Animal Models of Hypoxic-Ischemic Brain Damage in the Newborn

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Controversy continues over which animal model to use as a reflection of human disease states. With respect to perinatal brain disorders, scientists must contend with a disease in evolution. In that regard, the perinatal brain is at risk during a time of extremely rapid development and maturation, involving processes that are required for normal growth. Interfering with these processes, as part of therapeutic intervention must be efficacious and safe. To date, numerous models have provided tremendous information regarding the pathophysiology of brain damage to term and preterm infants. Our challenges will continue to be in identifying those infants at greatest risk for permanent injury, and adapting therapies that provide more benefit than harm. Using animal models to conduct these studies will bring us closer to that goal.

The series of papers presented in this issue of Seminars in Pediatric Neurology pertain to the clinical entity of cerebral palsy (CP), a group of disorders of the central nervous system manifested by aberrant control of movement or posture, present since early in life and not the result of a recognized progressive disease. Prevalence rates for this disorder continue to be 1.0 to 2.4/1000 live births. Therefore, despite a continuing drop in perinatal deaths and sharp decreases in “birth asphyxia,” there has been no substantive decline in the occurrence of CP among term infants. Moreover, despite the fact that the prevalence of CP is increasing in the infant born prematurely (in large part due to their greater survivability), term infants continue to represent more than 50% of the population of children with CP.

The purpose of this review is to highlight those models currently in most common use for studying perinatal asphyxia. Clearly, the outcome of CP or any of the developmental disabilities (ie, mental retardation, specific learning problems, attention deficit disorder, pervasive developmental disorders, language delay) may or may not have their origins in an asphyxial event. Nonetheless, intrapartum asphyxia, resulting in hypoxic-ischemic encephalopathy, remains an important contributor to the developmental disabilities and cerebral palsy. Badawi et al found that intrapartum hypoxia alone correlated with a picture of moderate to severe neonatal encephalopathy in 4% of their series of 164 term encephalopathic infants; however, hypoxia was a compounding feature for the production of neonatal encephalopathy (NE) in an additional 25% of cases. Others have suggested that fetal exposure to asphyxia may be as high as 23% of all pregnancies.

Significant sequelae of term neonatal hypoxia-ischemia have been documented in as many as 50% to 75% of children, and as much as 10% of cases of idiopathic mental retardation may be related to intrapartum asphyxia. Interestingly, most infants with mild NE appear to develop normally, with only those with moderate to severe encephalopathy exhibiting significant neurologic morbidity. Hence Robertson et al studied 145 8-year-old children, all of whom had experienced NE (based on Sarnat staging) after term birth asphyxia, and found a mortality rate of 13% to age 8 years. The incidence of impairment, classified as CP, blindness, cognitive delay, epilepsy, and severe hearing loss, was 16%. Among those children categorized as “nonimpaired,” the percentage with moderate encephalopathy who tested more than one school grade level below expected was an additional 25%, whereas 100% of survivors in the severe NE category tested below grade level. Moster et al similarly found significantly higher incidences of neurodevelopmental impairment and learning difficulties at adolescence among term infants who were born with evidence of a hypoxic-ischemic encephalopathy at birth but did not develop CP. Of the antepartum events that have been found to contribute significantly to the risk of NE, fetal growth restriction (FGR) ranks high. A large population-based, case-controlled study supported the contention that infants with birth weights less than the third percentile have a substantially in-

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creased relative risk for NE (adjusted odds ratio of 38.23). 17 The role of FGR as it relates to outcome is poorly understood. Although FGR is a common risk factor for NE, whether it contributes to poor adverse neurologic morbidity remains to be determined. Furthermore, whether FGR, either in isolation or in combination with hypoxia at birth, is detrimental to outcome is also unknown. As a single indicator of moderate to severe NE, FGR was the most strongly associated “marker” found in the population-based study of Badawi et al. 17 Other studies have clearly shown a relationship between FGR and learning deficits and behavioral problems of inattention and anxiety in 48% of children assessed between age 9 and 11 years, 18 and poor school performance at age 12 and 18 years. 19 Strong associations between FGR and CP have also been demonstrated. 20,21 Perhaps of even greater importance is the fact that FGR places the infant at a much higher risk for intrapartum asphyxia than infants of appropriate weight for gestational age. In one study, 35% of FGR infants exhibited fetal heart rate characteristics indicative of distress; other studies have shown significant increases in acid-base abnormalities and serum lactate concentrations. 16,22-24 More insight into the role of hypoxemia as a confounding factor for newborns with FGR has come from the National Collaborative Perinatal Project. 25 Assessment at age 7 years revealed that in the absence of hypoxia-related factors, neither symmetric nor asymmetric FGR children were at higher risk for neurologic morbidity compared with those without FGR. In the presence of hypoxia, however, FGR children were more likely to be neurologically abnormal (CP or mental retardation) compared with controls, and children with symmetric FGR (in which body and brain weight are reduced compared with brain weight being preserved) were at a greater risk than those with asymmetric FGR.

Increasingly, the literature is reporting a very strong role for chorioamnionitis as a frequent cause of acquired brain damage in the perinatal period. 26 A recent meta-analysis of chorioamnionitis and CP indicated a positive association among preterm and full-term infants, with a relative risk of 4.7 for the latter group. 27 Similarly, maternal fever has been linked with an increased incidence of neonatal encephalopathy. 28,29 Nelson et al 30 examined the blood of 31 children known to have CP and found significantly higher concentrations of interleukin (IL)-1, -6, -8, and -9; tumor necrosis factor (TNF), and various other cytokines compared with controls. Reviews of the epidemiologic and cytokine literature by Dammann and Leviton 31,32 clearly support a role for inflammation/cytokines in neonatal brain injury.

Recognizing that the underlying causes of CP and the developmental disabilities are not completely understood, the aforementioned predisposing conditions (ie, antepartum and intrapartum asphyxia plus or minus FGR and infection) have clearly been identified as risk factors for perinatal neurologic morbidity. Each of these conditions is reflective of an underlying cerebrovascular compromise to the brain, alone or in conjunction with additional complicating factors. Each of these conditions also has a spectrum of severity and, in turn, manifests phenotypic outcomes ranging from profound mental retardation and spastic quadriplegia to poor school performance and attention difficulties.

Models of perinatal injury are therefore meant to mimic this broad and as-yet poorly understood condition of the newborn human infant. In doing so, the goals of an animal models are to (1) contribute to our knowledge of the underlying mechanisms of injury, (2) improve our understanding of the evolution of injury and its outcome, and (3) provide a template on which to develop and test therapeutic strategies. To adequately meet these goals, the animal model must have certain characteristics reflective of its target. For the newborn infant who has experienced a cerebrovascular compromise to the brain, models should (1) mimic the etiologic basis through which these injuries occur, (2) reflect the histopathologic spectrum of injury to the developing brain, and (3) ideally express the functional outcomes seen in the human newborn infant and child. That the immature brain is in constant biologic evolution during its potential exposure to cerebrovascular compromise clearly complicates the development of animal models reflecting this human condition.

Accordingly, the goals of this article are to (1) review some of the relevant aspects of development as they pertain to models of perinatal hypoxic-ischemic brain damage, (2) review commonly used models of hypoxic-ischemic injury to the newborn brain, and (3) suggest future directions of investigation that may help provide answers to an area of pediatrics whose consequences have such a profound effect on families and society in general.
THE HUMAN NEWBORN CONDITION

Development of the Newborn Brain

An understanding of normal human brain development is essential to evaluating the validity of animal models developed to study the pathogenesis of cerebrovascular compromise to the newborn brain. In circumstances whereby the immature brain is rapidly evolving, assimilating this knowledge with research relating to the development of the animal model allows us to better correlate the importance of our findings as they relate to the human newborn.

The major events of neurulation (occurring at 3 to 4 weeks gestation), neuronal proliferation and migration (at 3 to 5 months gestation), organization (at 5 months to years), and myelination (at birth to years), are well outlined in many texts and review articles and thus are not detailed here. This part of the review focuses on those aspects related to neocortical development that may be influenced by or altered in the pathogenesis of hypoxic-ischemic brain damage.

In humans, developmental processes in the nervous system occur over periods of weeks to months; in comparison, in the rodent the same processes occur in a matter of days (Fig 1). Proliferation and migration of neurons in the human brain occurs between 4 and 24 weeks of gestation (ie, in midgestation), with neurogenesis occurring first in the spinal cord and brain stem structures and progressing rostrally thereafter, having been largely completed by midgestation. The same events in the rodent progress over a period of days (gestational day 11 to 16) for the spinal cord and brain stem, but extend to postnatal day 15 for the neocortical and limbic system and hippocampal structures. In humans, synaptogenesis, the neurobiological substrate for cell-to-cell communication, begins gradually during the first few months of gestation but does not reach its peak until the first 1 to 2 years of life, and matures over several years to adolescence. In rodents, this process occurs somewhat rapidly during the later part of gestation through to the first 3 weeks of postnatal life, corresponding to weaning of the rat pup from its dam.
Recently, attention has focused on the subplate neuron. Subplate neurons are situated beneath the cortical plate and are among the first cells formed in neocortical development. Because they form a population of cells at the interface of the developing cortex with the intermediate zone (i.e., primordial white matter), the subplate neurons are in a position to interact with later-generated neurons as they migrate into the cortex, and also to act with many of the afferent projections to the neocortex as they grow through the subplate layer. Several studies have provided evidence that subplate neurons receive temporary synaptic connections and serve as guides to thalamocortical axons during development, and also to cortical efferents. Finally, given its unique position in the developing nervous system, the subplate neuron may be only transiently functional and may no longer play a role postnatally.40 In humans, subplate neuron development peaks at around 24 weeks gestation and declines thereafter. In rats, this event corresponds more closely to the end of gestation and the early postnatal days of life to approximately postnatal day 40, at which point the number of cells has reduced by slightly less than half.40,41

The process of myelination deserves particular mention, given the sensitivity of the oligodendrocyte to vascular compromise42-44 and the prevalence of periventricular leukomalacia (PVL) among preterm infants. Differentiation of oligodendrocytes lags substantially behind the initial waves of neurogenesis and follows axonogenesis. In humans, myelination begins in midgestation and progresses over a protracted period to late childhood and early adolescence. In rats, this process occurs during the first 2 days of life and extends to well beyond weaning.45-48 In contrast studies of sheep fetus suggest that myelination begins in midgestation and is very near completion by term.49,50

Ontogenesis of Neurotransmitters

Neurotransmitters have qualitatively different functions in the newborn brain during development than in the adult brain. In the adult, neurotransmitters are traditionally thought of as mediating or modulating synaptic transmission between cells. In the maturing nervous system, however, these same neurotransmitters play fundamental roles in the normal physiological processes of brain growth and differentiation (Fig 2). Their role is no less important in the pathophysiology of injury to the newborn brain51-53 and presents a complicated scenario in the development of therapeutic interventions for neonatal hypoxic-ischemic encephalopathy.

In humans, development of the excitatory amino acid receptor (NMDA) begins in the region of the hippocampus and entorhinal cortex at midgestation and peaks quickly at around 24 weeks, to levels above those normally found in adults.54,55 In rats, this corresponds to a 150% to 200% overshoot in NMDA receptor binding sites at 6 to 14 days postnatal development.56-58 The non-NMDA binding sites in humans also begin to develop in the hippocampus, neocortical, and basal ganglia regions around 24 weeks of gestation and peak above normal adult levels near term, after which they decline and remain at adult levels.34 In rats, the quinolinic acid receptor develops early and reaches adult levels by 7 days postnatal age. The kainate receptor, on the other hand, does not begin to develop significantly until around 10 days postnatal age, and gradually increases until 21 days, where it attains adult values.

\[\gamma\text{-aminobutyric acid (GABA)},\] the predominant inhibitory neurotransmitter in the brain, begins to show expression in humans as early as 17 weeks, and rapidly increases in density during midgestation to reach around 60% of adult concentrations at term.59 Both excitatory and inhibitory GABA receptors have been identified in rats. Hence, during gestation, activation of GABA_A receptors causes depolarization. This maturational excitatory func-

Fig 2. Graph depicting the timelines for the development of excitatory amino acid development in the human and rat using birth as a reference point. Adapted from Hattori et al, Sanchez et al, and Rice and Barone. See text for details. Note the shift to the right for neurotransmitter development in the rat compare to human.
tion persists for the first postnatal week and is gradually transformed the more mature hyperpolarizing function over the first 3 weeks of life.60

**Neuropathology of Perinatal Asphyxia**

What we know of the human newborn condition stems largely from neuroradiologic investigations of preterm and term infants who sustained asphyxial events,61-63 as well as from necropsy studies of newborns who did not survive.64-66 From this information, it is quite clear that the pattern of injury seen in human newborns is highly dependent on the gestational age and on the duration and severity of the insult. Hence we see a continuum of neuropathologic lesions in the immature brain ranging from periventricular white matter injury in the preterm infant to parasagittal cerebral injuries in the more mature term infant (Fig 3A and B). Selective neuronal necrosis involving all regions of the brain can occur regardless of gestational age.33

Involvement of the deep cortical gray-matter structures and brain stem pathology appears to depend on the severity of injury. In that regard, Pasternak and Gorey67 reviewed their cases of acute near-total asphyxia in term infants and found a consistent pattern of injury in the subcortical brain nuclei, including the thalamus, basal ganglia, and brainstem, with almost complete sparing of the cortical gray- and white-matter structures. In contrast, infants with injury predominantly to cortical gray- and white-matter structures tend to have insults characterized by partial or prolonged hypoxia-ischemia. Hence Sie et al63 reviewed the records and magnetic resonance imaging (MRI) studies of 104 children with hypoxic-ischemic brain damage, and found 3 different patterns of MRI images: (1) PVL in preterm infants experiencing subacute or chronic hypoxia-ischemia, (2) basal ganglia and thalamic lesions in infants who experienced an acute profound asphyxial event, and (3) multicystic encephalopathy in term infants with perhaps prolonged mild to moderate hypoxia-ischemia superimposed by an acute or subacute event. Others have found similar patterns of injury based on the timing and duration of the insult.68,69

Recent advances in MRI have provided important information on injury to the newborn brain, particularly in relation to white matter injury. Inder et al70 studied 34 infants (20 preterm and 14 term) and reported for the first time the association between white-matter injury and a reduction in cortical gray-matter volume, suggesting an anatomical correlate for the intellectual deficits often seen in children with PVL. Subsequent studies have confirmed not only the sensitivity of MRI in detecting white-matter lesions,71,73 but also the efficacy of MRI techniques in correlating injury with intellectual and behavioral outcome.74,75 Others have reported on advanced MRI techniques using the apparent diffusion coefficient (ADC) (the amount of water movement) and anisotropy (the direction of water movement) to determine abnormalities of white-matter development in prematurely born infants.76 Miller et al76 studied a cohort of 23 newborns, of whom 11 were classified as normal, 7 had minimal white-matter injury, and 5 had moderate white-matter injury. They found significant abnormalities in ADC in those infants with moderate injury compared with the other 2 groups, and in anisotropy in the frontal white matter in both groups with white-matter damage. Importantly, this study demonstrated the sensitivity that these techniques can achieve in detecting injury in children previously thought to be normal. The findings are in keeping with recent reports indicating that infants born prematurely are at higher risk not only for abnormalities in motor function (eg, CP), but also for the more subtle abnormalities of learning and behavior. Bhutta et al77 published a meta-analysis that reviewed the literature on the effect of preterm birth on cognition and behavior. Their results indicated that of the 1556 cases reviewed, children born prematurely were at twice the relative risk for developing attention deficit hyperactivity disorder (ADHD) and had significantly lower cognitive scores compared with controls.

**ANIMAL MODELS OF INJURY**

The study of perinatal brain injury has seen tremendous advances, and numerous models have been adapted in the pursuit of enhancing our knowledge, particularly at the biochemical and molecular levels (Fig 4). In discussing this broad and complicated area, my intention is not to be all-inclusive, but rather to focus on those models that appear to comprehensively cover the topic.

**Monkey**

The classic studies of Myers et al78-80 categorized the patterns of neuronal injury and correlated them with systemic, perhaps causative, abnormalities. Importantly, these investigations were done in primates. Term monkey fetuses were exposed to true asphyxia (cessation of respiratory gas ex-
change) by covering the head with a rubber sac and clamping the umbilical cord at delivery. This led to an immediate rise in fetal blood pressure due to an increase in peripheral vascular resistance, followed within 20 seconds by profound fetal bradycardia and an accompanying decline in arterial pressure. The blood pressure slowly dropped to become pressure-passive at about 12 to 14 minutes postin-

Fig 3. (A) Cartoon depicting the evolution of neuropathologic injury during the latter half of pregnancy in human newborn. Note that white matter injury in the form of "periventricular eukomalacia" typically occurs in the premature infant, whereas cortical and deep grey matter structures are more prone to injury later in gestation. (B) MRI images depicting evolution of damage and change in topographical sensitivity of the newborn brain to hypoxic-ischemic injury. (a) DWI image of premature infant depicting enhancement of white matter adjacent to lateral ventricles. (b and c) T1 weighted images of term infant indicating hyperintensity of deep grey matter nuclei (b) and peri-rolandic fissure (c), in keeping with hypoxic-ischemic injury to the more mature brain. (Photographs courtesy of Dr. J. Cure MD and Dr. A.J. Barkovich MD).
sult. Despite this, the fetal heart rate remained at approximately 60 beats per minute for up to at least 35 minutes. In conjunction with these changes were a rapid decline in fetal oxygen content, a rise in carbon dioxide, and a fall in pH to approximately 6.9 during the first 12.5 minutes. Interestingly, fetuses resuscitated after 20 minutes had extremely high mortality. However, at least 12 minutes of total asphyxia was required to produce any signs of neuropathologic injury. These results coincide with the findings from clinical studies that examined the duration of prolonged fetal heart rate deceleration required for neurologic morbidity and found that a period of at least 17 minutes was required for morbidity to occur. Neuropathologically, these monkey fetuses displayed damage predominantly within the brainstem.

In models of partial ischemia, monkey fetuses remained in utero, and mothers were manipulated such as to render them hypotensive. These studies indicated that term fetuses can tolerate arterial oxygen pressure reductions to 30% of normal, but reductions to 10% of normal for periods of up to 5 hours cause them to become increasingly bradycardic and hypotensive. Physiologically, these fetuses were profoundly hypoxic and acidemic to pH < 7.0. At birth, they often displayed opisthotonus and decerebrate posturing as well as generalized convulsions. Pathologically, the brains showed widespread cortical tissue necrosis, and those who survived for longer periods before sacrifice displayed evidence of parasagittal infarction, and porencephaly.

Based on investigations over 2 decades, Myers et al described 4 patterns of brain damage relating to the degree of hypoxia/anoxia and whether or not it was combined with acidemia. In addition to the cohorts described earlier, fetal term monkeys exposed to severe hypoxia in the absence of acidemia developed predominantly white-matter injury, whereas those experiencing partial prolonged asphyxia combined with a terminal total
asphyxial event experienced damage focused on the basal ganglia and thalamus. Thus both the clinical and pathological changes produced in these term monkeys by various degrees of intrauterine asphyxia closely resemble the changes observed in perinatally damaged humans.

Sheep

Gray Matter

The sheep has served as an effective large animal model for the study of perinatal asphyxia. Gunn et al.\textsuperscript{83-86} described the neuropathologic consequences after umbilical cord occlusion in near-term fetal sheep. Brief (10-minute) periods of cord occlusion resulted in transient asphyxia accompanied by hypotension and bradycardia, together with prolonged neuronal depression demonstrated on electroencephalography. Histologically, areas of selective neuronal necrosis were found in the hippocampus. These studies were subsequently extended so that near-term sheep fetuses were exposed to repeated brief episodes of in utero hypoxia-ischemia for 3 10-minute intervals, separated by 1 or 5 hours. The findings were compared with results from a single 30-minute episode of hypoxia-ischemia. Repeated episodes at 1-hour intervals resulted in a greater degree of neuronal injury; however, episodes separated by 5-hour intervals produced a shifted distribution of injury involving the striatum almost exclusively. When episodes were repeated at much shorter intervals (every 2.5 to 5.0 minutes) but far more frequently (until arterial pH reached 6.8), the damage was diffuse and extensive, causing infarction of the parasagittal cortex, thalamus, and cerebellum in 40% of the animals and diffuse selective neuronal necrosis in the remainder.\textsuperscript{87}

Experiments in which near-term fetal sheep were exposed to prolonged hypoxia-ischemia of 30, 60, or 120 minutes’ duration were done to correlate the duration of insult with histopathologic injury.\textsuperscript{84} Uterine artery occlusion in this setting produced severe hypoxemia, hypercarbia, acidosis, and bradycardia. Neuronal injury in this model was inversely correlated with blood pressure during the insult, such that the lower the blood pressure, the greater the damage, but, interestingly, no association with hypoxemia was found. Areas of greatest sensitivity included the parasagittal cortex, the CA1–3 regions of the hippocampus, the striatum, and the thalamus. These findings were also confirmed by Williams et al.\textsuperscript{83}

In a similar model of near-term fetal asphyxia, Bagenholm et al.\textsuperscript{88} measured the concentration of free-radical production in the venous effluent of term sheep exposed to 30 minutes of acute asphyxia. They found a more than 2-fold increase in the production of free radicals during the early stages of reperfusion compared with nonischemic control animals.

White Matter

Recent years have brought an increasing focus on models of periventricular white-matter damage. Ting et al.\textsuperscript{89} were the first to elucidate this in sheep. These investigators developed a model whereby midgestational (ie, 68 to 85 days gestation) sheep fetuses were exposed to 10% oxygen for 2 hours. Of the 38 fetuses subjected to hypoxia, 29 were concomitantly rendered hypovolemic. The ewes and fetuses were then allowed to recover for 3 days, at which time they were delivered and sacrificed for neuropathologic assessment. Of the 38 fetuses, 10 died before delivery, and only 8 showed evidence of gross and microscopic brain injury. In that regard, the hemispheric white matter was most severely damaged, with some brains showing evidence of hemorrhage and cystic degeneration. It should be noted that damage was also noted in the basal ganglia of these animals, as well as in the dorsolateral regions of the cortex, although to a lesser extent than white-matter damage. Of particular note was the finding that only those fetuses in which mean arterial blood pressure fell below 30 mmHg showed brain damage, whereas none of those who maintained their blood pressure did, irrespective of hypoxia.

Petersson et al.\textsuperscript{90} detailed the neuropathologic injury to white matter in 126-day (0.85) gestation ovine fetuses after carotid artery occlusion for 30 minutes and recovery for either 48 or 72 hours. These investigators found both gray- and white-matter involvement, the latter of which was characterized by a reactive gliosis and the loss of myelin basic protein in oligodendrocytes. Reddy et al.\textsuperscript{91} compared the neuropathologic consequences of cerebral hypoperfusion for 30 minutes in 0.65 gestation and 0.9 gestation fetal sheep and confirmed the topographical specificity of white-matter injury. However, both ages of sheep displayed parasagittal cortical damage and selective neuronal necrosis in the thalamus and striatum. The preterm fetuses developed subcortical infarcts with more
rapidly evolving necrosis of the white matter compared with those closer to term.

Further investigations in the sheep have begun to outline the pathogenic mechanisms of injury to the immature fetal sheep brain. Ikeda et al\textsuperscript{92,93} measured levels of thiobarbiturate-reactive substances (TBARS) within gray and white matter after 60 minutes of umbilical cord occlusion and found significantly higher levels in the frontal and parietal white matter than in gray matter. Significantly higher concentrations of glutamate were also detected using intracerebral microdialysis in the white matter of fetal sheep at 0.85 gestation compared with those closer to term.

Most recently, several laboratories have developed models of white-matter injury in sheep after systemic endotoxemia.\textsuperscript{95,96} In this regard, Mallard et al\textsuperscript{96} compared the use of systemic asphyxia to endotoxemia for inducing injury resembling PVL in fetal sheep of age 93 to 96 days (or 0.65 of gestation). Asphyxia was promoted by umbilical cord occlusion for 25 minutes, whereas systemic endotoxemia was caused by intravenous injection of \textit{Escherichia coli} lipopolysaccharide. Interestingly, the white matter appeared particularly sensitive in both models, as characterized by microglial infiltration, loss of oligodendroglia, and damage to astrocytes. In contrast, however, whereas systemic endotoxemia caused selective injury to white matter, umbilical cord occlusion was less specific and also resulted in neuronal necrosis in subcortical regions including the striatum and hippocampus. Duncan et al\textsuperscript{95} also used a model of systemic injection of lipopolysaccharide over 5 days in fetal sheep at 0.65 gestation, and found that after the injection, particularly over the first 2 days, there was an acute decrease in both mean arterial blood pressure and partial oxygen pressure. This was accompanied by an increase in lactate and acidosis. Although statistically significant only over the first 2 days of injection, these data clearly show that these alterations occurred over 4 days of injection. Interestingly, the data indicate that in fact the endotoxemia model of white-matter injury is a model that combines both inflammation and ischemia as part of its pathophysiologic contribution to white-matter injury. These investigators also found an elevation of IL-6 during the first 6 hours of injection, and indicated a similar acute increase in TNF-\textit{z} within the first 2 hours. Histopathologically, diffuse white-matter injury was seen in the majority, with specific periventricular involvement occurring in 1/3 of the animals. Dalitz et al\textsuperscript{97} further demonstrated the significant influence of endotoxin by administering lipopolysaccharide to 11 catheterized fetal sheep at 0.7 gestation, then measuring fetal cerebral blood flow and placental flow using microspheres. Their findings showed that although fetal cerebral blood flow did not decrease, oxygen delivery did. Specifically, both cortical and white-matter oxygen delivery decreased by 36\% of control at 4 hours postinjection and by 28\% of control at 8 hours postinjection, and that placental blood flow decreased by 54\% at 4 hours postinjection and by 43\% at 8 hours postinjection. These data clearly support the role of infection in causing not only an inflammatory response, but a hypoxic response as well.

\section*{Rats}

By far the most commonly used animal for models of perinatal asphyxia is the rat. In the immature animal, this model was introduced by Vannucci’s group in the early 1980s and used the combination of unilateral common carotid artery ligation with 8\% oxygen in a 7-day-old rat pup. The authors described the pathological consequences of this insult, particularly within gray-matter structures, as columnar regions of selective neuronal necrosis through to infarction. Chronically, cystic infarction of the cerebral cortex may be seen within the distribution of the middle cerebral artery territory, resembling the formation of a porencephalic cyst. Although the myelogenic zones of vulnerability were discussed in this report, until recently these findings had largely been ignored.

\section*{Gray Matter}

Perhaps one of the main advantages of the rat model is that it has been so well characterized over the years, largely by Vannucci and colleagues.\textsuperscript{53} In this regard, the 7-day-old rat pup has been variously likened to a 32- to 34-week-old human infant. The physiological parameters of the model have shown that during the insult, the rat pup becomes hypoxic in combination with being hypocapnic as a result of hyperventilation. This results in a compensated metabolic acidosis and allows for a normal pH despite the lactic acidemia. Mean systemic blood pressure declines by approximately 25\% during the hypoxic-ischemic episode.\textsuperscript{99,100} Regional cerebral blood flow measure-
ments indicate a reduced blood flow to between 17% and 40% of control, with those areas most vulnerable to damage displaying the lowest blood flow.\textsuperscript{101,102} Cerebral metabolic correlates indicate a depletion of intracellular glucose, accompanying lactic acidosis, and a near-complete loss of high-energy phosphates within the hemisphere ipsilateral to the common carotid artery ligation.\textsuperscript{99,103} During recovery, adenosine triphosphate replenishes rapidly, although a secondary decline occurs within the first 24 to 48 hours of recovery. The findings appear consistent with a relative lack of substrate (glucose) compared with oxygen as an underlying causative mechanism of cell death, in keeping with the findings that the contralateral hemisphere appears normal even though it has been exposed to hypoxia in the absence of ischemia.\textsuperscript{103-107} Others have documented an increase particularly in the striatum of excitatory amino acid release,\textsuperscript{108} and the accumulation of intracellular calcium that arises during the terminus of the insult and into recovery.\textsuperscript{109} Pathologically, cerebral edema evolves over a period of several days, peaking at 72 hours in those animals ultimately having the most significant damage.\textsuperscript{110} Histologically, a gradation of injury is observed that correlates in a linear fashion with the duration/severity of the insult.\textsuperscript{111-113} Hence damage commences after 60 minutes of hypoxia-ischemia and progresses to produce infarction by 90 minutes. Neocortical damage often appears in a columnar distribution. There is also evidence of necrosis of the subcortical gray-matter structures and periventricular myelinogenic foci.

Hagberg et al\textsuperscript{114} nicely delineated the proinflammatory response that occurs during recovery in this model. In this regard, a distinctive IL-1 and TNF-\(\alpha\) response was seen in the first 24 hours, accompanied by chemokines and macrophage inflammatory protein. In the next phase, neutrophils transiently invade the lesion between 12 and 24 hours, followed by microglia/macrophages and astrocytes. The latter group persists for upwards of 42 days, with natural killer cells being evident from 24 hours and lymphocytes beginning to infiltrate at the end of the first week after the insult.

Several others have developed modifications of the Rice-Vannucci model. Renolleau and colleagues\textsuperscript{115-117} studied a model of transient unilateral hypoxic-ischemic injury in 7-day-old rats and found that with reperfusion, the inflammatory response was much more robust and occurred in a shorter time frame, augmenting the extent of injury. Similar results were reported by Derugin et al\textsuperscript{118} and Ashwal et al.\textsuperscript{119} Schwarz used a model of bilateral common carotid artery ligation that reportedly produced a more uniform and severe neocortical infarction of greater reproducibility to the unilateral model. Unfortunately, neuropathology was described at only 3 days of recovery, and in this investigator’s experience, the bilateral ligation model has an extremely high mortality rate beyond 72 hours (Yager et al, unpublished data).

**White Matter**

As for gray-matter injury models, recent years have seen the development of numerous rat models focusing on white-matter injury. In our laboratory we have developed a model of transient bilateral common carotid artery ligation for 5- to 10-minute periods. Assessment of the neuropathologic findings at 72 hours of recovery show evidence of cystic infarction involving the periventricular regions of the brain, reminiscent of those seen in PVL in the human neonate (Fig 5).\textsuperscript{48} Further elaboration of this model has shown that the cells most sensitive to the ischemic injury are O4 oligodendroglial progenitors, which are particularly sensitive to the development of reactive oxygen species during reperfusion.

Models of periventricular white-matter injury involving a hypoxic/ischemic insult in rats have also come from Uehara et al\textsuperscript{120} who induced white-matter injury after permanent bilateral common carotid artery ligation in P5 rats, and Cai et al,\textsuperscript{121} who studied permanent bilateral artery ligation induced in P1 rats and assessed the neuropathologic consequences on days P7 and P14 of recovery. The latter group found a reduction in O4 staining cells, an increase in microglia/macrophages, and a reduction in myelin basic protein on P7 but not on P14, specifically within white-matter structures, again indicating the vulnerability of the oligodendrocyte to damage from ischemia at this stage of development.

Back et al\textsuperscript{14,57,122} has done the important work of delineating the rat oligodendroglial cell lineage and correlating this with the human lineage, to identify the correct timing for using this model as one of PVL. This group has identified that the window of vulnerability for white-matter injury precedes myelination and coincides with a time when the late oligodendrocyte progenitor is the major target. In humans, this coincides with the 24-
to 32-week time frame during which PVL most commonly occurs in humans and the P2 to P5 time frame for rats, recognizing that white-matter injury does occur outside these age groups in both humans and rats.

Infectious/inflammatory models of PVL have also been developed, given the epidemiologic data suggesting a role for clinical and subclinical chorioamnionitis as an etiological factor in the development of cerebral palsy in children.126,127,128 Yoon et al126 created an ascending infection and chorioamnionitis model using *E. coli* in timed pregnant (0.70 of gestation) rabbits. White matter lesions were found in about 1/2 of those fetuses infected, but in none of those treated with saline. Histologically there was evidence of karyorrhexis and disorganization of white matter, along with evidence of apoptosis. In more recent studies by Debillon et al,127,128 maternal inoculation of rabbits with *E. coli* at 0.80 gestation resulted in consistent white-matter injury, with 25% of the brains exhibiting evidence of periventricular white-matter cysts. Interestingly, like Yoon’s experiments, this study pointed out the importance of treating the pregnant rabbits with antibiotics, because mortality was almost 100% if no treatment was provided. In this model focal white-matter cysts, accompanied by a robust inflammatory response and diffuse cell death, which mimic the white-matter damage seen in extremely preterm infants, occur in the absence of a detectable neocortical inflammatory response.

**FETAL GROWTH RETARDATION**

Although not specifically the topic of this review, the contribution of FGR to neonatal neurologic morbidity is significant and is clearly a major complicating feature in those infants presenting with a neonatal encephalopathy. FGR also enhances the likelihood of asphyxia occurring around the time of birth. Moreover, clearly one of the major etiologies of FGR is chronic placental insufficiency or hypoxemia. In this regard, Many et al130 examined the neurologic and intellectual outcomes of FGR infants born to mothers with and without preeclampsia. They found a significant difference between the 2 groups, with average IQs of 85.5 in the preeclamptic group and 96.9 in the non-preeclamptic group. Unfortunately, no normal controls were used in this study. Toft et al131 measured gray-matter volumes in FGR infants with MRI and found them significantly decreased compared with controls.

Several animal models of FGR as it relates to neurologic outcome have been developed; I will touch on a few of these here. Trescher et al132 induced growth retardation in newborn rats by uterine artery ligation. Once born, the pups were
allowed to nurse normally with their dam for 7 days, at which time a hypoxic-ischemic insult was induced. These investigators found that the FGR rat pups had less brain damage compared with controls. Unfortunately, once delivered, the pups were no longer restricted. In the fetal lamb, Mal- lard et al\textsuperscript{133} induced chronic fetal hypoxemia from gestational days 120 to 140. After sacrifice, the fetal brains exhibited evidence of severe gliosis and decreased myelination. A reduced number of Purkinje neurons was seen in the cerebellum. This same group of investigators then assessed the learning ability and behavior of the animals between 2 and 6 weeks after birth.\textsuperscript{134} In general, the low birth weight lambs did more poorly than their counterparts, but it was difficult to conclude whether this was a product of their preterm birth.

In the guinea pig, unilateral uterine artery ligation at 30 days’ gestation (term is 60 days) resulted in significantly larger cerebral ventricles and reduced cortical, striatal, and hippocampal volumes compared with controls (the latter of which was due to a reduced number of neurons in both the hippocampi and the cerebellum).\textsuperscript{135} In the hippocampus, evidence also suggests a decrease in dendritic spine outgrowth as a result of chronic placental insufficiency.\textsuperscript{136} These findings have raised questions about the possible relationship between growth restriction in the newborn and onset of schizophrenia later in life.\textsuperscript{137}

CONCLUSIONS

Controversy continues over the appropriate model to use when attempting to mimic the clinical and pathophysiologic aspects of human perinatal brain injury.\textsuperscript{138} It is clear, however, that tremendous progress has been made regarding the underlying mechanisms of perinatal brain injury, and that all models have contributed to this progress. Clearly the larger animal models, such as sheep, provide better access to the ongoing physiological parameters that arise during in utero or antenatal events. These issues will become increasingly important as we develop methods for ongoing monitoring, and in determining the physiological consequences to the fetus during chronic intrauterine ischemia. Small animal models clearly have an advantage when it comes to a better understanding the biochemical consequences of perinatal brain injury, as well as assessing longstanding neuro-pathologic endpoints and behavioral outcomes.\textsuperscript{139,140}

Yet despite all of the advances, adaptation of neuroprotective drugs to the clinical setting has been unsuccessful. Of the more than 49 agents tested in more than 114 clinical stroke trials, none has demonstrated proven benefit.\textsuperscript{141}

In the newborn infant, drug trials (except for those related to hypothermia) have essentially been nonexistent. This is because in the human newborn, one is faced with the much more complicated scenario of treating perhaps not only the fetus, who is in a state of rapid development, but also the mother. Once the insult has occurred, treatment may involve applying therapeutic strategies that may interfere with the normally and rapidly developing brain of the immature newborn, perhaps causing more harm than good. Under these circumstances, we must be very clear that the benefit of therapy outweighs any risk to either the patient or mother.

This is the challenge for physicians caring for newborns in the future. Irrespective of how great our understanding of the mechanisms of injury, we must be able to identify, with a high degree of specificity, those infants who are indeed at risk for subsequent neurologic morbidity and evaluate what that morbidity might be. Animal models hold great promise in this application.

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