Introduction
The consequences of injury to the fetal brain are influenced by factors unique to this initial phase of the lifespan. Fetal brain development unfolds across gestation through a sequence of overlapping phases, each with a specific period of peak activity. These events occur in different cell types and different regions of the brain in a complex, highly programmed manner. The regions with the most active development under normal conditions are also those that are at greatest risk for injury under adverse conditions. Regional injury during critical phases of development may derail subsequent developmental events, in and around the region of injury, as well as remotely in future projection fields of the injured area (Limperopoulos et al. 2005, Limperopoulos and du Plessis 2008). In summary, the topography, and consequently the long-term manifestations, of brain injury depend not only on the nature of the insult but also on its timing; ‘when’ is as important as ‘what’. Although the developing brain is more susceptible to injury, its immature state also underlies its sometimes remarkable ability to compensate for injury through the incompletely understood phenomenon of ‘plasticity’. Thus, the mechanisms of injury to the fetal brain, as well as the long-term structural and functional sequelae, are inextricably linked to normal developmental events in the brain. Although these developmental events will be reviewed briefly to provide context, the reader is referred to excellent published reviews for additional detail (e.g. Johnston et al. 2009).

Normal fetal brain development may be disrupted by primary disturbances in its genetic blueprint, or by internal and external environmental factors. Antenatal influences may also predispose the fetal brain to injury during the intrapartum period. A review of primary brain dysgenesis is beyond the scope of this chapter (Volpe 2008a), and intrapartum brain injury has been reviewed exhaustively elsewhere (du Plessis 2005, Volpe 2008b).

The focus of this chapter is therefore confined to fetal brain injury occurring prior to intrapartum events.

Overview of normal fetal brain development
Nervous system development in the fetus progresses through a series of events, starting several days after conception. During the embryonic period the principal phases of development are definition of the neural axis and formation of the neural tube (dorsal induction). The neural tube then comes under the influence of regional gene product gradients, which promote certain developmental processes and suppress others (Jessell 2000). After the neural tube closes at around 4 weeks after conception, three vesicles begin to form at its rostral end. These are the prosencephalon, mesencephalon, and rhombencephalon that will form the future forebrain, midbrain, and hindbrain respectively. This rostral region of the neural tube then goes through a series of folds in the sagittal plane forming the cervical, pontine, and cephalic flexures. The central canal, which, at its rostral end, will form the ventricular system of the brain, is surrounded by a layer of cells that form the primary neuroepithelium, the origin of all neuronal and glial cell lineages (Rakic et al. 2007, Bystron et al. 2008). The neuroepithelium becomes divided into dorsal and ventral segments. The dorsal neuroepithelium is the source of excitatory pyramidal neurons (that will form the future projection pathways), as well as the radial glial cells. The ventral neuroepithelium develops two thickened regions, the ganglionic eminences, which give rise to the future interneuronal population, which become critical for localized cortex-to-cortex circuits. The main neurotransmitter of these interneurons is gamma-aminobutyric acid (GABA), which has an initial excitatory influence, but ultimately becomes the principal inhibitory neurotransmitter in the brain. Neurons reach the developing cerebral cortex via two major paths of neuronal migration. Neurons from the dorsal ventricular zone...
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undergo radial migration along radial glial cells acting as guide-wires to the surface of the brain. During this migration the six-layered neocortex is formed in an inside-out manner, with later waves of migration passing through previous layers to settle on their outer-side. A critical part of this process is development and subsequent regression of the subplate zone (Kostovic and Jovanov-Milosevic 2008). The transient subplate zone acts as a ‘waiting station’ below the future cortex, where it is thought to ‘fine tune’ the itinerary of axons of thalamic neurons seeking to make appropriate connections in the developing cortex. Successful completion of these thalamocortical pathways establishes the fundamental neural scaffolding connecting the internal and external environment to the sensory cortex, and in so doing enables the development of conscious experience.

During initial formation of the cerebral hemispheres an overabundance of neural structures are formed, including neurons, dendrites, dendritic spines, and synapses. This occurs under the influence of ‘spontaneous’ bursts of electrical activity that are endogenously generated, i.e. are not activated by incoming stimuli. Once development of the neural apparatus connects the peripheral sensory nerves to the cerebral cortex through connection in the thalamus, a reorganization of the cortex occurs under experience-driven influences. This phase coincides with normal regressive events occurring in the developing brain. Specifically, ‘unstable’ synapses are pruned back, unless they are stabilized by an appropriate level of activation. Such neural activation also releases local growth factors and activates gene programs that support the survival of neurons. On a ‘use-it-or-lose-it’ basis, redundant neurons are culled by active energy-dependent cell death, or apoptosis. These regressive events and the early abundance of neural structures might underlie the compensatory plasticity of the immature nervous system after injury. Myelination is a relatively late phase of development and by term gestation has proceeded only into the brainstem, cerebellum, and the posterior limb of the internal capsule. The subsequent pattern of myelination does not commence uniformly across the cerebral hemispheric white matter, but rather it proceeds in a predictable spatial and temporal sequence (Kinney et al 1988, Drobyshovsky et al 2005).

At a cellular level, the consequences of an insult are heavily influenced by the maturational level of the neuronal and glial lineages at the time of the insult. As a broad statement, neurons in regions of the most active development at any one time have a physiology that promotes depolarization, making it most sensitive to incoming stimuli that then activate receptors responsible for controlled influx of enzyme-activating calcium. In order to finely titrate this process and to keep it localized to establish the most regionally discrete connectivity, the neurotransmitter is then rapidly cleared from the synaptic cleft by reuptake channels. During insults such as hypoxia and hypoglycemia, this arrangement turns hostile as neurotransmitter release becomes uncontrolled and reuptake mechanisms fail, resulting in sustained neurotransmitter activity at the synapse with neurotoxic levels of calcium influx. From this brief outline it can be seen that during critical periods of development the fetal brain might be particularly vulnerable to injury, which, in turn, might disrupt the complex sequence of subsequent developmental events.

The oligodendrocyte lineage goes through a series of maturational steps, from a progenitor phase to the mature myelin-generating form. Across this period, the oligodendrocyte lineage passes through a developmental phase (the late oligodendrocyte progenitor, or preOL) phase, during which it is highly vulnerable to hypoxia–ischemia and other insults (Back et al 2007, Segovia et al 2008); before and after this preOL phase the oligodendrocyte lineage is relatively resistant to insults. Data from animal models correspond with the peak period of white matter injury in preterm infants between 26 and 32 weeks’ gestation (Baud et al 2004, Back et al 2007). In fact, both the temporal and topographic distribution of the preOL overlaps with the timing and topography of preterm birth-related white matter injury (Buser et al 2010). During oligodendrocyte development there is a shift in glutamate receptor and transporter density, limitation of antioxidant defenses, and cytotoxic cytokine receptors (Back 2006, Volpe 2009), making the preOL particularly vulnerable to insults such as hypoxia–ischemia and infection–inflammation. A synopsis of published data suggests that hypoxia has different effects on the oligodendrocyte lineage at different stages of development. In the very early oligodendrocyte precursors, hypoxia may result in accelerated maturational events (Akundi and Rivkees 2009). In the somewhat more mature preOL, chronic hypoxia causes either delayed preOL degeneration or developmental arrest in the vulnerable preOL phase, and thus it is primed for injury from subsequent insults (Segovia et al 2008). Finally, in its mature myelinating phase the oligodendrocyte has a relatively elevated threshold to injury.

Similar phases of increased vulnerability occur during neuronal development. Immature neurons in rapidly developing brain regions have membranous and intracellular features that facilitate depolarization and promote influx of calcium for activation of growth-promoting enzymes. During earlier phases of development, neurons are maintained in a relatively hypopolarized state by the neurotransmitter GABA. At this early stage of
development, GABA has an excitatory influence (as opposed to its later inhibitory action) related to the ambient chloride gradients across the immature neuronal membrane (Staley et al 1995, Dzhala et al 2005). Furthermore, a paucity of reuptake transporters allows GABA to accumulate in the extracellular space, where it then acts in a more diffuse paracrine manner by maintaining a field of hypopolarization, which in turn releases the magnesium blockade at the N-methyl-D-aspartic acid (NMDA) receptor channel, permitting greater calcium influx for maturational processes. However, during insults such as hypoxia–ischemia both the hypopolarized membranous state and high density of glutamate receptors in regions of accelerated brain development predispose to excitotoxic injury with necrotic and/or apoptotic cell death.

The maturational development of astroglial cells is also relevant to the current discussion. Specifically, the typical response to brain injury seen in later stages of development and in the mature brain, i.e. reactive astrogliosis, does not occur before about 20 to 24 weeks gestational age (Kinney and Armstrong 1997). Consequently, tissue destruction occurring prior to this point in gestation triggers very little cicatricial response. As a result, the resulting lesions may have minimal gliosis and resemble malformations rather than encephaloclastic lesions.

The nature and severity of environmental insults may disrupt brain development in a number of different ways. Milder insults may trigger subtle pathologic processes through epigenetic programming pathways. Progressively more severe insults may result in arrested or disrupted development, selective cellular injury and loss, and frank pancellular destruction (infarction) of the structural scaffolding required for normal brain development. These different pathways likely act in concert during and after insults. Furthermore, below the threshold of injury, insults may also increase (sensitize) or decrease (pre-conditioning) sensitivity to subsequent insults.

The timing and nature of insult and injury might also influence the subsequent efficiency of compensatory processes, so-called plasticity. In the same way that early injury may disrupt subsequent brain development, so too may it affect the normal regressive processes of ‘pruning’ back and reorganization during later phases of brain development. As discussed above, earlier processes in fetal brain development produce an excess of neural structures, which are subsequently ‘pruned back’ by energy-dependent apoptosis, a process that occurs in part through competition for trophic factors. One theory of plasticity is that regional brain injury reduces competition for trophic factors and substrate, allowing surviving tissue to compete successfully.

Normal function of the maternal placenta–fetal interface
During the antenatal period the principal contact between the fetus and the environment is through the placenta. The placenta serves as the only nutrient and clearance system for the fetus. In addition, the placenta serves as a barrier to potentially noxious agents. The placenta also has a critical endocrine role in fetal growth and development. Placental development begins with ‘placentation’ several days after implantation of the embryo. The normal physiology of implantation includes trophoblast invasion of the endovascular layers of the spiral arteries, with disruption of the muscular media (Nanaev et al 1995), and conversion of the normally small caliber spiral arteries into distended flaccid vessels with limited vasoconstrictive capability. In so doing the uteroplacental system is converted to a low-resistance, low-pressure, and high-volume circulation. Exchange across the placenta occurs across an interface composed of the syncytiotrophoblast, a basal membrane, and the fetal endothelium. Such exchange occurs in several different ways: bulk flow down hydrostatic and osmotic gradients; diffusion down concentration gradients; transporter protein-mediated transfer (e.g. glucose, amino acids); and endo- or exocytosis (e.g. immunoglobulin G). Therefore, in addition to perfusion of the uteroplacental and fetoplacental circulations, the supply of nutrients across the placenta is also dependent upon the functional surface area, as well as the membrane-bound transporter activity on either side of the maternal–fetal interface. Transporter-mediated transfer is adaptively regulated via cellular homeostatic mechanisms, which change transporter function in response to substrate levels, and thereby maintain placentostatic mechanisms, which change transporter function in response to substrate levels, and thereby maintain placentostatic mechanisms, which change transporter function in response to substrate levels, and thereby maintain placental transport efficiency. Fetal brain development is dependent upon the appropriate delivery of nutritional elements for structural accretion, and of energy substrate to support enzyme function. The appropriate availability of energy substrate is
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particularly important during the third trimester, when energy-dependent neuronal activation is critical for establishing and consolidating neuronal circuitry.

Restriction of specific nutrients essential for nervous system development

The classic association between specific nutrient deficiency and disturbed neurodevelopment is that between folate deficiency and disturbances in neural tube closure. Maternal folate deficiency may result from inadequate dietary intake as well as malabsorption conditions (after gastric bypass surgery) (Haddow et al 1986). The precise cellular mechanisms by which folate supplementation prevents neural tube defects remains unknown; the current understanding is reviewed in detail elsewhere (Haddow et al 1986). Disturbances in cholesterol availability have been implicated in disruption of prosencephalic development. Specifically, the Sonic hedgehog (SHH) protein plays a central role in the development of the face, brain, and genitalia. In the brain the SHH gene product is critical for ventral induction and patterning, with formation of the cerebral hemispheres and the midline structures, most notably the corpus callosum. For the SHH protein to be activated it must bind to cholesterol. When cholesterol availability is limited, lesions such as holoprosencephaly (Fig. 1.1) and agenesis of the corpus callosum may develop. The classic example of cholesterol deficiency is 7-dehydrocholesterol reductase deficiency, the autosomal recessive Smith–Lemli–Opitz syndrome (ACOG 2000).

Restricted energy substrate for normal brain development

Under normal conditions the developing brain enjoys a privileged supply of oxygen and energy substrate. In fact, normal oxygen and glucose delivery to the fetal brain significantly exceeds demands. If this supply decreases, a number of systemic and cerebral compensatory mechanisms are activated to optimize cerebral supply and demand and to maintain a normal cerebral metabolic rate until hypoxemia is severe (Richardson 1993). In fetal sheep with a 50% decrease in oxygen delivery to the placenta, cerebral oxygen consumption was maintained for at least 24 hours (Bocking et al 1992). In another experimental model in which maternal oxyhemoglobin saturation was maintained below 30% (by decreasing inspired oxygen concentration), cerebral oxidative metabolism was maintained for more than 4 days (Richardson 1993). In animal models of fetal hypoxemia, induced by decreasing uterine artery blood flow, the first fetal response was an increase in umbilical blood flow and an increase in oxygen extraction, followed by increased shunting of umbilical venous return from the placenta through the ductus venosus and foramen ovale. Sympathetic activation causes peripheral vasoconstriction, while intrinsic autoregulatory vasodilation in the brain reduces resistance and increases cerebral blood flow. These adaptations in the fetal circulation divert the most highly oxygenated perfusion to vital organs including the brain (‘centralization’ or the so-called ‘brain-sparing effect’). In addition, there are physiologic responses aimed at decreasing energy demand. Myocardial energy utilization is reduced through a chemoreceptor-mediated fetal bradycardia. Cerebral metabolism is decreased by active suppression of neuronal activation; this is achieved by adenosine, an adenosine triphosphate (ATP) breakdown product that inhibits synaptic activity by blocking the presynaptic A1 receptor (Blood et al 2003). Chronic compensated fetal hypoxemia may cause epigenetic changes in fetal programming and, by sublethal neuronal suppression, may disrupt activity-driven processes in brain development. Pure hypoxemia (with intact perfusion) may be tolerated

Fig. 1.1 Holoprosencephaly semilobar with large dorsal cyst in a 36-week gestational age fetus: (a) MRI midline sagittal and (b) coronal T2 images.
for sustained periods of time, by adjustments in demand, redirected blood flow, and alternative energy pathways such as anaerobic metabolism and utilization of alternative energy sources (e.g. lactate and ketones). The efficacy of these compensatory mechanisms at preventing destructive brain injury is dependent upon multiple factors such as the ‘dose’, nature, and delivery of the insult, the maturation state and sex of the fetus, and pre-existing conditions in the fetal milieu (e.g. preceding energy restriction, infection). These and other processes confine the destructive brain injury caused by substrate restriction to a ‘very narrow window between intact survival and death’ (Bennet and Gunn 2009). When hypoxemia and hypoperfusion occur in combination (i.e. hypoxia–ischemia) these compensatory mechanisms rapidly collapse, in part because the interruption of glucose supply limits anaerobic metabolism and lactate formation, and destructive pathways are unleashed.

**Mechanisms of fetal energy substrate deprivation**

Limitation of substrate supply to the fetal brain may originate at the maternal, uteroplacental, fetoplacental, or fetal level. *Maternal starvation level deprivation* is uncommon in the developed world but remains a problem in underdeveloped regions and those ravaged by natural and man-made disasters. Although substrate concentrations in the maternal circulation may restrict fetal supply, the more common scenario is *limitation of uteroplacental perfusion*, by lesions such as abnormalities of placentation, infarction, and hemorrhage. Impaired uteroplacental perfusion may stem from abnormalities at the level of the uterine arteries, the spiral arteries, or the uteroplacental vascular bed. Failure of spiral artery transformation and vasodilation results in impaired perfusion of the placental intervillous spaces, setting the stage for compromised fetal oxygenation. Failure of normal placentation may result in spontaneous miscarriage, isolated fetal growth restriction (FGR), and pre-eclampsia with or without growth restriction. In pre-eclampsia the muscular media not only persists but may even hypertrophy.

**Placental mechanisms of fetal substrate restriction** constitute a major pathway for morbidity during the fetal period, with effects that extend through the neonatal period and beyond. However, the system has considerable reserve, and fetal growth is not impaired until approximately 30% of placental function is lost. Placental dysfunction, leading to FGR, may result from a spectrum of different etiologies, leading to a common end result. Broadly speaking, these placental pathologies may be considered in three categories: abnormal vascular development, inflammatory processes, and acquired degeneration, usually with thrombotic changes. Inflammatory changes in the intervillous space of the placenta may be caused by infections, such as toxoplasmosis, cytomegalovirus, and other presumed viral infections, as well as autoimmune conditions, such as the antiphospholipid antibody syndrome. However, fibrin deposition and inter villous thrombi may also be seen in up to half of placentas from normal-outcome pregnancies. In addition, some studies have failed to identify an association between maternal or neonatal thrombophilic polymorphisms and an increased risk of FGR (Infante-Rivard et al 2002, Infante-Rivard et al 2005).

The *fetoplacental circulation* normally receives almost half the fetal cardiac output. Signaling between fetal and maternal placental vessels couples fetal to uteroplacental blood flow (Talbert and Sebire 2004). However, this coupling may be disrupted by vasoconstrictive or occlusive placental lesions. The major fetal vessels in the chorionic plate perfuse large segments of the placenta, called cotyledons. The vascular territories of these large arteries do not overlap; the cotyledons have no collateral supply, and these vessels are neither innervated nor do they autoregulate. Fetoplacental blood flow is locally controlled entirely through fetal endocrine and placental paracrine systems (Poston 1997, Benoit et al 2008). Fetoplacental vascular pathology may cause significant elevation in fetal peripheral vascular resistance. In fact, sustained hypoperfusion of the fetoplacental circulation may actually lead to constriction of these vessels (Rockelein et al 1990). Fetoplacental thromboinflammatory lesions associated with adverse neurodevelopmental outcomes include fetal thrombotic vasculopathy and villitis of unknown etiology, which is associated with chronic inflammation and, ultimately, avascular distal villi (Redline 2004). Longstanding meconium exposure may cause vascular necrosis through apoptotic death of vascular smooth muscle cells and vasospasm.

**Intrinsic fetal conditions** may adversely affect fetal brain development. As discussed above, the normal fetal circulation is arranged such that there is an optimal oxygen-substrate delivery to the developing brain. In certain forms of fetal cardiac malformation this arrangement may be disrupted with potential restriction of cerebral oxygen-substrate delivery. For example, the oxygen content of aortic (and hence brain) blood flow is decreased in conditions such as transposition of the great arteries. Conversely, volumetric cerebral blood flow may be compromised by conditions such as aortic and left ventricular hypoplasia, which, in severe cases, leaves the brain dependent on retrograde perfusion from the ductus arteriosus across the aortic isthmus. A study comparing fetal volumetric brain growth in fetuses with heart lesions with controls showed that despite similar brain volumes at the end of the
second trimester, there occurred a significant and progressive fall-off in brain growth among the congenital heart disease fetuses (Limperopoulos et al 2010a). This brain growth failure was most pronounced among fetuses with the greatest expected oxygen delivery to the brain, as well as with the presence of cerebral lactate on fetal magnetic resonance spectroscopy, suggesting the development of anaerobic metabolism in these most affected participants (Catherine Limperopoulos, Children’s National Medical Center, personal communication, 2010).

The oxygen-carrying capacity may also become impaired in conditions that cause severe anemia, such as Rhesus incompatibility and fetal infections, especially with parvovirus (see below).

Assessing perfusion of the uteroplacental and fetoplacental circulations by Doppler ultrasound measures of blood flow velocity and vascular resistance is now standard in the management of suspected FGR. When FGR is associated with placental infarction and/or hemorrhage on the maternal side of the circulation, uterine artery Doppler indices will show an increase in resistance. Similarly, in the fetoplacental circulation, a characteristic sequence of perfusion and resistance changes develop during progressive placental failure. Particularly concerning is the development of decreased or reversed diastolic flow in the umbilical arteries, which becomes apparent only when about 50% or more of placental function is lost.

The so-called brain-sparing effect is misleading because, in many cases, the attempted compensatory response does not spare the brain. Fetuses with evidence of brain sparing on Doppler studies are almost always growth restricted (Arduini et al 1987).

Abnormal development of brain structure in fetal growth restriction

In animal studies, placental insufficiency has a broad range of effects on the developing brain, often with decreased gray matter volumes and impaired myelination (Mallard et al 1998). Experimental models of FGR have shown regional decreases in growth factor levels in the fetal brain (Duncan et al 2004), which in turn leads to apoptosis and activation of pro-apoptotic pathways.

It has been proposed that the patterns of structural brain abnormality in human fetuses with FGR are dependent on the gestational age, although there is lack of consensus on this issue (Bassan et al 2011).

In humans with FGR the range of structural brain changes is broad; findings between studies are not consistent. It is likely that many different factors influence this relationship, including gestational age at onset of FGR, gestational age at delivery, and postnatal age at the time of study. Microcephaly is a known complication of severe FGR. Some studies of preterm growth-restricted fetuses have suggested a preferential catch-up of head growth over the first few years (Jordan et al 2005, Westerberg et al 2010). At follow-up, many individuals have head sizes similar to appropriately grown ex-preterm infants. Padilla et al (2010) found no difference between growth-restricted and appropriately grown preterm infants in head circumference, total brain volume, or the volumes of gray or white matter (by three-dimensional magnetic resonance imaging [MRI]) at 12 months corrected age. Other studies of preterm FGR have shown decreased intracranial volumes and decreased cortical gray matter volumes after preterm birth, persisting at term, but no significant differences with appropriately grown ex-preterm infants at later ages (Tolsa et al 2004, Dubois et al 2008). Although overall brain size may not differ significantly, neuroimaging studies have detected lobar or regional decreases in the frontal lobe (Geva et al 2006a,b, Figueras et al 2008a; Hernandez-Andrade et al 2008), hippocampus (Geva et al 2006a, Lodygensky et al 2008), and insular lobes (Padilla et al 2010) in preterm growth-restricted infants.

Other more subtle differences in cerebral cortical development gyrification (Dubois et al 2008, Esteban et al 2010); with decreased gray matter density (Tolsa et al 2004, Lodygensky et al 2008), have been described. Magnetic resonance spectroscopy studies have detected elevated cerebral lactate (Leth et al 1996, Kok et al 2002, Wolfberg et al 2007) (suggestive of anaerobic metabolism), and elevated inositol–choline ratios (Sanz-Cortes et al 2010), suggestive of reactive astrogliosis. Diffusion-weighted imaging has shown significantly higher apparent diffusion coefficient values in the pyramidal tracts (Sanz-Cortes et al 2010).

Abnormal development of brain function in fetal growth restriction

A number of studies have demonstrated the increased risk for adverse neurodevelopmental and behavioral outcome in survivors of FGR (Low et al 1992, Kok et al 1998, Monset-Couchard et al 2002, Tideman et al 2007). However, there are major inconsistencies in the reported prevalence and manifestations of these sequelae. Both preterm and term (Oros et al 2010) growth-restricted fetuses appear to be at risk; some studies have suggested that the clinical profile of these two groups may differ (Figueras et al 2008b), but this has not been consistent (Bassan et al 2011). Padilla et al (2010) suggested that preterm FGR was associated with worse neurodevelopmental outcome, especially in the fine motor domain. FGR in late preterm and term infants may be associated with a distinct clinical picture with impaired cognition and executive function (Fattal-Valevski et al 1999, Geva et al 2004).
et al 2006a,b, 2008). FGR in preterm infants increases the risk for neurodevelopmental sequelae in some, but not all, studies (Lodygensky et al 2008, Padilla et al 2010).

For term growth-restricted infants the risk for cerebral palsy increases four- to sixfold compared with those born between the 25th and 75th centiles (Larciprete et al 2005). Others have shown a significantly greater risk of subsequent cerebral palsy for growth-restricted infants born between 34 and 37 weeks’ gestation compared with those born before 33 weeks (Blair and Stanley 1990, 1992). Monset-Couchard et al (2002) showed an almost twofold increase in behavioral abnormalities when preterm infants born growth restricted compared with those appropriately grown. The need for later special education services was significantly greater for growth restricted than for appropriately grown preterm infants in some (Kok et al 1998), but not other (Schaap et al 1999) studies.

**Fetal Cerebrovascular Injury**

Currently, the majority of cerebrovascular injury diagnosed in newborn infants is considered to be of perinatal origin. Although both ischemic and hemorrhagic lesions are more easily diagnosed by modern fetal imaging, distinguishing between antepartum, intrapartum, and neonatal-onset stroke may be difficult when imaging is delayed. Arterio-occlusive stroke of the immature brain has a relatively limited acute presentation, with seizures being the most obvious clinical change. If seizures are the heralding sign of stroke, these usually occur over a period of days, then recede whether or not they have been treated. Hereafter, there is commonly a latent period with a paucity of physical signs until around 4 to 6 months, when normally emergence of purposeful movements reveals motor asymmetry. Early stroke, including intrauterine stroke, without detected seizures, may have delayed motor asymmetry as the first indication of a focal brain lesion. By this chronic phase of the injury even advanced MRI may be unable to distinguish between antenatal, perinatal, or neonatal-onset stroke. Early intrauterine stroke occurring before mid-gestation may show little, if any, residual evidence of tissue destruction as reactive gliosis is usually minimal (see above). In fact, the resulting lesion may be misdiagnosed as a primary dysgenetic lesion. Current clinical fetal MRI techniques remain relatively limited in their ability to detect fetal hemorrhagic lesions.

For all of the above reasons our understanding of the true incidence and pathogenesis of fetal arterial stroke remains poorly characterized (Curry et al 2007). Reported associations have included twin pregnancies (especially monochorionic with co-twin demise), fetal congenital heart disease, thrombophilias, and intrauterine infections. Strokes occurring in the perinatal period have a strong predilection for the middle cerebral artery distribution, especially on the left. Pregnancy is a naturally occurring hypercoagulable state, resulting from elevated circulating prothrombotic factors, decreased natural anticoagulants, and reduced fibrinolytic activity. The placenta has been implicated as a source for embolic phenomena in neonatal stroke, and thus presumably also fetal stroke (Burke et al 1997, Kraus and Acheen 1999). The most commonly implicated placental lesions include fetal thrombotic vasculopathy and fetal vasculitis (in intrauterine infection).

As a major proportion of the normal fetal venous return from the placenta passes through the foramen ovale and from there into the major cerebral arteries, a thromboembolic source in the placenta has direct access to the fetal brain arteries. Such thromboembolic sources in the placenta may result from several different processes, including inflammatory processes such as infection, thrombosis, and abnormal arteriovenous connections between monochorionic twins, especially in cases of co-twin demise and twin–twin transfusion syndrome (Fig. 1.2). Monochorionic twin pregnancies have placental connections; the twin circulations remain balanced in all but 10% to 15%. Stroke risk is significantly increased in twin–twin transfusion syndrome; when there is co-twin demise (which is probably underestimated) thromboplastin material is transferred from the dead twin.

An association between maternal thrombophilias and adverse pregnancy outcome was first suggested by Kupferminc and colleagues in 1999 (Kupferminc et al 1999). Proposed thrombophilia-related complications have included recurrent miscarriages, fetal demise, intrauterine growth retardation, pre-eclampsia, and fetal-neonatal stroke. Thrombophilias have been implicated in up to 70% of neonatal arterial strokes (Volpe 2008c), including factor V Leiden, prothrombin 20210A mutation, MTHFR mutation, protein C deficiency, protein S deficiency, antithrombin deficiency, antiphospholipid antibody syndrome, and elevated lipoprotein A (Golomb et al 2001, Mercuri et al 2001, Curry et al 2007, Suppiej et al 2008, Simchen et al 2009). Conversely, a recent study for a broad range of genetic thrombophilia polymorphisms failed to show an association with arterial stroke in newborn infants (Miller et al 2006). In many cases with an association between thrombophilia and neonatal stroke, it has been in the setting of other potentially prothrombotic conditions such as sepsis, chorioamnionitis, and pre-eclampsia. Infection is known to predispose to a hypercoagulable state, in part through endothelial injury and cytokine generation, with downregulation of thrombomodulin. In summary, the association (especially a causative association) between thrombophilia and arterial stroke in the fetus remains inconsistent at best.
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Patterns of intracranial hemorrhage in the fetus resemble those described in the preterm newborn infant. These hemorrhages are likely due to an underlying anatomic and physiologic immaturity, with an intrinsic fragility of the vasculature (reviewed in more detail elsewhere) (Volpe 2008d). Hemorrhages in the fetus include the typical germinal matrix-intraventricular hemorrhage lesion, and its complications including periventricular hemorrhagic infarction (Fig. 1.3) and posthemorrhagic hydrocephalus (Fig. 1.4). Similarly, cases of fetal cerebellar hemorrhage have been reported that probably result from rupture of the fragile vessels in the germinal matrices of the immature cerebellum. Such hemorrhages may result in disruptions of cerebellar development (Glenn et al 2007), and may mimic primary dysgenetic lesions such as cerebellar clefts (Poretti et al 2009) and Dandy–Walker spectrum lesions (Fig. 1.4 and 1.5) (Limperopoulos et al 2010b). Fetal intracranial hemorrhage has been associated with fetal thrombocytopenia (e.g. in parvovirus infection and alloimmune thrombocytopenia; Fig. 1.6), maternal ingestion of agents such as aspirin and cocaine, and thrombophilic conditions including factor V Leiden and MTHFR mutations (Petaja et al 2001, Aronis et al 2002, Ramenghi et al 2005). Another proposed mechanism underlying fetal intracranial hemorrhage is cerebral venous thrombosis, which tends to recanalize rapidly, leaving only features of hemorrhage and its complications.

Fig. 1.4 Fetal brain showing the distended fourth and lateral ventricles with low-signal hemorrhage layering along the dependent side of the dilated right lateral ventricle (arrow). Axial MRI single-shot fast spin-echo T2-weighted image.
Brain Injury in the Fetus

Maternal Toxins
A multitude of substances may have toxic effects in the developing nervous system, with both teratogenic and destructive consequences. Only select examples are discussed here, and are confined to drugs of abuse (alcohol and cocaine) and the effect of phenylalanine on the fetus in asymptomatic maternal phenylketonuria. Understanding the mechanisms of fetal brain injury associated with maternal drug abuse is complicated by confounding psychosocial factors and frequent multisubstance abuse.

Alcohol remains the most commonly implicated teratogenic toxin. Fetal alcohol exposure may result in a spectrum of outcomes, related in large part to the timing and dose of alcohol exposure. The classic fetal alcohol syndrome has well-known facial and somatic features (Clarren and Smith 1978, Erb and Andresen 1978), and almost universal microcephaly and mental impairment. In addition to the full-blown fetal alcohol syndrome, lower fetal exposure may be associated with less obvious somatic features but with some level of cognitive impairment. The precise mechanisms by which alcohol induces its effects on the developing brain are not well established, and probably include a variety of mechanisms related to the maturational stage at exposure, as well as the confounding socioeconomic, nutritional, and multi-toxin exposures. Putative mechanisms have included the following: a decrease in uterine blood flow, possibly due to vasoconstriction of the uterine arteries leading to fetal hypoxemia; fetal hypoglycemia; fetal zinc deficiency; an effect on NMDA receptors with pro-apoptotic effects; and impaired vitamin A synthesis. Fetal alcohol exposure has been implicated in disruption of all the major phases of brain development. The most common lesions described

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Fig. 1.6 Massive cerebral hemorrhage in 32-week gestational age fetus with alloimmune thrombocytopenia (axial T2-weighted MRI).
have been neural tube defects, and disturbances in neural proliferation and migration, with cell–cell adhesion disturbances being implicated. Examples of neuropathologic lesions include schizencephaly and polymicrogyria, as well as disturbances in midline prosencephalic development, including agenesis of the corpus callosum and septum pellucidum.

Cocaine is another maternal intoxicant with potentially devastating impacts on the fetal brain. Fetal cocaine exposure has been associated with a spectrum of structural and functional neurologic sequelae. Cocaine readily crosses the placenta and fetal blood–brain barrier. Once in the fetal brain, cocaine blocks presynaptic catecholamine reuptake, leading to sustained catecholaminergic activity in the developing brain. Under normal conditions, the immature nervous system is in autonomic imbalance with delayed parasympathetic maturation favoring relatively unopposed sympathetic tone, a scenario further amplified by the action of cocaine.

Disturbances in cognition, affect, attention, visual–motor and visual–spatial function, and behavioral regulation have been described after fetal cocaine exposure, even in the absence of obvious structural lesions. Several mechanisms have been proposed for these effects, including neurochemical, cerebrovascular, and non-specific ‘stress’ effects. During fetal life, endogenous catecholamines form part of a regulating signal system that influences many of the major processes of brain development, including neurogenesis, neuronal differentiation, neural migration, and cortical organization (Gressens et al 1992, Garg et al 1993, Lipton et al 1999, Lidow and Song 2001). The unregulated neurotransmitter increase during cocaine exposure may disrupt development at any or all of these developmental pathways, as seen in the spectrum of developmental brain lesions associated with fetal cocaine exposure ranging from microcephaly, neuronal migration defects, disorders of prosencephalic development (including agenesis of corpus callosum and septo-optic dysplasia), and neuronal migration abnormalities (Domínguez et al 1991, Handler et al 1991, Gieron-Korthals et al 1994, Addis et al 2001, He and Lidow 2004, Salisbury et al 2009). Cocaine and its metabolites may also cause vasoconstriction in the maternal, placental, umbilical (especially umbilical vein), and fetal (Zhang and Dyer 1991, Schreiber 1995, Patel et al 1999, Robinson et al 2000) circulation disrupting fetal oxygen-substrate supply. Although this mechanism of cocaine-mediated brain injury has been challenged, hypoxic–ischemic/reperfusion injury and hemorrhagic destructive brain lesions are well described in these infants (Fig. 1.7). Another proposed pathogenetic mechanism of developmental cocaine toxicity relates to the features it shares with the general fetal stress response (Lester and Padbury 2009). The early part of the fetal response to cocaine, specifically the elevation of catecholamines, is common to the fetal stress response. Therefore, it is possible that the more downstream events known to occur after fetal stress also play a role in survivors of fetal cocaine exposure. Specifically, the secondary effects of catecholamine on the hypothalamic–pituitary–adrenal axis include an elevation in circulating glucocorticoids, which in turn have potent effects on fetal and placental genetic programming, with potential health effects into adult life and across generations (Lester and Padbury 2009).

Maternal phenylketonuria may have major toxic effects on the fetal brain, with more than 75% of offspring having an intellectual disability. During normal development phenylalanine hydroxylase expression begins as early as the sixth week of gestation; however, early function of the immature enzyme may be incapable of handling a phenylalanine level that is elevated but asymptomatic in the mother. Elevated phenylalanine levels in the fetal circulation may have dose-dependent teratogenic effects on the fetal brain (Levy and Ghavami 1996, Levy et al 1996), including microcephaly, hypomyelinated white matter, and callosal dysgenesis. Although a precise mechanism(s) for these fetal effects remains unclear, possible pathways include disruption of essential nutrient transport by the placenta or phenylalanine’s direct oligodendrocyte toxicity resulting in hypomyelination.

Fetal infections

The developmental neuropathology of fetal infection may be categorized broadly into two, often overlapping, forms. Specifically, fetal encephalitis may cause disruption of
normal pathways for brain development and/or destructive brain lesions, often with prominent inflammatory features. It is likely that multiple mechanisms may operate with direct viral injury, vascular and inflammatory injury, and possibly viral disruption of genetic mechanisms. It may be difficult to establish congenital infection early in gestation as neither humoral inflammatory nor reactive astroglial responses in the fetus becomes evident until mid-gestation. Although many clinical and imaging features are common to the fetal encephalitides, certain features are more suggestive of certain agents (Bale 2009). For example, microcephaly is common to congenital encephalitis due to cytomegalovirus (CMV), rubella, herpes simplex (HSV) type II, and varicella zoster (VZV) infections, whereas congenital toxoplasmosis may be associated with hydrocephalic macrocephaly. Congenital infections are commonly associated with ocular findings, with chorioretinitis occurring in approximately 75% of infants with congenital toxoplasmosis, but infrequently in congenital CMV (20%) (Bale 2009). Sensorineural hearing loss is common in congenital rubella and CMV (Fowler et al 1997, Grosse et al 2008), and less common in congenital toxoplasmosis. Neuroimaging findings common to fetal encephalitis include periventricular hyperechoic and/or cystic lesions with atrophic ventriculomegaly and intracranial calcifications. The distribution of calcifications tends to be periventricular in congenital CMV, more scattered in fetal toxoplasmosis, and basal ganglia–thalamic in fetal HSV encephalitis (Hutto et al 1987). While cerebral cortical malformations such as schizencephaly, pachygyria/ lissencephaly, and hydranencephaly may develop after fetal HSV, CMV, and VZV encephalitis (Wright et al 1997, Bonthius et al 2007, Bale 2009), polymicrogyria is highly suggestive of CMV, particularly when associated with cerebellar hypoplasia (Bonthius et al 2007, Volpe 2008e).

In this chapter we focus on the neurologic sequelae of fetal CMV and parvovirus infections because they illustrate certain themes and are more common than other forms of viral infections with neurologic complications. Most cases of HSV encephalitis are acquired in the perinatal and neonatal periods, with only about 10% of HSV-II infections acquired in utero (Kimberlin 2004a,b). Congenital rubella syndrome, a potentially catastrophic transplacental infection associated with necrotizing encephalopathy with marked inflammation, has become rare in developed countries since the advent of widespread vaccination.

Congenital CMV infection remains the most common viral infection affecting the fetus, with approximately 1% of neonates in the USA infected at birth; of these 10% (or 4000 individuals) per year will be symptomatic at birth, with a significant mortality (Istas et al 1995). Humans are the only reservoir and transfer is through salivary or genital secretions and breast milk. Fortunately, fetal transfer rates are low, occurring in only 2% of individuals. Most fetal CMV infections occur after primary maternal infections, but may also occur after maternal reinfection or reactivation. The best predictor of adverse outcome in fetal CMV is neuroimaging evidence for brain involvement. The CMV is tropic to rapidly proliferating cells and to endothelial cells. The tropism toward rapidly proliferating cells likely underlies the microcephaly, often progressive. Cerebellar hypoplasia is present in around 50% of individuals with symptomatic CMV, which is likely to be related to the protracted development of the cerebellum with its primary and secondary germinal matrices for cell proliferation. Inflammatory changes are prominent and diffuse in CMV encephalitis, and involve the periventricular white matter, germinal matrices, and cerebral cortex. The white matter pathology may mimic periventricular leukomalacia (PVL) with its predilection for hypomyelination and periventricular cysts, particularly in a parietal distribution (Fig. 1.8a). However, unlike PVL, CMV encephalopathy commonly also involves the anterior temporal white matter (Fig. 1.8b). Another feature that distinguishes CMV from other forms of fetal encephalitis is the often striking involvement of gray matter, ranging from neuronal migration defects, particularly when the infection occurs earlier in gestation. In fact, in individuals with white matter abnormalities but normal cortical gray matter, the likely onset of CMV encephalitis is in the third trimester (Barkovich and Girard 2003). The cortical gray matter lesions include heterotopias, schizencephalies, pachygyria–lissencephaly (suggesting infection at between 16 and 18 weeks), and polymicrogyria (suggested infection at between 18 and 24 weeks). The predilection of CMV for endothelial cells underlies the mineralizing vasculopathy (‘candelabra sign’, Fig. 1.9) of the basal ganglia–thalamus vessels evident in one-third of individuals with congenital CMV encephalitis. The prominent inflammatory changes may underlie the atrophic ventriculomegaly, porencephaly, hypomyelination, and cystic changes of the periventricular and subcortical white matter in congenital CMV encephalitis.

Parvovirus B19 infection has a spectrum of potentially catastrophic effects during pregnancy, including neurodevelopmental disability in one-third of survivors (Nagel et al 2007). ‘Fifth disease’ is the most common form of parvovirus infection in childhood, and is transmitted by respiratory droplets and blood products. Transmission of the virus from mother to fetus is vertical. Parvovirus is tropic for erythroid lineage cells and may cause severe fetal anemia and thrombocytopenia, resulting in anemic hypoxia and hemorrhage, as well as
non-immune hydrops and fetal death, especially when transmitted between 17 and 24 weeks’ gestation. Fetal brain involvement ranges from perivascular calcifications in the cerebral cortex, subcortical gray matter, and germinal matrix layers to ventriculomegaly (Katz et al 1996), polymicrogyria, cerebellar hemorrhage, and hypoplasia of the cerebellar hemispheres and vermis (Glenn et al 2007, Nagel et al 2007, Pistorius et al 2008). In animal models, fetal parvovirus infection is associated with destruction of the external granular layer and cerebellar hypoplasia.

**Fetal errors of metabolism**

Inborn errors of metabolism in the fetus are usually due to enzyme or co-factor deficiencies.

These conditions may exert injurious effects on the developing brain through accumulation of neurotoxic by-products or a deficiency in substances essential for normal brain development (Nissenkorn et al 2001). Broadly speaking, the neuropathology in these conditions may reflect disruption of cellular energetics, disturbed development of the cell membrane, and abnormal signaling between cells. The fetal brain lesions may manifest in several ways. First, the metabolic defect may cause ‘acquired’ disruption of brain development or destructive lesions, often in combination. In certain conditions (e.g. sulfite oxidase deficiency, nonketotic hyperglycinemia, pyruvate dehydrogenase deficiency) this combination of developmental disruption and encephaloclastic changes may be prominent (Dobyns 1989, Schiaffino et al 2004).

A broad range of nervous system malformations (Nissenkorn et al 2001, Prasad et al 2007, Prasad et al 2009) has been described in infants with inborn metabolic errors. Certain developmental lesions are more common in certain metabolic conditions. This reflects a stage-dependent interaction between the ‘product’ of the metabolic defect and the concurrent events in brain development. Malformations of the developing nervous system from the early stages of neurulation, through prosencephalic and callosal development, to the late stages of cerebral cortical development have been described in children with inborn errors of metabolism.

All phases of brain development during the fetal period (rapid neuronal proliferation, differentiation and neuronal migration, and the synaptically mediated development of normal circuitry) are, to some extent, energy dependent. It is therefore not surprising that *inborn disturbances in energy metabolism*, such as pyruvate dehydrogenase deficiency (Shevell et al 1994, Nissenkorn et al 2001) and mitochondrial respiratory chain defects (Shevell et al 1994, Rotig and Munnich 2003, von Kleist-Retzow et al 2004).
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2003, Sarnat and Marin-Garcia 2005), are associated with a spectrum of developmental brain anomalies. In these cases intrauterine somatic and cerebral growth restriction is common. White matter lesions, often cystic, ranging from germinal matrix and other periventricular cysts to extensive cystic encephalopathy with calcifications have been described, together with ventriculomegaly and callosal dysgenesis (Samson et al 1994). Gray matter lesions have included cerebral cortical malformations such as polymicrogyria and heterotopias in these disorders of energy metabolism, as have a variety of cerebellar lesions, including cerebellar (von Kleist-Retzow et al 2003) and pontocerebellar hypoplasia (Lincke et al 1996), and ‘Dandy–Walker malformation’. Callosal dysgenesis may be seen in a wide variety of inborn metabolic disorders including pyruvate dehydrogenase deficiency, nonketotic hyperglycinemia, as well as mitochondrial and peroxisomal disorders. White matter lesions may occur as areas of cystic destruction and/or hypomyelination due to cytotoxic death of oligodendrocyte precursors with subsequent failure of myelination.

Certain inborn metabolic conditions exert their effect on brain development by disrupting normal cell-to-cell signaling. One example is the Smith–Lemli–Opitz syndrome, a multisystem dysmorphic condition resulting from 7-dehydrocholesterol reductase deficiency (Porter 2003, 2008), and hence very low cholesterol levels. The SHH gene plays a central role in development of the brain, cranium, face, and other organs (Opitz et al 1987, Penchasazadeh 1987, Porter 2008). The effect of SHH is mediated by a cholesterol-dependent gene product that is critical for cell–cell membrane signaling (Ingham 2001). Insufficient cholesterol results in failed SHH signaling and results in the lesions described in SLOS, such as craniofacial (e.g. cyclopia and cebocephaly) and forebrain development (failure of cerebral prosencephalic ‘cleavage’ or holoprosencephaly) (Porter 2003).

Congenital disorders of glycosylation (CDG) result in multisystem malformation syndromes including the brain and face (Krasnewich and Gahl 1997, Baric et al 1998). The most common subtype, CDG type 1a, is associated with cerebellar hypoplasia, hypotonia, fetal hypokinesia, and intellectual disability (Krasnewich and Gahl 1997, Baric et al 1998), as well as inverted nipples, aberrant fat distribution, and disturbed coagulation and endocrine function (Baric et al 1998). An associated cardiomyopathy may result in non-immune hydrops fetalis (Hertz-Pannier et al 2006, van de Kamp et al 2007, Malhotra et al 2009). Peroxisomal disorders in the Zellweger syndrome spectrum are associated with the whole spectrum of neuronal migration anomalies including lissencephaly, pachgyria, polymicrogyria, and heterotopias, as well as callosal hypoplasia and cerebellar anomalies (Fig. 1.10) (Volpe and Adams 1972, Barkovich and Peck 1997). Nonketotic hyperglycinemia is associated with callosal dysgenesis, dysmyelination, and cortical anomalies (Press et al 1989, Paupe et al 2002).

**Conclusions**

The fledgling clinical field of fetal neurology has advanced dramatically in recent years. Factors playing a major role in these developments have been the advances in fetal imaging and the accelerated understanding of neurogenetic mechanisms underlying normal and abnormal fetal brain development. Advances in these areas have provided invaluable insights into the phenotype–genotype associations in fetal neurology. Likewise, as highlighted in this chapter, a broad

![Fig. 1.10](image_url) Cytomegalovirus encephalitis in a 36-week gestation infant showing striking white matter abnormality and cortical malformation, with prominent polymicrogyria particularly in the fronto-parietal and Sylvian regions: axial (a) and coronal (b) T2-weighted MRI.
spectrum of environmental influences may affect brain development. The ability of advanced in vivo fetal brain imaging to detect and measure the effect of these ‘insults’ on the developing brain will, in future, be critical for the design of clinical trials aimed at preventing irreversible derangements in brain development.

REFERENCES


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