the lateral ventricle can be seen in this section. The caudate nucleus lies below the floor of the frontal horn of the lateral ventricle and the thalamus lies behind and below it. The caudate nucleus is usually more echogenic than the thalamus. If the scan head is angled further laterally, a plane tangential and superficial to the lateral ventricle is imaged. The Sylvian fissure is seen best in this view, and in less mature infants the insula is visualized as it has not been fully covered by the opercula.

Appendix 3.

Lesions Detected on Ultrasound Cranial Sonography in Hypoxic Ischemic Encephalopathy (HIE)

Cerebral edema. Cerebral edema identified by sonography is the earliest imaging manifestation of HIE. Generalized increased echogenicity of the cerebral parenchyma due to increased in brain water produced a "bright brain" (Skeffington, & Pearse, 1983), or "snowstorm speckling" of parenchyma (Babcock, & Ball, 1983; Martin et al., 1983). The ventricular cavities may be obliterated and there is bihemispheric loss of gyral markings. Similar findings may be seen in meningitis. The appearance may resolve completely leaving no sequelae, rendering the diagnosis of cerebral edema of no prognostic value.

Ischemic and hemorrhagic lesions. The thalamus is particularly vulnerable to insult from acute total asphyxia as it has a high metabolic demand (Myers, 1975; Pasternak, 1991). Hyperechoiec foci within or replacing the thalami were noted in preterm and term asphyxiated neonates. Recent MR studies have revealed that thalamic damage maybe more common than previously suspected. However, only a minority of the affected infants developed athetoid cerebral palsy (Rutherford, Pennock, Murdoch-Eaton, Cowan, & Dubowitz, 1992; Martin, & Barkovich, 1995). Intracranial hemorrhage and MCA infarction are also recognized complications of

asphyxia (Martin et al., 1983; Levene, 1995).

White matter injury and multicystic encephalomalacia. The early appearances are more obvious on ultrasound than CT (Hope et al., 1988), though subtle damage may be missed. As cerebral edema resolves, generalized patchy, fluffy periventricular echodensities or widespread periventricular flares may be seen. The areas gradually become cystic over a few weeks. MR may be more reliable than sonography or CT in detecting basal ganglia damage, focal areas of hemorrhage or infarction, and early white matter infarction.

Lesions Typical of Term Infants

Intracranial Hemorrhage. In utero hemorrhage as a result of alloimmune thrombocytopenia (Zalneraitis, Young, & Krishnamoorthy, 1979) remains the most important cause of an intracranial hemorrhage present at birth in a term infant. Other coagulopathies such as factor V and VII deficiency have been associated with in utero intracranial hemorrhage (Whitelaw, Haines, Bolsover, & Harris, 1984), as well as hydrops fetalis (Bose, 1978). Congenital hydrocephalus (Jackson, & Blumhagen, 1983; Mintz, Arger, & Coleman, 1985; Leidig, Dannecker, Pfeiffer, Salinas, & Peiffer, 1988) has been observed following prenatal intracranial hemorrhage. Twins are eight times at higher risk of cerebral palsy than singleton births and this risk increases to 1 in 100 when the co-twin has died in utero (Petterson, Nelson, Watson, &

Stanley, 1993; Grether, Nelson, & Cummins, 1994). White matter injury may be sustained when the surviving twin experiences a period of hypotension acting as a pump twin during the agonal phase of the intrauterine partner (Fusi, McFarland, Fisk, & Wigglesworth, 1991). Maternal collapse as a result of anaphylactic shock, infection, or trauma, has caused cerebral damage in utero (Erasmus, Blackwood, & Wilson, 1982; Kim, & Elyaderani, 1982). Arterio-venous malformations and congenital aneurysms can result in hemorrhage presenting in the perinatal period (Lee, Kandall, & Ghali, 1978).

Subdural hemorrhage. Subdural hemorrhage usually occurs as a consequence of birth trauma, bleeding diathesis, or rupture of dural vascular malformation. It often co-exists with HIE. Drainage can reduce the risk of cerebral atrophy. Subdural hemorrhage may lead to occlusion of the arterial tree and vasospasm, producing an associated ischemic lesion.

Growing skull fracture / leptomeningeal cyst. This condition is extremely rare and requires a dural tear with an underlying brain insult causing edema. A skull fracture is not required. The edema disrupts the dura and prevents healing, hence the dural fracture fails to heal. The bone edges deviate, leading eventually to the formation of a pulsatile CSF-containing swelling on the surface of the skull (Hansen, Pedersen, & Petersen, 1987).

Focal subarachnoid hemorrhage / convexity hemorrhage. Term infants, several of whom had required exchange transfusion for rhesus isoimmunization, were found to have large subarachnoid hemorrhages (Morgan, Hensey, & Cooke, 1983). Similar hemorrhage has resulted from alloimmune thrombocytopenia and sepsis (Govaert, 1993). Hemostatic failure with disseminated intravascular coagulation resulting in focal subarachnoid hemorrhage has a predilection for the temporo-parietal convexity (Chessells, & Wigglesworth, 1970).

Thalamic hemorrhage. Isolated thalamic hemorrhage without associated IVH may occur in term neonates. Extension of IVH into the thalamus is often seen in preterm infants. There may be underlying thrombosis of the internal cerebral vein (Govaert, Achten, Vanhaesebrouck, De Praeter, & Van Damme, 1992). These infants present with seizures and may have eye signs including sunset sign, deviation, and upward gaze palsy (Roland, Flodmark, & Hill, 1990; De Vries, Eken, & Dubowitz, 1992). Onset is quite sudden with bulging fontanel, vomiting and jitteriness. Thalamic lesions have been described in infection (Roland, Flodmark, & Hill, 1990; Govaert, Achten, Vanhaesebrouck, De Praeter, & Van Damme, 1992), coagulopathy, and asphyxia (Kreusser, Schmidt, Shackleford, & Volpe, 1984; Cabañas et al., 1991). Recently MRI has confirmed previous pathological evidence that

thalamic lesions occur very frequently after birth asphyxia, and are not always hemorrhagic (Rutherford, Pennock, & Dubowitz, 1994).

Hemorrhagic arterial cerebral infarction. Neonatal strokes are rare, with an estimated incidence of 1 in 10,000 deliveries (Uvebrant, 1988). Arterial occlusion can result from an embolus, hyperviscosity, sepsis, stretching and damage to the artery from birth trauma or secondary to edema associated with birth asphyxia. Sonography reveals focal hyperdensity in the territory of the affected artery. Color Doppler shows absence of arterial pulsations. CT shows a corresponding low-density area (Hill, Martin, Daneman, & Fitz, 1983).

Intraventricular hemorrhage. This can occur in mature infants but is more typical of preterm infants.

Cerebellar hemorrhage. This is more common in preterm than term infants, and is associated with a grave prognosis (Scotti, Flodmark, Harwood-Nash, & Humphries, 1981).

Choroid plexus hemorrhage. The normal choroid often appears bright and bulky, particularly in preterm babies. A diagnosis of choroid plexus hemorrhage is made if there is marked asymmetry of the choroid in the absence of IVH (as blood from an IVH often settles around the choroid). The assumption that irregularity in the shape of the choroid plexus indicates hemorrhage is incorrect.

Lesions Typical of Preterm Infants

The frequent association of a unilateral parenchymal lesion with IVH may be explained by the premise that a germinal matrix hemorrhage reduces perfusion of the adjacent white matter by obstructing venous drainage. This type of venous infarct should be regarded a complication of IVH (Gould, Howard, Hope, & Reynolds, 1987). Bleeding can also occur into areas of the brain previously rendered ischemic (Rushton, Preston, & Durbin, 1985). This condition, hemorrhagic periventricular leukomalacia, complicates about 15% of periventricular leukomalacia and is often bilateral (Armstrong, & Norman, 1974).

Germinal matrix hemorrhage-intraventricular hemorrhage (GMH-IVH). The germinal matrix is unique to the preterm infant and regresses after 32 weeks gestation. Bleeding starts in the outer region of the matrix, not the region bordering the ventricle (Paneth, Rudelli, Kazam, & Monte, 1994). Bleeding occurs most frequently at the caudothalamic groove at the head of the caudate nucleus. Hemorrhage destroys the glia and is eventually replaced by a cavity, seen on sonography as a subependymal cyst.

Germinal plate hemorrhages were found in 75% of preterm infants and appear to originate in the deep terminal veins of the vena galen system. A metabolic or hypoxic basis has been postulated as the most likely etiology. Towbin

(1969a,b, 1970) suggested that infarction of the germinal plate precedes the hemorrhages. Anoxia and asphyxia lead to venous congestion and stasis, followed by thrombosis. However, in the series studied by Grunnet, Curless, Bray, & Jung (1974) only 1 infant had venous thrombi in the germinal area and no infant showed evidence of recent infarction. Apnea, cyanosis, hypotension and a sudden drop in hematocrit are correlated with severe intracerebral hemorrhage in the pre-term infant.

Philip, Allan, Toto, & Wheeler (1989) reported a decline in the incidence of GMH-IVH and parenchymal lesions in very low birth weight infants (< 1500g) from 39% in 1980 to 25% in 1987. Rennie, Wheater, & Cole (1996) also reported a decline from 35% to 15% since 1985. There was an associated increased use of antenatal steroids during this period.

There is consensus that in 75% cases GMH-IVH has occurred by 72 hours of age (Dolfin, Skidmore, Fong, Hoskins, & Shennan, 1983). In 10-20% of cases further progression takes place over the next 24-48 hours (Levene, & De Vries, 1984). The optimal time to perform a single sonogram to screen for GMH-IVH in very low birth weight infants is therefore 4-7 days after birth (Partridge, Babcock, Steichen, & Han, 1983). Repeat scans at 14 days and at discharge are required to detect periventricular

leukomalacia.

The main factors predisposing to GMH-IVH are prematurity and the presence of respiratory distress syndrome. The best unifying hypothesis is that GMH-IVH occurs because of a combination of hemodynamic instability together with a propensity to bleed, which is intrinsic to the newborn. Change in cerebral blood flow can occur in response to hypercarbia, hypoglycemia, or hypoxia. Changes in systemic blood pressure are more likely to be transmitted to the cerebral circulation if autoregulation is disturbed.

Paneth, Rudelli, Kazam, & Monte (1994) found a poor sensitivity for detection of lesions smaller than 0.2 cm, with only 29% being diagnosed. This number increased to 50% when the child survived long enough to have three or more scans. Hemorrhages larger than 1 cm were all detected. Hope et al. (1988) studied 56 cases and reported a sensitivity and specificity of 61% and 78% for GMH, 91% and 81% for IVH.

GMH-IVH has been classified into four grades (Papile, Burstein, Burstein, & Koffler, 1978): (a) grade 1, hemorrhage confined to germinal matrix; (b) grade 2, blood in the ventricular cavity with no distension; (c) grade 3, blood filling and distending ventricles; (d) grade 4, bleeding into parenchyma of brain.

Levene & de Crespigny (1983) classified hemorrhage into five grades, ventricular dilation into five grades, and

parenchymal lesions into three grades. Eken, Toet, Groenendaal, & De Vries (1995) modified the classification suggested by Levene, Fawer, and Lamont (1982), and proposed the following scale: (a) grade 1, small hemorrhage, restricted to the germinal layer; (b) grade 2a, germinal layer hemorrhage extending into the basal ganglia, with a small IVH; (c) grade 2b, IVH with > 50% ventricles filled with blood clot; (d) grade 3, IVH associated with parenchymal involvement from venous or hemorrhagic infarction.

Ischemic lesions in preterm neonates.

Periventricular leukomalacia (PVLM). The term was introduced by Banker & Larroche (1962) to denote white matter softening. This consists of small grossly visible areas of necrosis about the ventricles anterior to the anterior horns, in the corona radiata, and in the internal and external sagittal strata of the temporal and occipital horns of the lateral ventricles. These are outside the usual areas of germinal plate hemorrhage. The lesions apparently occur at the boundary zone between ventriculofugal and ventriculopetal circulation (Takashima, & Tanaka, 1978; Takashima, Armstrong, & Becker, 1978). The etiology is varied, but the mechanism consists of hypoperfusion, with toxin or excitatory amino acid induced reduction in local blood flow and energy supply.

Unlike GMH-IVH that occurs in the first week after birth, PVLM may develop up to 11 weeks after birth. The incidence is about 2% in inborn very low birth weight infants (Rennie, 1989). The areas of increased echogenicity appear within 24-48 hours after birth. Cysts evolve 2-4 weeks later, with median time to cyst development being 21 days (Trounce, Rutter, & Levene, 1986). The cysts remain visible for several weeks, and eventually disappear leaving glial scars or generalized cerebral atrophy.

Based on sonographic characteristics PVLM was classified by De Vries, Eken, & Dubowitz, (1992) as follows: (a) grade 1, periventricular echodensity persistent for > 7 days; (b) grade 2, periventricular echodense areas evolving into localized small frontoparietal cysts; (c) grade 3, periventricular echodense areas evolving into multiple cysts in the parieto-occipital white matter; (d) grade 4, echodense areas in the deep white matter evolving into multiple subcortical cysts.

Sonographic characteristics of PVLM.

Grades 1 & 2 PVLM. Periventricular echodensities are called flares. To distinguish flares from the normal periventricular blush, these echodensities must be present in two planes for longer than 48 hours.

Post mortem studies have shown that flares correspond to areas of necrosis of the premyelin cells with a glial

cell inflammatory response. 50% flares resolve within 10 days and only 10% evolve into small cysts after 2-4 weeks. The area of PVLM may be replaced by a glial scar. More often there is delayed myelination and ventricular dilation. These cases may have normal development or only mild dystonia.

Grade 3 PVLM. 2% of infants having BW < 1500g may develop multiple cysts. Significant flares always become cystic if the baby survives. Multiple cysts mark poor prognosis, as most of the children followed up have developed cerebral palsy.

Grade 4 PVLM. This severe white matter injury is more typical of hypoxic-ischemic damage in the term infant. The initial appearance is that of bright thalami and slit-like ventricles, which evolve into ventriculomegaly and cystic change over the next few weeks.

REFERENCES

Achor, L. J., & Starr, A. (1980a). Auditory brainstem response in the cat: I. Intracranial versus extracranial recordings. Electroencephalography Clinical Neurophysiology, 48, 154-173.

Achor, L. J., & Starr, A. (1980b). Auditory brainstem response in the cat: II. Effects of lesions. Electroencephalography Clinical Neurophysiology, 48, 174-190.

Adams, J. H., Brierley, J. B., Connor, R. C., & Treip, C. S. (1966). The effects of systemic hypotension upon the human brain. Clinical and neuropathological observations in 11 cases. Brain, 89, 235-268.

Adhikari, M., Moodley, M., & Desai, P. K. (1990). Mannitol in neonatal cerebral oedema. Brain Development, 12, 349-351.

AIUM (1988). Bioeffects considerations for the safety of diagnostic ultrasound. Journal of Ultrasound Medicine, 7(9), [Suppl.].

Amadeo, M., & Shagass, C. (1973). Brief latency click-evoked potentials during waking and sleep. Psychophysiology, 10, 244-250.

Amochaev, A., Johnson, R. C., Salamy, A., & Shah, S. N. (1979). Brain stem auditory evoked potentials and myelin changes in trichtylin-induced edema in young adult rats. Experimental Neurology, 66, 629-635.

Archer, L. N. J., Evans, D. H., Paton, J. Y., & Levene, M. I. (1986). Controlled hypercapnia and neonatal cerebral artery Doppler ultrasound waveforms. Pediatric Research, 20, 218-21.

Archer, L. N. J., Levene, M. I., & Evans, D. H. (1986). Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. Lancet, ii, 1116-18.

Armstrong, D., & Norman, M. G. (1974). Periventricular leukomalacia in neonates: complications and sequelae. Archives of Disease of Childhood, 49, 367-75.

Ashwal, S., Dale, P. S., & Longo, L. D. (1984). Regional cerebral blood flow: Studies in the fetal lamb during hypoxia, hypercapnia, acidosis, and hypotension. Pediatric Research, 18, 1309-1316.

Ashwal, S., Majcher, J. S., & Longo, L. D. (1981). Patterns of fetal lamb regional cerebral blood flow during and after prolonged hypoxia: Studies during the posthypoxic recovery period. American Journal of Obstetrics and Gynecology, 139, 365-372.

Ashwal, S., Majcher, J. S., Vain, N., & Longo, L. D. (1980). Patterns of fetal lamb regional cerebral blood flow during and after prolonged hypoxia. Pediatric Research, 14, 1104-1110.

Auen, E. L., Bourke, R. S., Barron, K. D., San Filippo, B. D., & Waldman, J. B. (1979). Alterations in cat cerebrocortical capillary morphometric parameters following K+-induced cerebrocortical swelling. Acta Neuropathologica (Berlin), 47, 175-81.

Auer, L. M., & Johannson, B. B. (1980). Dilatation of pial arterial vessels. Acta Physiologica Scandinavica, 109, 249-251.

Azzarelli, B., Caldmeyer, K. S., Phillips, J. P., & DeMyer, W. E. (1996). Hypoxic-ischemic encephalopathy in areas of primary myelination: a neuroimaging and PET study. Pediatric Neurology, 14(2), 108-16.

Babbs, C. F., Ralston, S. H., Badylak, S. F., Nichols, M., Cull, F. A., & Gale, M. S. (1986). Evidence of preventable lipid peroxidation in brain and heart four hours after cardiac arrest and reperfusion in rats [Abstract]. American Journal of Emergency Medicine, 4, 414.

Babcock, D. S., & Ball, W. (1983). Postasphyxial encephalopathy in full term infants: ultrasound diagnosis. Radiology, 148, 417-23.

Bakhle, Y. S. (1983). Synthesis and catabolism of cyclo-oxygenase products. British Medicine Bulletin, 39, 214.

Banker, B. Q., & Larroche, J. C. (1962). Periventricular leukomalacia of infancy: a form of neonatal hypoxic encephalopathy. Archives of Neurology, 7, 386-410.

Barkhof, F., & Valk, S. (1988). "Top of the basilar" syndrome: a comparison of clinical and MR findings. Neuroradiology, 30, 293-298.

Batton, D. G., Hellman, J., & Hernandez, M. D. (1983). Regional cerebral blood flow, cerebral blood flow velocity

and pulsatility index in newborn dogs. Pediatric Research, 17, 908-12.

Beal, M. F. (1998). Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. Annals of Neurology, 44, [3 Suppl. 1], S110-4.

Beckman, J. S., Ye, Y., Chen, J., & Conger, K. (1996). The interaction of nitric oxide with oxygen radicals and scavengers in cerebral ischemic injury. Advances in Neurology, 71, 339-350.

Behrman, R., Lees, M., & Peterson, E. (1970). Distribution of the circulation in the normal and asphyxiated fetal primate. American Journal of Obstetrics and Gynecology, 108, 956-969.

Békésy, G. V. (1960). Experiments in Hearing. New York: McGraw Hill Book Company.

Benveniste, H., Drejer, J., Schousboe, A., & Diemer, N. H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. Journal of Neurochemistry, 43, 1369-1374.

Betz, A. L., Keep, R. F., Beer, M. E, & Ren, X. (1994). Blood-brain barrier permeability and brain concentration of sodium, potassium, and chloride during focal ischemia. Journal of Cerebral Blood Flow and Metabolism, 14, 29-37.

Bode, H. (1988). Physiological influences on cerebral hemodynamics. In: H. Bode (Ed.), Pediatric applications of transcranial Doppler sonography (pp. 84-93). New York: Springler-Verlag Wien.

Bode, H., & Eden, A. (1989). Transcranial Doppler sonography in children. Journal of Child Neurology, 4, S68-S76.

Bond, A., O'Neill, M. J., Hicks, C. A., Monn, J. A., & Lodge, D. (1998). Neuroprotective effects of a systematically active group II metabotropic glutamate receptor agonist LY354740 in a gerbil model of global ischemia. Neuroreport, 9(6), 1191-1193.

Borgstrom, P., Grande, P. O., & Mellander, S. (1984). An evaluation of the metabolic interaction with myogenic vascular reactivity during blood flow autoregulation. Acta Physiologica Scandinavica, 122, 275-284.

Bose, C. (1978). Hydrops fetalis and in utero intracranial hemorrhage. Journal of Pediatrics, 93, 1023-4.

Brann, A. W. (1985). Factors during neonatal life that influence brain disorders. In: J. H. Freeman (Ed.), Prenatal and perinatal factors associated with brain disorders. PHS, NIH Publication 85-1149 (pp. 263). Washington, DC: Department of Health and Human Services.

Brann, A. W., Jr., & Myers, R. E. (1975). Central nervous system findings in the newborn monkey following severe in utero partial asphyxia. Neurology, 25, 327-338.

Braughler, J. M., Pregenzer, J. F., Chase, R. L., Duncan, L. A, Jacobsen, E. J., & McCall, J. M. (1987). Novel 21-amino steroids as potent inhibitors of iron-dependent lipid peroxidation. Journal of Biology and Chemistry, 262, 10438-440.

Brawn, K., & Fridovich, I. (1981). DNA strand scission by enzymatically generated oxygen radicals. Archives of Biochemistry and Biophysiology, 206(2), 414-9.

Broman, S. H., Nicholas, P. L., & Kennedy, W. A. (1975). Preschool IQ: Prenatal and early developmental correlates. Hillsdale, NJ: Erlbaum.

Bromont, C., Marie, C., & Bralet, J. (1989). Increased lipid peroxidation in vulnerable brain regions after transient forebrain ischemia in rats. Stroke, 20, 918-924.

Brough, R. B. Jr., Cowley, A. W., Jr., & Guyton, A. C. (1975). Quantitative analysis of the acute response to hemorrhage of the renin-angiotensin-vasoconstrictor loop in areflexic dogs. Cardiovascular Research, 9, 722-733.

Buchwald, J. S. (1985). Generators. In: J. T. Jacobson (Ed.), The auditory brainstem response (pp. 161-164). San Diego, CA: College-Hill Press.

Buchwald, J. S., & Huang, C. (1975). Far field acoustic response: origins in the cat. Science, 189, 382-384.

Bunting, S., Moncada, S., & Vane, J. R. (1983). The prostacyclin-thromboxane A2 balance: pathophysiological and therapeutic implications. British Medical Bulletin, 39(3), 271-6.

Burns P. N. (1995a). Hemodynamics. In: K. J. W. Taylor, P. N. Burns, & P. N. T. Wells (Eds.), Clinical applications of

Doppler ultrasound (pp. 35-53). New York: Raven Press.

Burns P. N. (1995b). Doppler examination: interpretation and analysis. In K. J. W. Taylor, P. N. Burns, & P. N. T. Wells (Eds.), Clinical applications of Doppler ultrasound (pp. 90-91). New York: Raven Press.

Busija, D. W., Heistad, D. D., & Marcus, M. L. (1981). Continuous measurements of cerebral blood flow in anesthetized cats and dogs. American Journal of Physiology, 241, H228-234.

Busse, R., Forstermann, U., Matsuda, H., & Pohl, U. (1984). The role of prostaglandins in endothelium-mediated vasodilatory response to hypoxia. Pflügers Archives, 401, 77-83.

Cabañas, F., Pellicer, A., Perez-Higueras, A., Garcia-Alix, A., Roche, C., & Ouro, J. (1991). Ultrasonographic findings in thalamus and basal ganglia in term asphyxiated infants. Pediatric Neurology, 7, 211-15.

Caldwell, M., O'Neill, M., Earley, B., & Leonard, B. (1994). N^G-Nitro-L-arginine protects against ischemia-induced increases in nitric oxide and hippocampal neuro-degeneration in the gerbil. European Journal of Pharmacology, 260, 191-200.

Carlezon, W. A., Mendrek, A., & Wise, R. A. (1995). MK-801 disrupts the expression but not the development of bromocriptine sensitization: a state-dependency interpretation. Synapse, 20, 1-9.

Carpenter, M. B. (1978). The pons. In: M. B. Carpenter (Ed.), Core text of neuroanatomy (pp. 112-139). Baltimore, MD: The Williams & Wilkins Co.

Cavazzuti, M., & Duffy, T. E. (1982). Regulation of local cerebral blood flow in normal and hypoxic newborn dogs. Annals of Neurology, 11, 247-257.

Cerchiari, E. L., Hoel, T. M., Safar, P., & Sclabassi, R. J. (1987). Protective effects of combined superoxide dismutase and deferoxamine on recovery of cerebral blood flow and function after cardiac arrest in dogs. Stroke, 18, 869-78.

Chan, P. H. (1996). Role of oxidants in ischemic brain damage. Stroke, 27(6), 1124-1129.

Chan, P. H., Epstein, C. J., Kinouchi, H., Imaizumi, S.,

Carlson, E., & Chen, S. F. (1993). Role of superoxide dismutase in ischemic brain injury: reduction of edema and infarction in transgenic mice following focal cerebral ischemia. Progress in Brain Research, 96, 97-104.

Chessells, J. M., & Wigglesworth, J. S. (1970). Secondary hemorrhagic disease of the newborn. Archives of Disease of Childhood, 45, 539-43.

Choi, D. W. (1987). Ionic dependence of glutamate neurotoxicity. Journal of Neuroscience, 7, 369-379.

Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. Neuron, 1, 623-634.

Chugani, H. T., Phelps, M. E., & Mazziotta, A. (1986). Patterns of focal cerebral metabolic rates for glucose in children with psychomotor retardation determined with positron emission tomography. [Abstract] Annals of Neurology, 20, 425.

Clancy, R., Legido, A., Newell, R., Bruce, D., Baumgart, S. & Fox, W. W. (1988). Continuous intracranial pressure monitoring and serial electroencephalographic recordings in severely asphyxiated term neonates. American Journal of Disease of Childhood, 142(7), 740-47.

Connolly, E. S., Winfree, C. J., Springer, T. A., Naka, Y., Liao, H., Yan, S. D., Stern, D. M., Solomon, R. A., Gutierrez-Ramos, J. C., & Pinsky, D. J. (1996). Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. Journal of Clinical Investigation, 97, 209-216.

Cooper, H. K., Zalewska, T., Kawakami, S., & Hossman K. A. (1977). The effect of ischemia and recirculation on protein synthesis in the rat brain. Journal of Neurochemistry, 28, 929-934.

Cowley, A. W., Jr. (1982). Vasopressin and cardiovascular regulation. In: A. C. Guyton, & J. E. Hall (Eds.), International Review of Physiology (vol. 26, pp. 189-242). Baltimore: University Press Park.

Crichton, R. R. (1979). Interactions between iron metabolism and oxygen activation. In: Oxygen free radicals and tissue damage. Ciba Foundation Symposium, 65, (pp. 57-66). New York: Excerpta Medica.

Davies, J. M., & Tweed, W. A. (1984). The regional distribution and determinants of myocardial blood flow during asphyxia in the fetal lamb. Pediatric Research, 18, 764-767.

Dawson, T. M., & Snyder, S. H. (1994). Gases as biological messengers: nitric oxide and carbon monoxide in the brain. Journal of Neuroscience, 14, 5147-5159.

De Vries, L. S., Eken, P., & Dubowitz, L. M. S. (1992). The spectrum of leukomalacia using cranial ultrasound. Behavioral Brain Research, 49, 1-6.

del Zoppo, G. J., Schmid-Schonbein, G. W., Mori, E., Copeland, B. R., & Chang, C. M. (1991). Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. Stroke, 22(10), 1276-83.

Diemer, K., & Henn, R. (1964). The capillary density in the frontal lobe of mature and premature infants. Biology of the Neonate, 7, 240-242.

Dienel, G. A., Pulsinelli, W. A., & Duffy, T. E. (1980). Regional protein synthesis in rat brain following acute hemispheric ischemia. Journal of Neurochemistry, 35, 1216-26.

Diener, H. C. (1998). Dipyridamole trials in stroke prevention. Neurology, 51, [Suppl.] S17-9.

DiPietro, M. A., Brody, B. A., & Teele, R. L. (1986). Peritrigonal echogenic 'blush' on cranial sonography: pathologic correlates. American Journal of Radiology, 146, 1067-71.

Dolfin, T., Skidmore, M. B., Fong, K. W., Hoskins, E. M., & Shennan, A. T. (1983). Incidence, severity and timing of subependymal and intraventricular hemorrhages in preterm infants born in a perinatal unit as detected by serial real-time ultrasound. Pediatrics, 71, 541-6.

Dykens, J. A., Stern, A., & Trenkner, E. (1987). Mechanism of kainate toxicity to cerebellar neurons in vitro is analogous to reperfusion tissue injury. Journal of Neurochemistry, 49(4), 1222-8.

Edgar, A. D., Strosznajder, J., & Horrocks, L. A. (1982). Activation of ethanolamine phospholipase A_2 in brain during ischemia. Journal of Neurochemistry, 39, 1111 -6.

Edvinsson, L., MacKenzie, E. T., & McCulloch, J. (1993). Functional circuitry. In: L. Edvinsson, E. T. MacKenzie, & J. McCulloch (Eds.), Cerebral blood flow and metabolism (pp. 92-110). New York: Raven Press.

Eken, P., Toet, M. C., Groenendaal, F., & De Vries, L. S. (1995). Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischemic encephalopathy. Archives of Disease of Childhood, 73, F75-80.

Eklof, B., Lassen, N. A., Nilsson, L., & Siesjo, B. K. (1973). Blood flow and metabolic rate for oxygen in the cerebral cortex of the rat. Acta Physiologica Scandinavica, 88, 587-89.

Ellenberg, J. H., & Nelson, K. B. (1988). Cluster of perinatal events identifying infants at high risk for death or disability. Journal of Pediatrics, 113, 546-552.

Enevoldsen, E. M., & Jensen, F. T. (1977). "False" autoregulation of cerebral blood flow in patients with acute severe head injury. Acta Neurologica Scandinavica, 64, [Suppl.], 514-515.

Erasmus, C., Blackwood, W., & Wilson, J. (1982). Infantile multicystic encephalomalacia after maternal bee-sting anaphylaxis during pregnancy. Archives of Disease of Childhood, 57, 785-7.

Ergander, U., Eriksson, M., & Zetterström, R. (1983). Severe neonatal asphyxia. Acta Paediatrica Scandinavica, 72, 321-325.

Evans, D. H. (1985). On measurement of mean velocity of flow using Doppler ultrasound. Ultrasound in Medicine and Biology, 11, 735-41.

Evans, D. H., Barrie, W. W., Asher, M. J., Bentley, S., & Bell, P. R. F. (1980). The relationship between ultrasonic pulsatility index and proximal arterial stenosis in a canine model. Circulation Research, 46, 470-475.

Faraci, F. M., & Heistad, D. D. (1990). Regulation of large cerebral arteries and cerebral microvascular pressure. Circulation Research, 66, 8-17.

Fenton, A. C., Woods, K. L., Evans, D. H., & Levene, M. I. (1992). Cerebrovascular carbon dioxide reactivity and

failure of autoregulation in preterm infants. Archives of Disease of Childhood, 67, 835-39.

Feuerstein, G. Z., Wang, X., & Barone, F. C. (1998). In: M. Ginsberg, & J. Bogousslavsky (Eds.), Cerebrovascular Disease: Pathophysiology, Diagnosis and Management (pp. 507-531). Blackwell Science.

Fitzgerald, G. A. (1987). Dipyridamole. New England Journal of Medicine, 316(20), 1247-1257.

Florence, G., & Seylaz, J. (1992). Rapid autoregulation of cerebral blood flow - A laser-Doppler flowmetry study. Journal of Cerebral Blood Flow and Metabolism, 12, 674-680.

Forster, C., Clark, C. B., Ross, M. E., & Iadecola, C. (1999). Inducible nitric oxide synthase expression in human cerebral infarcts. Acta Neuropathologica (Berl), 97, 215-220.

Fredholm, B. B., & Hedqvist, P. (1980). Modulation of neuro-transmission by purine nucleotides and nucleosides. Biochemical Pharmacology, 29, 1635-1643.

Freeman, J. M. (1985). Preface. In: J. H. Freeman (Ed.), Prenatal and perinatal factors associated with brain disorders. PHS, NIH Publication 85-1149. Washington, DC: Department of Health and Human Services.

Frewen, T. C., Kissoon, N., Kronick, J., Fox, M., Lee, R., Bradwin, N., & Chance, G. (1991). Cerebral blood flow, cross-brain oxygen extraction, and fontanelle pressure after hypoxic-ischemic injury in newborn infants. Journal of Pediatrics, 118, 265-271.

Friede, R. L. (1972). Ponto-subicular lesions in perinatal anoxia. Archives of Pathology, 94, 343-354.

Friedman, W. F., & Kirkpatrick, S. E. (1977). Fetal cardiovascular adaptation to asphyxia. In: L. Gluck (Ed.), Intrauterine asphyxia and the developing fetal brain. Chicago: Year Book.

Fusi, L., McFarland, P., Fisk, N., & Wigglesworth, J. S. (1991). Acute twin-twin transfusion: a possible mechanism for brain-damaged survivors after intrauterine death of a monochorionic twin. Obstetrics and Gynecology, 78, 517-20.

Garcia, J. H., Mitchem, H. L., Briggs, L., Morawetz, R., Hudetz, A. G., Hazelrig, J. B., Halsey, Jr., J. H., &

Conger, K. A. (1983). Transient focal ischemia in subhuman primates. Neuronal injury as a function of local cerebral blood flow. Journal of Neuropathology and Experimental Neurology, 42(1), 44-60.

Garthwaite, G., & Garthwaite, J. (1986). Neurotoxicity of excitatory amino acid receptor agonists in rat cerebral slices: dependence on calcium concentration. Neuroscience Letters, 66, 193-198.

Gordon, K. E., Simpson, J., Statman, D., & Silverstein, F. S. (1991). Effects of perinatal stroke on striatal amino acid efflux in rats studied with in vivo microdialysis. Stroke, 22, 928-32.

Gosling, R. G., King, D. H., Newman, D. L., & Woodcock J. P. (1969). Transcutaneous measurement of arterial blood velocity by ultrasound. In: Ultrasonics for industry conference papers (pp. 16-32). Guildford, England: IPC.

Gould, S. J., Howard, S., Hope, P. L., & Reynolds, E. O. R. (1987). Periventricular intraparenchymal cerebral hemorrhage in preterm infants: role of venous infarction. Journal of Pathology, 151, 197-202.

Govaert, P. (1993). Deep hemorrhage. In: P. Govaert (Ed.), Cranial hemorrhage in the term newborn infant (pp. 101-61). London: MacKeith Press.

Govaert, P., Achten, E., Vanhaesebrouck, P., De Praeter, C., & Van Damme, J. (1992). Deep cerebral venous thrombosis in thalamo-ventricular hemorrhage of the term newborn. Pediatric Radiology, 22, 123-127.

Granger, H. J., Goodman, A. H., & Granger, D. N. (1976). Role of resistance and exchange vessels in local microvascular control of skeletal muscle oxygenation in the dog. Circulation Research, 38, 379-385.

Grant, A. (1989). Monitoring the fetus during labor. In: A. Chalmers, M. Enkin, M. J. N. C. Kersie (Eds.), Effective care in pregnancy and childbirth (vol. 2, p. 848). Oxford: Oxford University Press.

Grant, E., Schellinger, D., Richardson, J., Coffey, M., & Smirniotopoulous, J. (1983). Echogenic periventricular halo: normal sonographic finding or neonatal cerebral hemorrhage. American Journal of Neuroradiology, 4, 43-6.

Greisen, G., & Trojaborg, W. (1987). Cerebral blood flow,

$PaCO_2$ changes and visual evoked potentials in mechanically ventilated, preterm infants. Acta Paediatrica Scandinavica, 76, 394-400.

Greisen, G., Johansen, K., Ellison, P. H., Frederiksen, P. S., Mali, J., & Friis-Hansen, B. (1984). Cerebral blood flow in the newborn infant. Comparison of Doppler ultrasound and Xe-133 clearance. Journal of Pediatrics, 104, 411-18.

Grether, J. K., Nelson, K. B., & Cummins, S. K. (1994). Twinning and cerebral palsy: experience in four northern California counties, births through 1983-1985. Pediatrics, 92(6), 854-8.

Grunnet, M. L., Curless, R. G., Bray, P. F., & Jung, A. L. (1974). Brain changes in newborns from an intensive care unit. Developmental Medicine and Child Neurology, 16, 320-328.

Gunn, A. J., Gunn, T. R., de Haan, H. H., Williams, C. E., & Gluckman, P. D. (1997). Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal sheep. Journal of Clinical Investigation, 99, 248-256.

Hagberg, H., Lehmann, A., Sandberg, M, Nystrom, B., Jacobson, I., & Hamberger, A. (1985). Ischemia-induced shift of inhibitory and excitatory amino acids from intra- to extracellular compartments. Journal of Cerebral Blood Flow and Metabolism, 5, 413-9.

Haggendal, E., & Johansson, B. (1965). Effects of arterial carbon dioxide tension and oxygen saturation on cerebral blood flow autoregulation in dogs. Acta Physiologica Scandinavica, 66, [Suppl. 258], 27-53.

Hall, E. D., & Yonkers, P. A. (1988). Attenuation of postischemic cerebral hypoperfusion by the 21-aminosteroid U74006F. Stroke, 10, 340-44.

Hall, J. E. (1982). Regulation of renal hemodynamics. In: A. C. Guyton, & J. E. Hall (Eds.), International Review of Physiology (vol. 26, pp. 243-321). Baltimore: University Park Press.

Hambleton, G., & Wigglesworth, J. S. (1976). Origin of intraventricular hemorrhage in the preterm infant. Archives of Disease of Childhood, 51, 651-59.

Hansen, K. N., Pedersen, H., & Petersen, M. B. (1987). Growing skull fracture-rupture of coronal suture caused by

vacuum extraction. Neuroradiology, 29, 502.

Hansen, N. B., Stonestreet, B. S., Rosenkrantz, T. S., & Oh, W. (1983). Validity of Doppler measurements of anterior cerebral blood flow velocity: Correlation with brain blood flow in piglets. Pediatrics, 72, 526-531.

Haring, H. P., Berg, E. L., Tsurushita, N., Tagaya, M., & del Zoppo, G. J. (1996). E-selectin appears in nonischemic tissue during experimental focal cerebral ischemia. Stroke, 27, 1386-91.

Harper, A. M., & Glass, H. I. (1965). Effects of alterations in the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressure. Journal of Neurology, Neurosurgery, Psychiatry, 28, 449-52.

Hauge, A., Thoresen, M., & Walloe, L. (1980). Changes in cerebral blood flow during hyperventilation and CO_2 breathing measured transcutaneously in humans by a bi-directional pulsed ultrasound Doppler blood velocitymeter. Acta Physiologica Scandinavica, 110, 167-73.

Hayashi, T., Ichiyama, T., Uchida, M., Tashiro, N., & Tanaka, H. (1992). Evaluation by color Doppler and pulsed Doppler sonography of blood flow velocities in intracranial arteries during the early neonatal period. European Journal of Pediatrics, 151, 461-65.

He, Y. Y., Hsu, C. Y., Ezrin, A. M., & Miller, M. S. (1993). Polyethylene glycol-conjugated superoxide dismutase in focal cerebral ischemia-reperfusion. American Journal of Physiology, 265, H252-256.

Hellström-Westas, L., Rosénn, I., & Svenningsen, N. W. (1995). Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. Archives of Disease of Childhood, 72, F34-38.

Hill, A., Martin, D. J., Daneman, A., & Fitz, C. R. (1983). Focal ischemic cerebral injury in the newborn: diagnosis by ultrasound and correlation with computed tomographic scan. Pediatrics, 71, 790-3.

Hope, P. L., Gould, S. J., Howard, S., Hamilton, P. A., Costello, A. M. de L., & Reynolds, E. O. R. (1988). Precision of ultrasound diagnosis of pathologically verified lesions in the brains of very preterm infants. Developmental

Medicine and Child Neurology, 30, 457-71.

Hossman, K. A., & Kleihues, P. (1973). Reversibility of ischemic brain damage. Archives of Neurology, 29, 375-384.

Hossman, K. A., Sakaki, S., & Zimmermann, V. (1977). Cation activities in reversible ischemia of the cat brain. Stroke, 8(1), 77-81.

Hossmann, K. A. (1996). Periinfarct depolarizations. Cerebrovascular and Brain Metabolism Reviews, 8, 195-208.

House, J. W., & Brackmann, D. E. (1979). Brainstem audiometry in neurotologic diagnosis. Archives of Otolaryngology, 105, 305-309.

Iadecola, C. (1997). Bright and dark sides of nitric oxide in ischemic brain injury. Trends in Neurosciences, 20, 132-139.

Iadecola, C., Salkowski, C. A., Zhang, F., Aber, T., Nagayama, M., Vogel, S. N., & Ross, M. E. (1999).The transcription factor interferon regulatory factor 1 is expressed after cerebral ischemia and contributes to ischemic brain injury. Journal of Experimental Medicine, 189, 719-727.

Iijima, T., Mies, G., & Hossmann, K. A. (1992). Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801: effect on volume of injury. Journal of Cerebral Blood Flow and Metabolism, 12, 727-733.

Irvine, R. F. (1980). How is the level of free arachidonic acid controlled in mammalian cells? Biochemical Journal, 204, 3-16.

Ivacko, J., Szaflarski, J., Malinak, C., Flory, C., Warren, J. S., & Silverstein, F. S. (1997). Hypoxic-ischemic injury induces monocyte chemoattractant protein-1 expression in neonatal rat brain. Journal of Cerebral Blood Flow and Metabolism, 17, 759-770.

Jackson, J. C., & Blumhagen, J. D. (1983). Congenital hydrocephalus due to prenatal intracranial hemorrhage. Pediatrics, 72, 344-6.

Jewett, D. L. (1970). Volume-conducted potentials in response to auditory stimuli as detected by averaging in the cat. Electroencephalography and Clinical Neurophysiology,

28(6), 609-618.

Jewett, D. L., & Romano, M. N. (1972). Neonatal development of auditory system potentials averaged from the scalp of rat and cat. Brain Research, 36, 101-115.

Jewett, D. L., & Williston, J. S. (1971). Auditory evoked far fields averaged from the scalp of humans. Brain, 94, 681-696.

Jewett, D. L., Romano, M. N., & Williston, J. S. (1970). Human auditory evoked potentials: Possible brainstem components detected on the scalp. Science, 167, 1517-1518.

Jeziorski, M., White, F. J., & Wolf, M. E., (1994). MK-801 prevents the development of behavioral sensitization during repeated morphine administration. Synapse, 16(2), 137-147.

Johannsson, H., & Siesjö, B. K. (1975). Cerebral blood flow and oxygen consumption in the rat in hypoxic hypoxia. Acta Physiologica Scandinavica, 93, 269-276.

Johnson, G. N., Palahnuik, R. J., Tweed, W. A., Jones, M. V., & Wade, J. G. (1979). Regional cerebral blood flow changes during severe fetal asphyxia produced by slow partial umbilical cord compression. American Journal of Obstetrics and Gynecology, 135, 48-52.

Johnson, M. A., Pennock, J. M., Bydder, G. M., Dubowitz, L., M., Thomas, D. J., & Young, I. R. (1987). Serial MR imaging in neonatal cerebral injury. American Journal of Neuroradiology, 8(1), 83-92.

Johnson, P. C. (1980). The myogenic response. In: D. F. Bohr, A. P. Somlyo, & H. V. Sparks, Jr. (Eds.), Handbook of physiology, Section 2: The cardiovascular system, vol II, Vascular smooth muscle (pp. 409-442). Bethesda: The American Physiological Society.

Johnson, P. C. (1986). Autoregulation of blood flow. Circulation Research, 59(5), 483-495.

Kaga, K., Kitazumi, E., & Kodama, K. (1979). Auditory brain stem responses of kernicterus infants. International Journal of Pediatric Otorhinolaryngology, 1, 255-264.

Karmel, B. Z., Gardner, J. M., Zapulla, R. A., Magnano, C. L., & Brown, E. G. (1988). Brainstem auditory evoked responses as indicators of early brain insult. Electroencephalography Clinical Neurophysiology, 71, 429-

442.

Katsura, K., Kristian, T., & Siesjö, B. K. (1994). Energy metabolism, ion homeostasis, and cell damage in the brain. Biochemical Society Transactions, 22, 991-996.

Kempski, O. S., & Volk, C. (1994). Neuron-glial interaction during injury and edema of the CNS. Acta Neurochirurgica, 60, [Suppl.] 7-11.

Kennedy, C., DesRosiers, M. H., Jehle, J. W., Reivich, M., Sharp, F., & Sokoloff, L. (1975). Mapping of the functional neural pathways by autoradiographic survey of local metabolic rate with [14C]deoxyglucose. Science, 187, 850-853.

Kennedy, C., Sakurada, O., Shinohara, M., Jehle, J., & Sokoloff, L. (1978). Local cerebral glucose utilization in the normal conscious macaque monkey. Annals of Neurology, 4, 293-301.

Kilney, P., Connelly, C., & Robertson, C. (1980). Auditory brainstem responses in perinatal asphyxia. International Journal of Pediatric Otorhinolaryngology, 2, 147-159.

Kim, M. S., & Elyaderani, M. K. (1982). Sonographic diagnosis of cerebroventricular hemorrhage in utero. Radiology, 142, 479-80.

Klatzo, I. (1994). Evolution of brain edema concepts. Acta Neurochirurgica, 60, [Suppl.] 3-6.

Kogure, K., Watson, B. D., Busto, R., & Abe, K. (1982). Potentiation of lipid peroxides by ischemia in rat brain. Neurochemical Research, 7, 437-54.

Kontos, H. A., Raper, A. J., & Patterson, J. L. (1977). Analysis of vasoactivity of local pH, pCO_2 and bicarbonate on pial vessels. Stroke, 8, 358-360.

Kontos, H. A., Wei, E. P., Raper, A. J. & Patterson, J. L. Jr. (1977). Local mechanism of CO_2 action of cat pial arterioles. Stroke, 8(2), 227-229.

Kontos, H. A., Wei, E. P., Raper, A. J., Rosenblum, W. I., Navari, R. M., & Patterson, J. L. (1978). Role of tissue hypoxia in local regulation of cerebral microcirculation. American Journal of Physiology, 234, H582-H591.

Krause, G. S., Joyce, K. M., Nayini, N. R., Zonica, C. L.,

Garritano, A. M., Hoehner, T. J., Evans, A. T., Indreri, R. J., Huang, R. R., & Aust, S. D. (1985). Cardiac arrest and resuscitation: Brain iron delocalization during reperfusion. Annals of Emergency Medicine, 14(11), 1037-43.

Krause, G. S., White, B. C., Aust, S. D., Nayini, N. R., & Kumar, K. (1988). Brain death following ischemia and reperfusion: a proposed biochemical sequence. Critical Care Medicine, 16, 714-726.

Kremkau, F. W. (1990a). Doppler effect. In: F. W. Kremkau (Ed.), Doppler ultrasound. Principles and instruments (pp. 85-92). Philadelphia, PA: W. B. Saunders Company.

Kremkau, F. W. (1990b). Hemodynamics. In: F. W. Kremkau (Ed.), Doppler ultrasound. Principles and instruments (pp. 53-67). Philadelphia, PA: W. B. Saunders Company.

Kremkau, F. W. (1990c). Spectral analysis. In: F. W. Kremkau (Ed.), Doppler ultrasound. Principles and instruments (pp. 121-144). Philadelphia, PA: W. B. Saunders Company.

Kreusser, K. L., Schmidt, R. E., Shackleford, G. D., & Volpe, J. J. (1984). Value of ultrasound for identification of acute hemorrhagic necrosis of thalamus and basal ganglia in an asphyxiated infant. Annals of Neurology, 16, 361-3.

Larroche, J. C. (1979). Intraventricular hemorrhage in the premature neonate. In: R. Korobkin, & C. Guilleminault (Eds.), Advances in perinatal neurology (vol 1). New York: S. P. Medical and Scientific Books.

Lassen, N. (1978). Brain. In: P. C. Johnson (Ed.), Peripheral Circulation (pp. 337-358). New York: Wiley and Sons.

Lassen, N. A. (1968). Brain extracellular pH: The main factor controlling cerebral blood flow. Scandinavian Journal of Clinical and Laboratory Investigation, 22, 247-251.

Lassen, N. A., & Christensen, M. S. (1976). Physiology of cerebral blood flow. British Journal of Anesthesia, 48, 719-734.

Laudignon, N., Farri, E., Beharry, K., & Aranda, J. V. (1991). Rapid effects of hypoxia on the cerebrospinal fluid levels of adenosine and related metabolites in newborn and one-month-old piglets. Biology of the Neonate, 59, 54-59.

Laudignon, N., Farri, E., Beharry, K., Rex, J., & Aranda, J.

V. (1990). The influence of adenosine on cerebral blood flow during hypoxic-hypoxia in the newborn piglet. Journal of Applied Physiology, 68, 1534-1541.

Lee, Y. J., Kandall, S. R., & Ghali, V. S. (1978). Intracerebral arterial aneurysm in a newborn. Archives of Neurology, 35, 171-2.

Lees, K. R. (1997). Cerestat and other NMDA antagonists in ischemic stroke. Neurology, 49, [5 Suppl. 4], S66-69.

Leidig, E., Dannecker, G., Pfeiffer, K. H., Salinas, R., & Peiffer, J. (1988). Intrauterine development of posthemorrhagic hydrocephalus. European Journal of Pediatrics, 147, 26-9.

Levene, M. I. (1995). The asphyxiated newborn infant. In M. I. Levene & R. J. Lilford (Eds.), Fetal and neonatal neurology and neurosurgery (pp. 450). Edinburgh: Churchill Livingstone.

Levene, M. I., & de Crespigny, L. C. (1983). Classification of intraventricular hemorrhage. Lancet, 1(8325), 643.

Levene, M. I., & De Vries, L. S. (1984). Extension of intraventricular hemorrhage. Archives of Disease of Childhood, 57, 631-6.

Levene, M. I., Evans, D. H., Forde, A., & Archer, L. N. (1987). Value of intracranial pressure monitoring of asphyxiated term newborn infants. Developmental Medicine and Child Neurology, 29(3), 311-319.

Levene, M. I., Fawer, C. L., & Lamont, R. F. (1982). Risk factors in the development of intraventricular hemorrhage in the preterm newborn. Archives of Disease of Childhood, 57, 410-417.

Levene, M. I., Fenton, A. C., Evans, D. H., Archer, L. N. J., Shortland, D. B., & Gibson, N. A. (1989). Severe birth asphyxia and abnormal cerebral blood flow velocity. Developmental Medicine and Child Neurology, 31, 427-434.

Levene, M. I., Shortland, D., Gibson, N., & Evans, D. H. (1988). Carbon dioxide reactivity of the cerebral circulation in extremely preterm infants: effects of postnatal age and indomethacin. Pediatric Research, 24, 175-9.

Lindsberg, P. J., Carpen, O., Paetau, A., Karjalainen-

Lindsberg, M. L., & Kaste, M. (1996). Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. Circulation, 94(5), 939-945.

Litt, L., Espanol, M. T., Hasegawa, K., Chang, L. H., Gregory, G. A., James, T. L., Beal, M. F., & Chan, P. H. (1999). NOS inhibitors decrease hypoxia-induced ATP reductions in respiring cerebrocortical slices. Anesthesiology, 90(5), 1392-401.

Loddick, S. A., & Rothwel, N. J. (1996). Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischemia in the rat. Journal of Cerebral Blood Flow and Metabolism, 16, 932-40.

Lou, H. C., Lassen, N. A., & Friis-Hansen, B. (1979). Impaired autoregulation of cerebral blood flow in the distressed newborn infant. Journal of Pediatrics, 94, 118-121.

Lou, H. C., Lassen, N. A., Tweed, W. A., Johnson, G., Jones, M., & Palahnuik, R. J. (1979). Pressure passive cerebral blood flow and breakdown of the blood-brain barrier in experimental fetal asphyxia. Acta Paediatrica Scandinavica, 68, 57-63.

Lou, H. C., Tweed, W. A., & Davies, J. M. (1985). Preferential blood flow increase to the brain stem in moderate neonatal hypoxia: Reversal by naloxone. European Journal of Pediatrics, 144, 225-227.

Lou, H. C., Tweed, W. A., & Davis, J. M. (1989). Endogenous opioids may protect the perinatal brain in hypoxia. Developmental Pharmacology and Therapeutics, 13, 129-133.

Low, J. A. (1997). The significance of fetal asphyxia in regard to motor and cognitive deficits in infancy and childhood. In: N. Tejani (Ed.), Obstetrical events and developmental sequelae (pp. 44-56). Boca Raton, FL: CRC Press.

Low, J. A., Froese, A. B., Galbraith, R. S., Smith, J. T., & Karchmar, J. E. (1993). Middle cerebral artery blood flow velocity in the newborn following delivery. Clinical and Investigative Medicine, 16(1), 29-37.

Low, J. A., Pancham, S. R., & Worthington, G. (1977). Intrapartum fetal heart rate profiles with and without fetal asphyxia. American Journal of Obstetrics and Gynecology, 127, 729-37.

Lupton, B. A., Hill, A., Roland, E. H., Whitfield, M. F., & Flodmark, O. (1988). Brain swelling in the asphyxiated term newborn: pathogenesis and outcome. Pediatrics, 82(2), 139-146.

MacDonald, H. M., Mulligan, J. C., Allen, A. C., & Taylor, P. M. (1980). Neonatal asphyxia. I. Relationship of obstetric and neonatal complications to neonatal mortality in 38,405 consecutive deliveries. Journal of Pediatrics, 96, 898-902.

MacKnight, A. D. C., & Leaf, A. (1975). Regulation of cellular volume. Physiology Review, 57, 510-573.

Marinkovic, S. V., & Gibo, H. (1993). The surgical anatomy of the perforating branches of the basilar artery. Neurosurgery, 33, 80-87.

Markwalder, T. M. Grolimund, P., Seiler, R. W., Roth, F., & Aaslid, R. (1984). Dependency of blood flow velocity in the middle cerebral artery on end-tidal carbon dioxide partial pressure - a transcranial ultrasound Doppler study. Journal of Cerebral Blood Flow and Metabolism, 4, 368-72.

Marnett, L. J., & Kalgutkar, A. S. (1999). Cyclo-oxygenase 2 inhibitors: discovery, selectivity and the future. Trends in Pharmacological Sciences, 20, 465-469.

Marshall, R. E., Reichert, T. J., Kerley, S. M., & Davis, H. (1980). Auditory function in newborn intensive care unit patients by auditory brainstem potentials. Journal of Pediatrics, 96, 731-735.

Martin, D. J., Hill, A., Fitz, C. R., Daneman, A., Havill, D. A., & Becker, L. E. (1983). Hypoxic/ischemic cerebral injury in the neonatal brain. Pediatric Radiology, 13, 307-12.

Martin, E., & Barkovich, A. J. (1995). Magnetic resonance imaging in perinatal asphyxia. Archives of Disease of Childhood, 72, F62-70.

Martin, R. G., Grant, J. L., Peace, D., Theiss, C., & Rhoton, A. L. Jr. (1980). Microsurgical relationships of the anterior inferior cerebellar artery and the facial-vestibulocochlear nerve complex. Neurosurgery, 6(5), 483-507.

Matsumoto, M., Yamamoto, K., Homburger, H. A., & Yanagihara,

T. (1987). Early detection of cerebral ischemic damage and repair process in the gerbil by use of an immunohistochemical technique. Mayo Clinic Proceedings, 62, 460-472.

Matthews, R. T., Ferrante, R. J., Klivenyi, P., Yang, L., Klein, A. M., Mueller, G., Kaddurah-Daouk, R., & Beal, M. F. (1999a). Creatine and cyclocreatine attenuate MPTP neurotoxicity. Experimental Neurology, 157(1), 142-9.

Matthews, R. T., Klivenyi, P., Mueller, G., Yang, L., Wermer, M., Thomas, C. E., & Beal, M. F. (1999b). Novel free radical spin traps protect against malonate and MPTP neurotoxicity. Experimental Neurology, 157(1), 120-6.

McCord, J. M. (1985). Oxygen-derived free radicals in post-ischemic tissue injury. New England Journal of Medicine, 312(3), 159-163.

McCord, J. M., Roy, R. S., & Schaffer, S. W. (1985). Free radicals and myocardial ischemia. The role of xanthine oxidase. Advances in Myocardiology, 5, 183-9.

Mchedlishvili, G. (1986). Blood supply to cerebral tissue. In: J. A. Bevan (Ed.), Arterial behavior and blood circulation in the brain (pp. 117-121). New York: Plenum.

McPhee, A. J., Kotagal, U. R., & Kleinman, L. I. (1985). Cerebrovascular hemodynamics during and after recovery from acute asphyxia in the newborn dog. Pediatric Research, 19, 645-650.

Menke, J., Michel, E., Rabe, H., Bresser, B. W., Grohs, B., Schnitt, R. M., & Jorch, G. (1993). Simultaneous influence of blood pressure, PCO_2, and PO_2 on cerebral blood flow velocity in preterm infants of less than 33 week's gestation. Pediatric Research, 34, 173-7.

Mies, G., Iijima, T., & Hossmann, K. A. (1993). Correlation between peri-infarct DC shifts and ischemic neuronal damage in rat. Neuroreport, 4(6), 709-711.

Mintz, M. C., Arger, P. H., & Coleman, B. G. (1985). In-utero sonographic diagnosis of intracerebral hemorrhage. Journal of Ultrasound in Medicine, 4, 375-6.

Morgan, M. E. I., Hensey, O. J., & Cooke, R. W. I. (1983). Convexity cerebral hemorrhage in the neonate: in vivo diagnosis. Archives of Disease of Childhood, 58, 814-8.

Myers, R. E. (1975). Four patterns of perinatal brain damage and their conditions of occurrence in primates. Advances in Neurology, 10, 223-34.

Neary, J. T., Rathbone, M. P., Cattabeni, F., Abbracchio, M. P., & Burnstock, G. (1996). Trophic actions of extracellular nucleotides and nucleosides on glial and neuronal cells. Trends in Neurosciences, 19(1), 13-18.

Nelson, K. B. (1988). What proportion of cerebral palsy is related to birth asphyxia? Journal of Pediatrics, 112(4), 572-4.

Nelson, K. B., & Broman, S. H. (1977). Perinatal risk factors in children with serious motor and mental handicaps. Annals of Neurology, 2(5), 371-7.

Nelson, K. B., & Ellenberg J. H. (1979). Neonatal signs as predictors of cerebral palsy. Pediatrics, 64(2), 225-32.

Nelson, K. B., & Ellenberg, J. H. (1981). Apgar scores as predictors of chronic neurologic disability. Pediatrics, 68(1), 36-44.

Nelson, K. B., & Ellenberg, J.H. (1986). Antecedents of cerebral palsy. Multivariate analysis of risk. New England Journal of Medicine, 315(2), 81-6.

Newell, D. W., & Aaslid, R. (1992). Transcranial Doppler-Clinical and experimental uses. Cerebrovascular and Brain Metabolism Reviews, 4(2), 122-143.

Nicholson, C., Bruggengate, G. T., Steinberg, R., & Stockle H. (1977). Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. Proceedings of the National Academy of Science, 74, 1287-1290.

Nowak, T. S., Fried, R. L., David Lust W., & Passonneau, J. V. (1985). Changes in brain energy metabolism and protein synthesis following transient bilateral ischemia in the gerbil. Journal of Neurochemistry, 44(2), 487-494.

O'Neill, L. A., & Kaltschmidt, C. (1997). NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends in Neurosciences, 20, 252-258.

O'Rourke, M. F. (1967). Input impedance of the systemic circulation. Circulation Research, 20(4), 365-80.

Orlowski, J. P., Nodar, R. H., & Lonsdale, D. (1979). Abnormal brainstem auditory evoked potentials in infants with threatened sudden death syndrome. Cleveland Clinical Quarterly, 46, 77-81.

Packer, M. A., & Murphy, M. P. (1995). Peroxynitrite formed by simultaneous nitric oxide and superoxide generation causes cyclosporin-A-sensitive mitochondrial calcium efflux and depolarization. European Journal of Biochemistry, 234, 231-239.

Paneth, N., Rudelli, R., Kazam, E., & Monte, W. (1994). Brain damage in the preterm infant. Clinics in Developmental Medicine, 131. Lavenham: MacKeith Press.

Papile, L. A, Burstein, J., Burstein, R., & Koffler, H. (1978). Incidence and evaluation of subependymal and intraventricular hemorrhage: a study of infants with birthweight < 1500g. Journal of Pediatrics, 92, 529-34.

Park, C. K., Nehls, D. G., Teasdale, G. M., & McCulloch, J. (1989). Effect of the NMDA antagonist MK-801 on local cerebal blood flow in focal cerebral ischemia in the rat. Journal of Cerebral Blood Flow and Metabolism, 9, 617-22.

Partridge, J. C., Babcock, D. S., Steichen, J. J., & Han, B. K. (1983). Optimal timing for diagnostic cranial ultrasound in low birth weight infants: detection of intracranial hemorrhage and ventricular dilatation. Journal of Pediatrics, 102, 281-7.

Pasternak, J. F. (1991). Neonatal asphyxia: vulnerability of the basal ganglia, thalamus, and brainstem. Pediatric Neurology, 7, 147-9.

Paulson, O. B., Strandgaard, S., & Edvinsson, L. (1990). Cerebral autoregulation. Cerebrovascular Brain Metabolism Review, 2, 161-192.

Perlman, J. M., Herscovitch, P., & Corriveau, S. (1985). The relationship of cerebral blood flow velocity, determined by Doppler, to regional cerebral blood flow, determined by positron emission tomography [Abstract]. Pediatric Research, 19, 357.

Peters, T. J., Golding, J., Lawrence, C. J., Fryer, J. G., Chamberlain, G. V., & Butler, N. R. (1984). Factors associated with delayed onset of regular respiration. Early Human Development, 9(3), 209-23.

Petterson, B., Nelson, K. B., Watson, L., & Stanley, F. (1993). Twins, triplets, and cerebral palsy in Western Australia in the 1980's. British Medical Journal, 307, 1239-43.

Philip, A. G., Allan, W. C., Toto, A. M., & Wheeler, L. R. (1989). Intraventricular hemorrhage in preterm infants: declining incidence in the 1980s. Pediatrics, 84, 797-801.

Picton, T. W., Hillyard, S. A., Kraug, H. I., & Galambos, R. (1974). Human auditory evoked potentials. I. Evaluation of components. Electroencephalography Clinical Neurophysiology, 36, 179-190.

Planas, A. M., Soriano, M. A., Berruezo, M., Justicia, C., Estrada, A., Pitarch, S., & Ferrer, I. (1996). Induction of Stat3, a signal transducer and transcription factor, in reactive microglia following transient focal cerebral ischemia. European Journal of Neuroscience, 8, 2612-18.

Plum, F., Gjedde, A., & Samson, F. E. (1976). Neuroanatomical functional mapping by the radioactive 2-deoxy-d-glucose method. Neurosciences Research Program Bulletin, 14, 457-518.

Pourcelot, L. (1976). Diagnostic ultrasound for cerebrovascular disease. In: J. Donald, & S. Levi (Eds.), Present and future diagnostic ultrasound. New York: Wiley.

Pourcyrous, M., Leffler, C. W., Bada, H. S., Korones, S. B., & Busija, D. W. (1993). Brain superoxide anion generation in asphyxiated piglets and the effect of indomethacin at therapeutic dose. Pediatric Research, 34(3), 366-369.

Pryds, O., Greisen, G., Lou, H., & Friis-Hansen, B. (1989). Heterogeneity of cerebral vasoreactivity in preterm infants supported by mechanical ventilation. Journal of Pediatrics, 115, 111-38.

Pryds, O., Greisen, G., Lou, H., & Friis-Hansen, B. (1990). Vasoparalysis associated with brain damage in asphyxiated term infants. Journal of Pediatrics, 117, 119-25.

Purves, M. J., & James, I. M. (1969). Observations in the control of cerebral blood flow in the sheep fetus and newborn lamb. Circulation Research, 25, 651-667.

Raju, T. N. K. (1991). Cerebral Doppler studies in the fetus and newborn infant. Journal of Pediatrics, 119, 165-174.

Ramaekers, V. T., & Casaer, P. (1990). Defective regulation of cerebral oxygen transport after severe birth asphyxia. Developmental Medicine and Child Neurology, 32(1), 56-62.

Rehncrona, S., Rosen, I., & Siesjö B. K. (1980). Excessive cellular acidosis: An important mechanism of neuronal damage in the brain? Acta Physiologica Scandinavica, 110(4), 435-7.

Rehncrona, S., Westerberg, E., Akesson, B., & Siesjö, B. K. (1982). Brain cortical fatty acids and phospholipids during and following complete and severe incomplete ischemia. Journal of Neurochemistry, 38(1), 84-93.

Reivich, M., Brann, A. W. Jr., & Shapiro, H. M. (1972). Regional cerebral blood flow during prolonged partial asphyxia. In: J. S. Meyer, M. Reivich, & H. Lechner (Eds.), Research on the cerebral circulation. Springfield IL: Thomas.

Rennie, J. M. (1989). Cerebral blood flow velocity variability after cardiovascular support in premature babies. Archives of Disease of Childhood, 64, 897-901.

Rennie, J. M., Wheater, M., & Cole, T. J. (1996). Antenatal steroid administration is associated with an improved chance of intact survival in preterm infants. European Journal of Pediatrics, 155, 576-9.

Rickey, D. W., & Fenster, A. (1996). Evaluation of an automated real-time spectral analysis technique. Ultrasound in Medicine and Biology, 22(1), 61-73.

Robinson, K., & Rudge, P. (1977). Abnormalities of the auditory evoked potentials in patients with multiple sclerosis. Brain, 100, 19-40.

Roland, E. H., Flodmark, O., & Hill, A. (1990). Thalamic hemorrhage with intraventricular hemorrhage in the full term newborn. Pediatrics, 85, 737-742.

Rosenberg, A. A. (1986). Cerebral blood flow and O2 metabolism after asphyxia in neonatal lamb. Pediatric Research, 20, 778-782.

Rosenberg, A. A., Murdaugh, E., & White, C. W. (1989). The role of oxygen free-radicals in postasphyxial cerebral hypoperfusion in newborn lambs. Pediatric Research, 26, 215-219.

Rosenberg, A. A., Parks, J. K., Murdaugh, E., & Parker, W.

D. Jr. (1989). Mitochondrial function after asphyxia in newborn lambs. Stroke, 20(5), 674-679.

Rothman S. (1984). Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. Journal of Neuroscience, 4, 1884-1891.

Rothwell, N. J., & Hopkins, S. J. (1995). Cytokines and the nervous system II: Actions and mechanisms of action. Trends in Neurosciences, 18, 130-36.

Rudolph, A. M. (1984). The fetal circulation and its response to stress. Journal of Developmental Physiology, 6, 11-19.

Rudolphi, K. A., & Schubert, P. (1996). In: L. Belardinelli, L., & A. Pelleg (Eds.), Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology (pp. 391-398). Kluwer Academic Press.

Rumpel, H., Nedelcu, J., Aguzzi, A., & Martin, E. (1997). Late glial swelling after acute cerebral hypoxia-ischemia in the neonatal rat: a combined magnetic resonance and histochemical study. Pediatric Research, 42(1), 54-59.

Ruscher, K., Isaev, N., Trendelenburg, G., Weih, M., Iurato, L., Meisel, A., & Dirnagl, U. (1998). Induction of hypoxia inducible factor 1 by oxygen glucose deprivation is attenuated by hypoxic preconditioning in rat cultured neurons. Neuroscience Letters, 254, 117-120.

Rushton, D. I., Preston, P. R., & Durbin, G. M. (1985). Structure and evolution of echodense lesions in the neonatal brain: a combined ultrasound and necropsy study. Archives of Disease of Childhood, 60, 798-808.

Rutherford, M. A., Pennock, J. M., & Dubowitz, L. M. S. (1994). Cranial ultrasound and magnetic resonance imaging in hypoxic ischemic encephalopathy: a comparison with outcome. Developmental Medicine and Child Neurology, 36, 813-25.

Rutherford, M. A., Pennock, J. M., Murdoch-Eaton, D. M., Cowan, F., & Dubowitz, L. M. S. (1992). Athetoid cerebral palsy with cysts in the putamen after hypoxic-ischemic encephalopathy. Archives of Disease of Childhood, 58, 509-11.

Salamy, A., & McKean, C. M. (1976). Postnatal development of human brainstem potentials during the first year of life. Electroencephalography Clinical Neurophysiology, 40, 418-

426.

Salamy, A., McKean, C. M., & Buda, F. B. (1975). Maturational changes in auditory transmissions as reflected in human brainstem potentials. Brain Research, 96, 361-366.

Sato, M., Hashimoto, H., & Kosaka, F. (1990). Histological changes of neuronal damage in vegetative dogs induced by 18 minutes of complete global brain ischemia: two-phase damage of Purkinje cells and hippocampal CA1 pyramidal cells. Acta Neuropathologica (Berlin), 80, 527-534.

Saugstad, O. D., & Schrader, H. (1978). The determination of inosine and hypoxanthine in rat brain during normothermic and hypothermic anoxia. Acta Neurologica Scandinavica, 57(4), 281-8.

Schweizer, M., & Richter, C. (1994). Nitric oxide potently and reversibly deenergizes mitochondria at low oxygen tension. Biochemical and Biophysical Research Communications, 204, 169-175.

Scott, H. (1976). Outcome of very severe birth asphyxia. Archives of Disease of Childhood, 51(9), 712-6.

Scotti, G., Flodmark, O., Harwood-Nash, D. C., & Humphries, R. P. (1981). Posterior fossa hemorrhages in the newborn. Journal of Computer Assisted Tomography, 5, 68-72.

Shah, S. N., Bhargava, U. K., Johnson, R. C., & McKean, C. M. (1978). Latency changes in brainstem auditory evoked potentials associated with impaired brain myelination. Experimental Neurology, 58, 111-118.

Shankaran, S., Kottamasu, S. R., & Kuhns, L. (1993). Brain sonography, computed tomography, and single-photon emission computed tomography in term neonates with perinatal asphyxia. Clinical Perinatology, 20, 379-394.

Shrontz, C., Dujovny, M., Ausman, J. I., Diaz, F. G., Pearce, J. E., Berman, S. K., Hirsch, E., & Mirchandani, H. G. (1986). Surgical anatomy of the arteries of the posterior fossa. Journal of Neurosurgery, 65(4), 540-544.

Shucard, D. W., Shucard, J. L., & Thomas, D. G. (1988). Neurophysiological studies of human cognitive development in premature infants: an approach to the study of maturational brain processes. Neurotoxicity, 9(3), 299-316.

Sidman, R. L., Dickie, M. M., & Appel, S. H. (1964). Mutant

(Quaking and Jimpy) with deficient myelination in the central nervous system. Science, 144, 309-311.

Siesjö, B. K. (1992). Pathophysiology and treatment of focal cerebral ischemia. I. Pathophysiology. Journal of Neurosurgery, 77, 169-174.

Skeffington, F. S., & Pearse, R. G. (1983). The 'bright brain'. Archives of Disease of Childhood, 58, 509-11.

Smoker, W. R., Price, M. J., Keyes, W. D., Corbett, J. J., & Gentry, L. R. (1986). High-resolution computed tomography of the basilar artery: I. normal size and position. American Journal of Neuroradiology, 7(1), 55-60.

Sohmer, H., & Gafni, M. (1979). Assessment of infants "at-risk" for hearing disorders by means of electric response audiometry. In S. Harel (Ed.), International congress series no. 492: The at risk infant. Amsterdam: Excerpta Medica.

Sohmer, H., & Student, M. (1978). Auditory nerve and brainstem evoked responses in normal, autistic, minimal brain dysfunction and psychomotor retarded children. Electroencephalography Clinical Neurophysiology, 44, 380-388.

Sohmer, H., Gafni, M., Tannenbaum, A., Szabo, G., Robinson, M. J., & Weitzmen, Z. (1979). Auditory nerve-brainstem response abnormalities in infants with developmental disorders. In: S. Harel (Ed.), The at risk infant. Amsterdam: Elsevier.

Sonesson, S-E., & Herin, P. (1988). Intracranial arterial blood flow velocity and brain blood flow during hypocarbia and hypercarbia in newborn lambs: a validation of range-gated Doppler ultrasound flow velocimetry. Pediatric Research, 24, 423-6.

Sonesson, S-E., Winberg, P., & Lundell, B. W. P. (1987). Early postnatal changes in intracranial arterial blood flow velocities in term infants. Pediatric Research, 22, 461-4.

Starr, A., & Achor, J. (1975). Auditory brainstem responses in neurological disease. Archives of Neurology (Chicago), 32, 761-768.

Starr, A., & Hamilton, A. E. (1976). Correlation between confirmed sites of neurological lesions and abnormalities by far field auditory brainstem responses. Electroencephalography Clinical Neurophysiology, 41, 595-

608.

Starr, A., Amlie, R. N., Martin, W. H., & Sanders, S. (1977). Development of auditory function in newborn infants revealed by auditory brainstem potentials. Pediatrics, 60, 831-839.

Steiner, H., & Neligan, G. (1975). Perinatal cardiac arrest. Quality of survivors. Archives of Disease of Childhood, 50(9), 696-702.

Stephens, R. B., & Stilwell, D. L. (1969). Arteries and veins of the human brain. Springfield, IL: Charles C. Thomas.

Stockard, J. J., & Rossiter, V. S. (1977). Clinical and pathological correlates of brainstem auditory response abnormalities. Neurology, 27, 316-325.

Symon, L., Branston, N. M., & Chikovani, O. (1979). Ischemic brain edema following middle cerebral artery occlusion in baboons: Relationship between regional cerebral water content and blood flow at 1 to 2 hours. Stroke, 10, 184-91.

Tagari, P., Du Boulay, G. H., Aitken, V., & Boullin, D. J. (1983). Leukotriene D4 and the cerebral vasculature in vivo and in vitro. Prostaglandins, Leukotrienes and Medicine, 11(3), 281-97.

Takashima, S. & Tanaka, K. (1978). Development of cerebral architecture and its relationship to periventricular leukomalacia. Archives of Neurology, 35, 11-16.

Takashima, S., Armstrong, D. L., & Becker, L. E. (1978). Subcortical Leukomalacia. Relationship to development of the cerebral sulcus and its vascular supply. Archives of Neurology, 35, 470-472.

Thomas, C. E., Morehouse, L. A., & Aust, S. D. (1985). Ferritin and superoxide dependent lipid peroxidation. Journal of Biological Chemistry, 260(6), 3275-80.

Towbin, A. (1969a). Mental retardation due to germinal matrix infarction. Science, 164(867), 156-61.

Towbin, A. (1969b). Cerebral hypoxic damage in fetus and newborn. Basic patterns and their clinical significance. Archives of Neurology, 20(1), 35-43.

Towbin, A. (1970). Central nervous system damage in the

human fetus and newborn infant. Mechanical and hypoxic injury incurred in the fetal-neonatal period. American Journal of Diseases of Children, 119(6), 529-42.

Tower, D. B. (1992). A century of neuronal and neuroglial interactions and their pathological interactions: an overview. Progress in Brain Research, 94, 3-17.

Trounce, J. Q., Rutter, N., & Levene, M. I. (1988). Periventricular leukomalacia and intraventricular hemorrhage in the preterm neonate. Archives of Disease of Childhood, 61, 1196-202.

Tullis, R. H., & Rubin, H. (1982). Calcium protects DNAseI from proteinase K: A new method for the removal of contaminating RNAse from DNAse I. Annals of Biochemistry, 107(1), 260-4.

Turski, L., Huth, A., Sheardown, M., McDonald, F., Neuhaus, R., Schneider, H. H., Dirnagl, U., Weigand, F., Jascobsen, P., & Ottow, E. (1998). ZK200775: a phosphonate quinoxalinedione AMPA antagonist for neuroprotection in stroke and trauma. Proceedings of the National Academy of Sciences of the USA, 95, 10960-10965.

Ultramark 9 HDI Reference Manual (1992). Setup (pp. 11-1 - 11-20). Bothell, WA: Advanced Technology Laboratories, Inc.

Ushijima, K., Miyazaki, H., & Morioka, T. (1986). Immunohistochemical localization of glutathione peroxidase in the brain of the rat. Resuscitation, 13, 97-105.

Uvebrant, P. (1988). Hemiplegic cerebral palsy: aetiology and outcome. Acta Paediatrica Scandinavica, [Suppl.], 345, 1-100.

van Bel, F., Hirasing, R. A., & Grimberg, M. T. (1984). Can perinatal asphyxia cause cerebral edema and affect cerebral blood flow velocity? European Journal of Pediatrics, 142, 29-32.

van Bel, F., van de Bor, M., Baan, J., & Ruys, J. H. (1988). The influence of abnormal blood gases on cerebral blood flow velocity in the preterm newborn. Neuropediatrics, 19, 27-32.

van Bel, F., van de Bor, M., Stijnen, T., Baan, J., & Ruys, J. H. (1987). Cerebral blood flow velocity patterns in healthy and asphyxiated newborns: a controlled study. European Journal of Pediatrics, 146, 461-7.

Vanderschuren, L. J., Schoffelmeer, A. N., & De Vries, T. J. (1997). Does dizocilpine (MK-801) inhibit the development of morphine-induced behavioral sensitization in rats? Life Sciences, 60, PL427-PL433.

Vannucci, R. C., & Plum, F. (1975). Pathophysiology of perinatal hypoxic-ischemic brain damage. In: G. E. Gaull (Ed.), Biology of brain dysfunction. New York: Plenum Press.

Volpe, J. J. (1990). Brain injury in the premature infant: Is it preventable? Pediatric Research, 27(6), [Suppl.] S28-33.

Volpe, J. J., & Pasternak, J. F. (1977). Parasagittal cerebral injury in neonatal hypoxic-ischemic encephalopathy: Clinical and neuroradiologic features. Journal of Pediatrics, 91, 472-476.

Volpe, J. J., Herscovitch, P., Perlman, J. M., Kreusser, K. L., & Raichle, M. E. (1985). Positron emission tomography in the asphyxiated term newborn: Parasagittal impairment of cerebral blood flow. Annals of Neurology, 17, 287-296.

Wahl, M., & Kuschinsky, W. (1976). The dilatory action of adenosine in pial arteries of cats and its inhibition by theophylline. Pflügers Archives, 362, 55-59.

Watson, B. D., Busto, R., Goldberg, W. J., Santiso, M., Yoshida, S., & Ginsberg, M. D. (1984). Lipid peroxidation in vivo induced by reversible global ischemia in rat brain. Journal of Neurochemistry, 42, 268-74.

Wei, E. P., Kontos, H. A., & Patterson, J. L., Jr. (1980). Dependence of pial arteriolar response to hypercapnia on vessel size. American Journal of Physiology, 238, H697-702.

White, B. C., Nayini, N. R., Krause, G. S., Aust, S. D., March, G. G., Bicknell, J. S., & Goosmann, M. (1988). Effect on biochemical markers of brain injury of therapy with deferoxamine or superoxide dismutase following cardiac arrest. American Journal of Emergency Medicine, 6, 569-576.

White, B. C., Rafols, J. A., DeGarcia, D. J., Skjaerlund, J. M., & Krause, G. S. (1992). Fluorescent histochemical localization of lipid peroxidation during brain reperfusion [Abstract]. Annals of Emergency Medicine, 21, 634.

Whitelaw, A., Haines, M. F., Bolsover, W., & Harris, E. (1984). Factor V deficiency and antenatal intracranial hemorrhage. Archives of Disease of Childhood, 59, 997-9.

Williams, C. E., Gunn, A. J., & Gluckman, P. D. (1991). The time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. Stroke, 22, 516-521.

Williams, C. E., Gunn, A. J., Synek, B., & Gluckman, P. D. (1990). Delayed seizures occurring with hypoxic-ischemic encephalopathy in the fetal sheep. Pediatric Research, 27, 561-565.

Winberg, P., Sonesson, S-E., & Lundell, B. (1990). Postnatal changes in intracranial blood flow velocity in preterm infants. Acta Paediatrica Scandinavica, 79, 1150-5.

Winn, H. R., Welsh, J. E., Rubio, R., & Berne, R. M. (1988). Brain adenosine production in rat during sustained alteration in systemic blood pressure. American Journal of Physiology, 239, H636-H641.

Yamasaki, Y., Matsuo, Y., Matsuura, N., Onodera, H., Itoyama, Y., & Kogure, K. (1995). Transient increase of cytokine-induced neutrophil chemoattractant, a member of the interleukin-8 family, in ischemic brain areas after focal ischemia in rats. Stroke, 26, 318-322.

Yanagihara, T., & McCall, J. T. (1982). Ionic shift in cerebral ischemia. Life Sciences, 30(22), 1921-5.

Yang, G., Chan, P. H., Chen, J., Carlson, E., Chen, S. F., Weinstein, P., Epstein, C. J., & Kamii, H. (1994). Human copper-zinc superoxide dismutase transgenic mice are highly resistant to reperfusion injury after focal cerebral ischemia. Stroke, 25, 165-170.

Yoshida-Shuto, H., Yashura, A., & Kobayashi, Y. (1992). Cerebral blood flow velocity and failure of autoregulation in neonates and their relation to outcome of birth asphyxia. Neuropaediatrics, 23, 241-4.

Young, R. S., Petroff, O. A., Aquila, W. J., Cheung, A., & Gore, J. C. (1992). Hyperglycemia and the rate of lactic acid accumulation during cerebral ischemia in developing animals: In vivo proton MRS study. Biology of the Neonate, 61(4), 235-42.

Young, W., Rappaport, Z. H., Chalif, D. J., & Flamm, E. S. (1987). Regional brain sodium, potassium, and water changes in the rat middle cerebral artery occlusion model of ischemia. Stroke, 18(4), 751-59.

Younkin, D., Delivoria-Papadopoulos, M., Reivich, M., Jaggi, J., & Obrist, W. (1988). Regional variations in human newborn cerebral blood flow. Journal of Pediatrics, 112(1), 104-108.

Zaleska, M. M., & Floyd, R. A. (1985). Regional lipid peroxidation in the rat brain in vitro: possible role of endogenous iron. Neurochemical Research, 10, 397-410.

Zalneraitis, E. L., Young, R. S., & Krishnamoorthy, K. S. (1979). Intracranial hemorrhage in utero as a complication of isoimmune thrombocytopenia. Journal of Pediatrics, 95, 611-14.

Zappulla, R. A., Karmel, B. Z., & Greenblatt, E. (1981). Discriminant analyses of brainstem evoked potential component latencies in acoustic neuroma patients. Neurosurgery, 9, 542-547.

Zhang, R., Chopp, M., Zhang, Z., Jiang, N. & Powers, C. (1998). The expression of P- and E-selectins in three models of middle cerebral artery occlusion. Brain Research, 785(2), 207-214.