# 6

# Hypoxic-Ischemic Encephalopathy: Biochemical and Physiological Aspects

Hypoxic-ischemic encephalopathy in the perinatal period is characterized by neuropathological and clinical features that constitute an important portion of neonatal neurology. In order to understand those features, which are discussed in Chapters 8 and 9, it is necessary to be cognizant of the biochemical and physiological derangements that lead to the structural and functional manifestations of this encephalopathy. In this chapter I deal with these derangements in detail, on a background of the normal biochemistry and physiology, the latter largely circulatory, of the perinatal brain. Most of what we know is based on experimental data; however, the advent of improved techniques, particularly in the areas of circulatory physiology and magnetic resonance (MR) spectroscopy, and the application of these techniques to the clinical setting, have provided information demonstrating the relevance of the lessons learned in the laboratory to the human situation.

## BIOCHEMICAL ASPECTS
## Major Pathogenetic Themes

The unifying disturbance to neural tissue in hypoxic-ischemic encephalopathy is a deficit in oxygen supply. Perinatal brain can be deprived of oxygen by two major pathogenetic mechanisms: *hypoxemia,* which is a diminished amount of oxygen in the blood supply, and *ischemia,* which is a diminished amount of blood perfusing the brain. The balance of experimental and clinical data (see later discussion and Chapter 8) leads to the conclusion that *ischemia is the more important of these two forms of oxygen deprivation.* Thus deprivation of *glucose as well as oxygen* is crucial in the genesis of injury. Moreover, the period of *reperfusion* now has been shown clearly to be the time of occurrence of many, if not most, of the deleterious consequences of ischemia on brain metabolism and, ultimately, structure (see later discussion). In most instances, during the perinatal period, hypoxemia or ischemia or both occur as a result of *asphyxia,* which refers to impairment in the exchange of respiratory gases, oxygen and carbon dioxide. Thus in asphyxia, the major additional feature is hypercapnia, which results in a number of other metabolic (e.g., additional acidosis) and physiological (e.g., initial increase in cerebral blood flow) effects. In the following sections, I discuss the biochemical changes in brain associated with hypoxemia, ischemia, and asphyxia, with an emphasis on carbohydrate and energy metabolism. The manner in which these biochemical changes are affected by other perinatal factors, e.g., the status of carbohydrate metabolism at the time of the insult, the state of brain maturation, and the process of birth, also is described. A final section synthesizes the burgeoning literature on the mechanism of cell death with oxygen deprivation and focuses on the critical importance of biochemical events beyond glucose and energy metabolism. Particular roles of increase in extracellular excitatory amino acids, cytosolic calcium, and generation of free radicals are emphasized.

## Normal Carbohydrate and Energy Metabolism

Because glucose and oxygen are the principal driving forces for energy production in brain, the major biochemical effects of well-established oxygen deprivation are exerted at the levels of carbohydrate and energy metabolism. A brief review of these areas of metabolism is appropriate here (Fig. 6-1) (see also Chapters 12 and 19). Detailed discussions are available from other, more specialized sources.[1-11]

### *Glucose Uptake*

Glucose from blood is taken up by the brain through a process of carrier-mediated, facilitated diffusion that allows transport of glucose faster than would be expected by simple diffusion. Specific glucose transporter proteins are involved (see Chapter 12). The transport process, however, is not energy dependent and thus differs in that critical fashion from active transport. Glucose transport across the blood-brain barrier utilizes the heavily glycosylated form of the facilitative glucose transporter protein, GLUT1 (55 kDA). Transport across glial membranes is facilitated by the lower molecular form of GLUT1 (45 kDA), and transport across the neuronal membrane is facilitated by GLUT3. The levels of these proteins are relatively low in the immature brain and are limiting to glucose transport and utilization.[7,8,11-16] Consistent with the experimental findings, elegant studies of human infants by positron emission tomography (PET) show that the cerebral metabolic rate for glucose in brain of preterm newborn infants is approximately one third of that in brain of adults and that this difference relates to a diminished transport capacity rather than a diminished affinity of the transporters for glucose.[17]

#### *Formation of Glucose-6-Phosphate*

Glucose in brain is phosphorylated to glucose-6-phosphate; the enzyme involved is hexokinase (Fig. 6-1). The activity of hexokinase is linked to glucose uptake by the

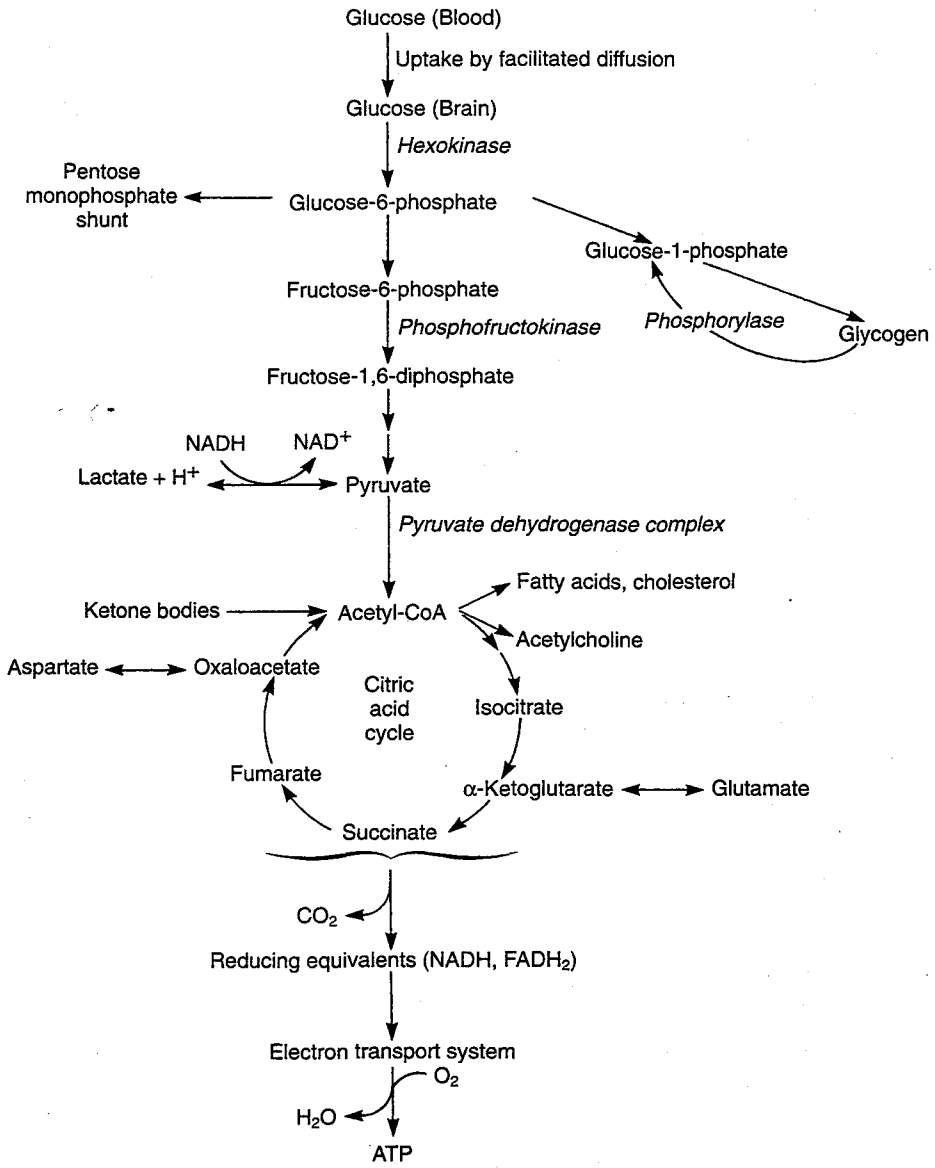


**Figure 6-1** Major features of carbohydrate and energy metabolism in brain. See text for details. *ATP,* Adenosine triphosphate; *NADH,* nicotinamide adenine dinucleotide; *NAD+,* oxidized nicotinamide adenine dinucleotide.

cell and is inhibited by the product of the reaction, glucose-6-phosphate. The activity of this enzyme is also lower in neonatal versus adult rat brain.[7,8,18] Glucose-6-phosphate is a pivotal metabolite in glucose metabolism, with three major fates: (1) glycolysis and, ultimately, energy production; (2) glycogen synthesis; and (3) the pentose monophosphate shunt for synthesis of lipids (via formation of reduced nicotinamide adenine dinucleotide phosphate [NADPH]) and nucleic acids.

### *Glycogen Metabolism*

Glycogen is found in relatively small concentrations in brain but represents an important storage form of carbohydrate. Glycogen synthesis proceeds through glucose-1-phosphate and then to glycogen via glycogen synthetase. Glycogen breakdown to glucose-1-phosphate via phosphorylase, and then to glucose-6-phosphate via phosphoglucomutase, is an important mechanism for generating oxidizable glucose (Fig. 6-1). Brain phosphorylase is activated by cyclic adenosine monophosphate (AMP), and levels of cyclic AMP are elevated by certain hormones, e.g., epinephrine. Epinephrine release is accentuated sharply with hypoxic, ischemic, and asphyxial insults. Although glycogen is broken down in perinatal brain under certain circumstances, the capacity of the perinatal degradative system, at least in the rodent brain, is considerably less than in the adult.[19,20]

### *Glycolysis*

The major portion of glucose-6-phosphate enters the glycolytic pathway to result ultimately in the formation of pyruvate. The major control step involves the conversion of fructose-6-phosphate to fructose-1,6-diphosphate; the rate-limiting enzyme involved is phosphofructokinase (Fig. 6-1). The major mechanism of control of this enzyme is via *allosteric* effects—involving conformational changes of component peptides—and thus are very rapid in onset. The activity of phosphofructokinase is inhibited by adenosine triphosphate (ATP), phosphocreatine (PCr), and low pH and activated by

$$\text{Glucose} + 2\,NAD^+ + 2\,ADP + 2\,P_i \longrightarrow 2\,\text{Pyruvate} + 2\,NADH + 2\,ATP$$

$$2\,\text{Pyruvate} + 2\,NADH + 36\,ADP + 36\,P_i + 6\,O_2 \longrightarrow 2\,NAD^+ + 6\,CO_2 + 44\,H_2O + 36\,ATP$$

**SUM:**

$$\text{Glucose} + 38\,ADP + 38\,P_i + 6\,O_2 \longrightarrow 6\,CO_2 + 44\,H_2O + \boxed{38\,ATP}$$

**Figure 6-2** Energy production from glucose under *aerobic* conditions. Contrast with production under anaerobic conditions (Fig. 6-6). *ADP,* Adenosine diphosphate; *ATP,* adenosine triphosphate; *NADH,* nicotinamide adenine dinucleotide; *NAD+*, oxidized nicotinamide adenine dinucleotide; *Pi,* inorganic phosphate.

adenosine diphosphate (ADP), inorganic phosphorus (Pi), cyclic AMP, and ammonium ion.

Under *aerobic* conditions the major product of glycolysis is pyruvate, which enters the mitochondrion and is converted via the pyruvate dehydrogenase complex to acetyl coenzyme A (acetyl-CoA) (Fig. 6-1). This mitochondrial enzyme is inhibited by an increase in the ATP/ADP ratio and is activated by a decrease in this ratio. Acetyl-CoA is utilized for fatty acid and cholesterol biosynthesis and for acetylcholine synthesis but particularly for entry into the citric acid cycle for energy production.

***Citric Acid Cycle and Electron Transport Chain***

Mitochondrial acetyl-CoA enters the citric acid cycle and undergoes oxidation to carbon dioxide (Fig. 6-1). The rate-limiting step is the conversion of isocitrate to alpha-ketoglutarate, catalyzed by the enzyme isocitrate dehydrogenase. A critical allosteric regulator of this enzyme is the ratio of ATP to ADP; an increase in the ratio causes a decrease in activity of the cycle, and a decrease in the ratio causes an increase in activity of the cycle. The electrons or reducing equivalents (nicotinamide adenine dinucleotide [NADH], flavin adenine dinucleotide [FADH]) generated by the citric acid cycle next enter the electron transport system.

The transport of electrons takes place through a multimember chain of electron carrier proteins and is associated with release of free energy, which is used to generate ATP from ADP and Pi. The free energy, in essence, is "captured" in this high-energy phosphate bond. ATP is generated at three steps in the scheme, and since the final electron acceptor is oxygen, the process is called "oxidative phosphorylation." Molecular oxygen is reduced, and water is the final product formed. The ATP generated by the citric acid cycle and the electron transport system is transported from the mitochondrion via a specific carrier and ultimately is utilized in brain primarily for *transport processes* (especially of ions and neurotransmitters for impulse transmission and for prevention of dangerous increases thereof (e.g., extracellular glutamate, cytosolic calcium) and for *synthetic processes* (especially of neurotransmitters, but also lipids and proteins, particularly in developing brain). The principal ions involved in ATP consumption are sodium, potassium, and calcium; in adult brain (under normal conditions) approximately 50% to 60% of ATP is utilized for maintenance of ion gradients of these three ions, primarily sodium and potassium.[1]

## Summary

The concerted action of glycolysis, the citric acid cycle, and the electron transport system, operative under aerobic conditions, results in the formation of 38 molecules of ATP for each molecule of glucose oxidized (Fig. 6-2). The glycolytic portion of the pathway occurs in the cytosol and generates only 2 of the 38 molecules of ATP; the bulk of the ATP is generated in the mitochondrial portion of the pathway, which begins with pyruvate. The ATP generated is transported from the mitochondrion via a specific carrier and is utilized in brain for two major purposes: *transport* and *synthetic processes*. Quantitatively, the most important transport processes involve ions in neurons for impulse transmission and maintenance of calcium homeostasis. Synthetic processes are important in developing brain and involve neurotransmitters, structural and functional proteins, and membrane lipids.

**TABLE 6-1**

**Effects of Hypoxemia on Carbohydrate and Energy Metabolism**

| |
|---|
| ↑ Glucose influx to brain |
| ↑ Glycogenolysis |
| ↑ Glycolysis |
| ↓ Brain glucose |
| ↑ Lactate production and tissue acidosis |
| ↓ Phosphocreatine |
| ↓ Adenosine triphosphate |

## Effects of Hypoxemia on Carbohydrate and Energy Metabolism

### Major Changes

Hypoxemia is accompanied by a number of effects on carbohydrate and energy metabolism in brain[7,8,10,21] (Table 6-1), which are understandable when viewed in the context of the normal metabolism just reviewed. Although it is likely that lack of oxygen is the major pathogenetic factor in these changes, it is difficult to produce hypoxemia experimentally without also causing other major metabolic changes that either accompany the hypoxemic insult or occur as a consequence of the insult, e.g., hypercapnia, acidosis, and hypotension. In most studies, however, these other changes either are prevented or are documented.

The quantitative and temporal aspects of the biochemical changes associated with a severe hypoxemic or anoxic insult, i.e., nitrogen breathing, in the newborn mouse are depicted in Figs. 6-3 to 6-5.[22] The earliest significant changes are a decrease in brain glycogen, an elevation in lactate, and a decrease in PCr. These are followed by a decrease in brain glucose and, finally, ATP. The changes appear to reflect principally the impaired production of high-energy phosphate, secondary to failure of the coupled mitochondrial system of the citric acid cycle and electron transport chain, in turn a consequence of the lack of the ultimate electron acceptor, oxygen. In response to the anaerobic state, glycolysis becomes the sole source of ATP production, and because lactate is the principal product of anaerobic glycolysis, only two molecules of ADP are generated for each molecule of glucose metabolized (Fig. 6-6). This number is clearly a serious difference from the 38 molecules gen-

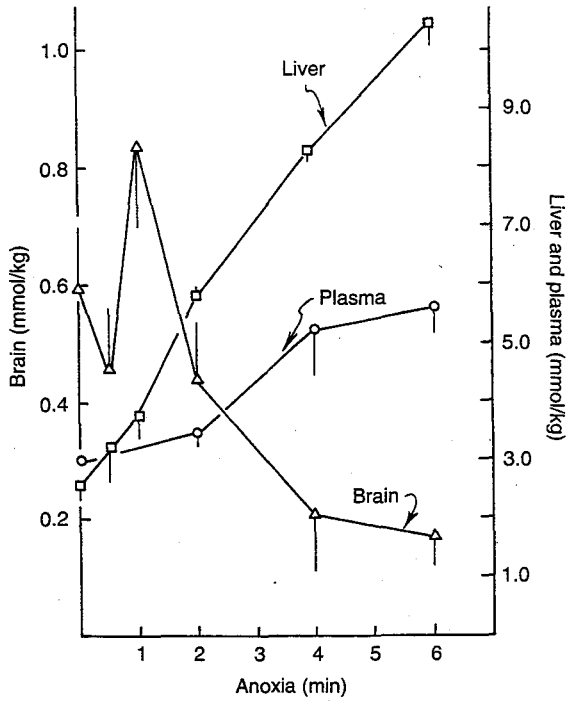


**Figure 6-3** Biochemical effects of hypoxemia. Concentrations of glucose in brain, liver, and plasma of newborn mice as a function of duration of anoxia ($N_2$ breathing). (From Holowach-Thurston J, Hauhart RE, Jones EM: *Pediatr Res* 7:691, 1973.)

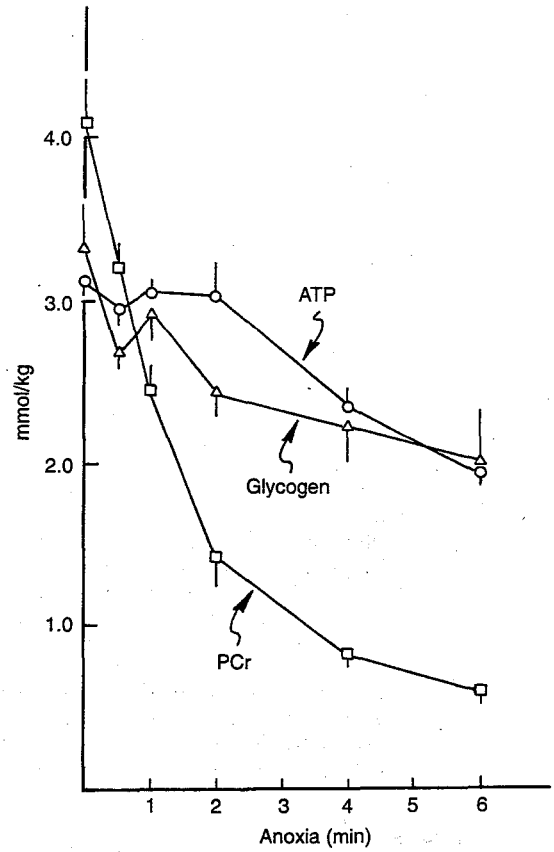


**Figure 6-4** Biochemical effects of hypoxemia. Concentrations of ATP, PCr, and glycogen in brain of newborn mice as a function of duration of anoxia ($N_2$ breathing). *ATP,* Adenosine triphosphate; *PCr,* phosphocreatine. (From Holowach-Thurston J, Hauhart RE, Jones EM: *Pediatr Res* 7:691, 1973.)

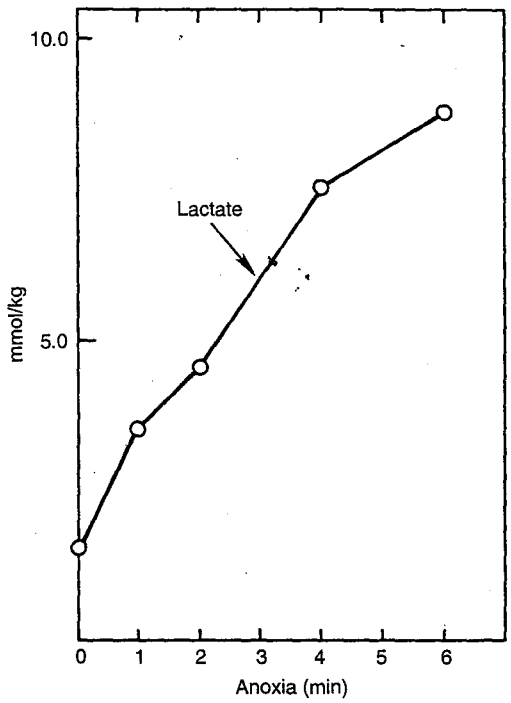


**Figure 6-5** Biochemical effects of hypoxemia. Concentrations of lactate in brain of newborn mice as a function of duration of anoxia ($N_2$ breathing). (Redrawn from Holowach-Thurston J, Hauhart RE, Jones EM: *Pediatr Res* 7:691, 1973.)

erated under aerobic conditions (Fig. 6-2). Glycolysis is accelerated fivefold to tenfold, and an attempt to meet this enhanced need for glucose is made by a combination of glycogenolysis and increased net uptake of glucose from blood.[22] (Glycogen contributes approximately one third of the cerebral energy supply under these conditions.[23]) Despite this acceleration, brain energy demands cannot be met, and ATP levels begin to fall after 2 minutes and decrease by 30% after 6 minutes.

The relationship between arterial oxygen delivery and brain PCr levels (expressed as the ratio of PCr to Pi determined by MR spectroscopy) has been clarified in studies of the neonatal dog.[24,25] Thus a crucial threshold decrease in PCr/Pi ratio of 50% occurred when arterial oxygen pressure decreased to 12 mm Hg (approximate arterial oxygen saturation, 20%).[25] The importance of the 50% decrease in PCr/Pi ratio relates to the finding in the neonatal piglet that at this level, brain lipid peroxidation and impaired $Na^+$, $K^+$-ATPase activity occur.[26] Interestingly, the critical value of $PaO_2$ required to lead to the 50% decline in the PCr/Pi ratio in the neonatal dog (12 mm Hg) was higher at 7 to 21 days (17 mm Hg) and higher in the adult (23 mm Hg).[25] This lower threshold value of $PaO_2$ in the neonatal animal correlated with in vitro data showing more efficient oxygen extraction in the neonatal animals (see later discussion). At any rate, it is clear that *marked* hypoxemia is required to produce serious changes in brain energy state in the neonatal animal.

$$\text{Glucose} + 2\,\text{ADP} + 2\,P_i \longrightarrow 2\,\text{Lactate}^- + 2\,H^+ + \boxed{2\,\text{ATP}}$$

**Figure 6-6** Energy production from glucose under *anaerobic* conditions. Contrast with production under aerobic conditions (Fig. 6-2). *ADP,* Adenosine diphosphate; *ATP,* adenosine triphosphate; *Pi,* inorganic phosphate.

Studies of the effect of hypoxemia on brain energy metabolism in the immature rat brain have delineated a particular window of vulnerability, characterized by *greater* vulnerability in the second postnatal week, comparable to the human brain at term, than in the first postnatal week, comparable to the human premature brain.[27] Thus the most marked declines in PCr and nucleoside triphosphates, defined by MR spectroscopy, occurred in the second postnatal week. This period of heightened vulnerability corresponds with the period of maximal susceptibility to excitotoxic neuronal injury and to epileptogenic effects of hypoxemia,[28-30] as well as with the period of maximal expression of specific excitatory amino acid receptors, incomplete maturation of inhibitory transmission, relatively low levels of $Ca^{2+}$ binding proteins, and incomplete maturation of $Na^+$-$K^+$ ATPase levels (see later discussion).[31] Taken together, these data suggest that the vulnerability of the immature rat in the second versus the first week of life relates to the increased propensity to develop with hypoxia, a hyperexcitable, hypermetabolic state in neurons, which leads to more marked declines in high-energy phosphates because of increased utilization. These considerations could help explain the greater likelihood of *neuronal* injury with hypoxia in the term brain than premature brain of the human.

Studies in the newborn dog have defined the *regional* changes in glucose and high-energy metabolism.[32] Thus animals subjected to acute hypoxemia ($Po_2$ of approximately 12 mm Hg) and studied by the autoradiographic 2-[$^{14}$C]deoxyglucose technique exhibited increased glucose utilization in most gray matter structures and every white matter structure. Moreover, the degree of hypoxemia was sufficient to cause accumulation of lactate in brain in both gray and white matter, but only in white matter did a decline in energy state occur (Table 6-2). *Thus it appears that anaerobic glycolysis with its accelerated glucose utilization was capable of preserving the energy state in gray matter but not in white matter.* Moreover, the fact that glucose levels declined more drastically in white matter than in gray matter (Table 6-2) suggests that glucose influx could not meet the increased demands for glucose in white matter. That the rate of glucose metabolism, in fact, was *limited by glucose influx from blood* is supported by the demonstration that local cerebral blood flow increased insignificantly to white matter but dramatically to gray matter.[33] The apparently limited vasodilatory capacity in white matter is discussed in the section on cerebral blood flow, but *this imbalance between glucose needs and glucose delivery may contribute to the propensity of neonatal cerebral white matter to hypoxic injury.*

### *Mechanisms*

The mechanisms for the biochemical effects relate to several factors (Table 6-3). ATP levels are preserved initially at the

**TABLE 6-2**

**Substrate Concentrations in Brain of Hypoxic Puppies (mmol/kg)**

| TISSUE | PHOSPHOCREATINE | ATP | GLUCOSE | LACTATE |
|---|---|---|---|---|
| **CONTROL** | | | | |
| Parietal cortex | 2.74 ± 0.08 | 2.30 ± 0.08 | 2.38 ± 0.25 | 1.08 ± 0.09 |
| Subcortical white matter | 1.85 ± 0.22 | 1.64 ± 0.06 | 2.14 ± 0.13 | 1.34 ± 0.07 |
| **HYPOXIA** | | | | |
| Parietal cortex | 2.56 ± 0.06 | 2.26 ± 0.02 | 1.64 ± 0.28 | 12.0 ± 1.4 |
| Subcortical white matter | 1.09 ± 0.19 | 1.40 ± 0.09 | 0.28 ± 0.04 | 13.4 ± 1.8 |

*ATP,* Adenosine triphosphate.
Data from Duffy TE, Cavazzuti M, Cruz NF, Sokoloff L: *Ann Neurol* 11:233-246, 1982.

**TABLE 6-3**

**Major Mechanisms for Biochemical Effects of Hypoxemia on Carbohydrate and Energy Metabolism**

**↑ GLUCOSE INFLUX TO BRAIN**
Link to accelerated glucose utilization

**↑ GLYCOGENOLYSIS**
Phosphorylase activation (↑ cAMP)

**↑ GLYCOLYSIS**
Phosphofructokinase activation (↑ cAMP, ↑ ADP, ↑ $P_i$, ↓ ATP, ↓ phosphocreatine)
Hexokinase activation (↑ cAMP)

**↓ BRAIN GLUCOSE**
Glucose utilization > glucose influx

**↑ LACTATE (AND $H^+$)**
Anaerobic glycolysis
Impaired utilization of pyruvate (via mitochondrial citric acid cycle–electron transport system)

**↓ PHOSPHOCREATINE**
↑ $H^+$ production via anaerobic glycolysis
↓ ATP, ↑ ADP

**↓ ATP**
↓ Oxidative phosphorylation

*ADP,* Adenosine diphosphate; *ATP;* adenosine triphosphate; *cAMP,* cyclic adenosine monophosphate; $P_i$, inorganic phosphate.

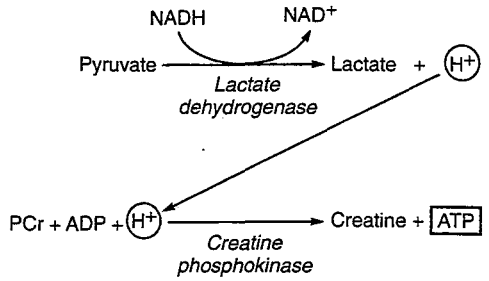


**Figure 6-7** Link between lactate production and hydrolysis of phosphocreatine. ATP formation is the result. *ADP,* Adenosine diphosphate; *ATP,* adenosine triphosphate; *NADH,* nicotinamide adenine dinucleotide; *NAD+,* oxidized nicotinamide adenine dinucleotide; *PCr,* phosphocreatine.

expense of PCr. The *initial* fall in PCr, the principal storage form of high-energy phosphate in brain, relates primarily to the shift in the creatine phosphokinase reaction induced by the hydrogen ion generated with lactate formation by anaerobic glycolysis (Fig. 6-7). Later the creatine phosphokinase reaction is driven by elevated concentrations of both ADP and hydrogen ion. The *initial acceleration of glycolysis and the glycogenolysis* may relate to primarily a rise in cyclic AMP levels in brain, demonstrated to be approximately threefold in the rat after only 30 seconds of nitrogen breathing.[34] Cyclic AMP leads to activation of phosphorylase for glycogenolysis and of phosphofructokinase (and hexokinase) for glycolysis.[5,35,36] Further activation of phosphofructokinase and, hence, glycolysis occurs as ATP levels fall and ADP and Pi levels rise. The fall in brain glucose occurs because the continued excessive utilization of glucose via anaerobic glycolysis, a most inefficient means of generating ATP, outstrips the capacity for glucose delivery from blood. Indeed, after 6 minutes, brain glucose levels had decreased by more than 70% while blood glucose levels had increased by nearly 100% (Fig. 6-3).[22] Thus blood glucose level no longer reflected the brain glucose level.

The accumulation of lactate and the associated hydrogen ion is worthy of additional emphasis because this accumulation *initially* is a *beneficial* adaptive response to oxygen deprivation, but *later* can be a serious *deleterious* factor. Thus, initially, the tissue acidosis leads to the generation of ATP from PCr (because of the shift in the creatine phosphokinase reaction) and also to an increase in cerebral blood flow (because of the local effect of elevated perivascular hydrogen ion concentration on vascular smooth muscle). However, with progression of lactate formation, severe tissue acidosis develops, and three deleterious effects ensue. The first is an impairment of vascular autoregulation and the potential for ischemic injury to brain when cerebral perfusion pressure falls (e.g., secondary to the often-associated myocardial injury). Second, phosphofructokinase activity is inhibited by low pH, and thus the brain's remaining source of ATP, i.e., glycolysis, is eliminated. Finally, advanced tissue acidosis leads directly to cellular injury and ultimately necrosis. A correlation between brain lactate concentration and cellular injury has been demonstrated in primate brain (see next section). Moreover, a correlation between the decline in fetal arterial pH with hypoxemia (primarily related to lactate) with the subsequent decline in cerebral oxygen consumption in the near-term fetal sheep has been shown.[37] Values of pH of 6.86, attained after 7.2 hours of hypoxemia of 13 to 14 mm Hg, induced from a baseline of 21 mm Hg, caused a more than 50% decline in cerebral oxygen consumption.

**TABLE 6-4**

**Effects of Ischemia on Carbohydrate and Energy Metabolism**

| |
|---|
| ↓ Glucose influx to brain |
| ↑ Glycogenolysis |
| ↑ Glycolysis |
| ↓ Brain glucose |
| ↑ Lactate production and tissue acidosis |
| ↓ Phosphocreatine |
| ↓ Adenosine triphosphate |
| ? Postischemic hypoperfusion |

## Effects of Ischemia on Carbohydrate and Energy Metabolism

### *Major Changes*

Ischemia is accompanied by effects on carbohydrate and energy metabolism in brain (Table 6-4) that exhibit important similarities to those observed with hypoxemia. Certain differences occur with ischemic insults (see later section). The most frequently utilized models with perinatal animals have included decapitation, severe hypotension, or occlusion of blood vessels supplying the cranium.[11,19,20,38-46] The most widely used model in the past decade has involved the Levine model of unilateral carotid artery ligation followed by systemic hypoxemia for generally 1 to 3 hours, a procedure that results in hypoxic-ischemic neuronal and white matter injury.[11] This clinically relevant model is discussed in the next major section.

The quantitative and temporal aspects of the biochemical changes associated with decapitation in the newborn mouse are depicted in Fig. 6-8.[20] In the neonatal piglet, studied by MR spectroscopy during hemorrhagic hypotension or carotid occlusion, similar decreases in high-energy phosphates and pH could be demonstrated in the intact animal.[43,46-49] Moreover, comparable decreases were observed in newborn lambs studied by MR spectroscopy after 10 to 15 minutes of ischemia produced by bilateral carotid occlusion.[42] Phosphorus and proton MR spectroscopy have been utilized in term newborns with apparent hypoxic-ischemic insults (related to perinatal asphyxia) to show both elevations in levels of lactate and declines in levels of high-energy phosphates in the hours after the insult (see next section).[50-54]

#### *Mechanisms*

The biochemical changes are compatible with accelerated anaerobic glycolysis with lactate accumulation and glycogenolysis (Fig. 6-8, *A*). Particular importance for an increased capacity for glucose uptake in the acceleration of glucose utilization has been shown by the demonstration of elevation in the levels of the glucose transporter proteins, GLUT1 (55 kDA) and GLUT3, for transport of glucose across the blood-brain barrier and the neuronal membrane respectively, in the brain of hypoxic-ischemic 7-day-old rat pups in the first 4 hours after the insult.[16] As with hypoxemia, a role for cyclic AMP in the induction of the glycolysis and glycogenolysis is suggested by marked rises (thirteenfold) in the levels of this mononucleotide in the first minutes after the onset of ischemia.[55] Initially, ATP levels are preserved at the expense of

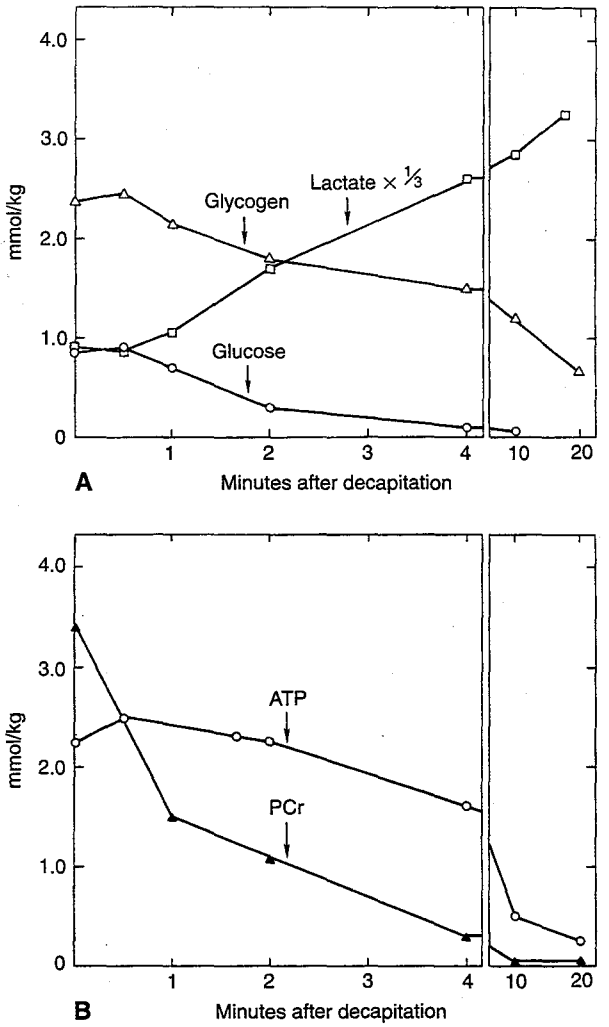


**Figure 6-8** Biochemical effects of ischemia. **A,** Concentrations of glycogen, glucose, and lactate and, **B,** concentrations of ATP and PCr after decapitation of newborn mice. *ATP,* Adenosine triphosphate; *PCr,* phosphocreatine. (Redrawn from Holowach-Thurston J, McDougal DB Jr: *Am J Physiol* 216: 348, 1969.)

PCr (Fig. 6-8, *B*). Brain glucose concentrations fall more severely than with the anoxia of nitrogen breathing; after 2 minutes of ischemia, glucose had decreased markedly whereas only a modest decrease occurred with nitrogen breathing after this time (compare Fig. 6-8 with Fig. 6-3). Of course, this difference relates to the impairment of cerebral blood flow and therefore glucose supply with ischemia. An additional difference between ischemia and hypoxemia, not shown in Fig. 6-8, is the more drastic change in intracellular pH with ischemia, because the circulation is interrupted.[21] The more severe tissue acidosis obtains because the impaired cerebral circulation results in (1) diminished clearance of accumulated lactate and (2) diminished buffering of tissue $CO_2$ via the bicarbonate buffering system.[21]

#### *Postischemic Relative Hypoperfusion*

Earlier studies of a variety of experimental models of ischemia in adult animals demonstrated, *after* the insult, i.e., with reperfusion, a period in which cerebral blood flow does not appear to keep pace with the renewed demands of energy

metabolism.[56-58] In some cases the impairments in cerebral blood flow were immediate, often termed the "no-reflow phenomenon," and in others, delayed, usually termed "secondary" or "delayed" hypoperfusion.[57,59-64] Mechanisms postulated for these states of hypoperfusion have included intravascular obstruction by platelets or other blood elements, constriction of arterioles by elevations of extracellular potassium, swelling of perivascular astrocytes or of endothelial cells (or both), and elevations of such vasoactive materials as various prostanoids (see later discussion).[56,60,65-70] These several events are well documented in experimental models of ischemia, as discussed in other sections of this chapter.

Only in recent years has the issue of postischemic defects in cerebral perfusion in *perinatal animals* been addressed. These defects are discussed later in this chapter in relation to changes in cerebral blood flow. *Suffice it to say here that whether such postischemic hypoperfusion plays a major role in the genesis of the structural injury with perinatal ischemic insults is unclear.*

## Effects of Hypoxic-Ischemic Insults on Carbohydrate and Energy Metabolism

The combination of hypoxemia and ischemia, i.e., hypoxic-ischemic insult, is most relevant to the situation in vivo in the human fetus and newborn, and the effects of such insult on carbohydrate and energy metabolism have been studied in detail in experimental models.[5,7,8,11,16,46,71-90] The biochemical features relative to carbohydrate and energy metabolism bear many similarities to those recorded previously for purely hypoxemic or ischemic insults (see earlier discussions). In the most commonly utilized model, the hypoxic-ischemic insult is produced in the 7-day-old rat (approximately analogous to a 34-week human newborn brain) by a combination of unilateral carotid occlusion and breathing of a low-oxygen (usually 8%) gas mixture. The *importance of ischemia* in the genesis of the brain injury in this model has been demonstrated by the findings that (1) carotid ligation alone does not lead to a decrease in cerebral blood flow to the ipsilateral hemisphere, (2) the addition of the hypoxemia leads to marked disturbances in regional blood flow to the ipsilateral hemisphere, and (3) the topography of the injury to this hemisphere correlates closely with the topography of the decreases in regional cerebral blood flow.[73] Vannucci and co-workers have defined most clearly the major biochemical changes.[5,7,8,11,16,71,72,74,75] Thus high-energy phosphate levels begin to decline within minutes, with the reservoir form, PCr, falling first (Fig. 6-9).[5] Histologic evidence for brain injury becomes apparent after approximately 90 minutes. Glucose levels in brain fall dramatically, lactate levels increase, and pH declines as anaerobic glycolysis attempts to compensate for the decrease in high-energy phosphate levels. The increased lactate/pyruvate ratio in the cytosol is reflected in increased reduction (i.e., decrease) of the $NAD^+$/NADH ratio. Interestingly, the latter ratio is more oxidized in the mitochondrion because of the limitation in cellular substrate (glucose) supply. (This important limiting role of brain glucose is discussed in more detail later concerning brain carbohydrate status and hypoxic-ischemic injury.)

The particular importance of *ischemia* in the genesis of the deleterious effects of hypoxic-ischemic insults has

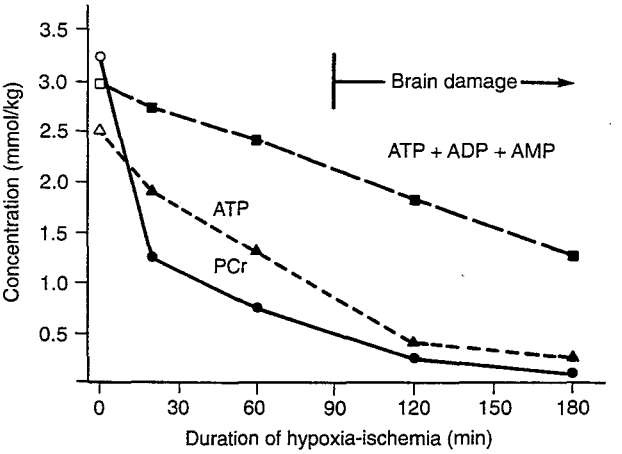


**Figure 6-9** Changes in cerebral high-energy phosphate reserves during hypoxia-ischemia in the immature rat. Seven-day-old postnatal rats were subjected to unilateral common carotid artery ligation followed by exposure to hypoxia with 8% oxygen at 37° C. Symbols represent means for ATP, PCr, and total adenine nucleotides (ATP + ADP + AMP). All values are significantly different from control (zero time point). Histological brain damage commences after 90 minutes of hypoxia-ischemia, with increasing severity thereafter. *ADP,* Adenosine diphosphate; *AMP,* adenosine monophosphate; *ATP,* adenosine triphosphate; *PCr,* phosphocreatine. (From Vannucci RC: *Pediatr Res* 27:317-326, 1990.)

been shown also in the fetal lamb and neonatal piglet.[45,78-80,91] In both animal models, marked hypoxemia did not result in brain injury unless hypotension supervened. In the piglet, hypotension appeared to be a particular consequence of cardiac dysfunction, and the latter was especially correlated with severe systemic acidosis. In the fetal lamb a pronounced decrease in brain glucose and in high-energy phosphate levels accompanied by an increase in lactate levels to as high as 16 to 24 mM were the principal biochemical effects on carbohydrate and energy metabolism. These effects were particularly pronounced in cerebral white matter (Table 6-5). *This regional predilection may be relevant to the propensity of white matter to exhibit injury with hypotension in the premature newborn* (see Chapter 8).

The temporal aspects of the changes in glucose and energy metabolism after hypoxic-ischemic insult in *the living animal* have been identified best by studies of the neonatal piglet with phosphorus and proton MR spectroscopy.[88-90] Thus, immediately after the insult, as expected, a marked increase in cerebral lactate levels and a marked decrease in high-energy phosphate levels were documented. High-energy phosphate levels recovered to baseline levels in 2 to 3 hours (Fig. 6-10); lactate levels improved but did not recover completely. Interestingly, a second decline in high-energy phosphate levels then occurred in the next 24 hours and was especially pronounced at 48 hours (Fig. 6-10). Importantly, this *"secondary energy failure"* and the earlier rise in cerebral lactate levels *have been documented in the human term newborn* subjected to apparent hypoxic-ischemic insult in the context of perinatal asphyxia (see Chapter 9).[51,52] Although the mechanisms underlying the secondary

**TABLE 6-5**

**Brain Metabolites in White Matter of Fetal Sheep Made Hypoxic With or Without Hypotension***

| FETAL CONDITION | WHITE MATTER INJURY | BRAIN METABOLITE† | | |
|---|---|---|---|---|
| | | LACTATE | PHOSPHOCREATINE | ADENOSINE TRIPHOSPHATE |
| Normoxic, normotensive | − | 3.2 | 0.7 | 0.7 |
| Hypoxic, normotensive | − | 9.9‡ | 0.5 | 0.9 |
| Hypoxic, hypotensive | + | 19.5‡ | 0.3‡ | 0.1‡ |

*Data for anterior white matter from Wagner KR, Ting P, Westfall MV, et al: *J Cereb Blood Flow Metab* 6:425-434, 1986.
†Concentrations are mmol/kg; values are rounded off.
‡$p < .05$ versus normoxic, normotensive.

energy failure are not known, they almost certainly include the cascade of biochemical mechanisms "beyond glucose and energy metabolism" discussed later. The severity of the "secondary energy failure" correlates closely with the degree of neuropathologic injury in the experimental studies and to the neonatal neurological features and the subsequent neurological deficits in the human studies (see Chapter 9).

The final result of the energy failure, particularly the secondary energy failure, is a cascade of events that includes accumulation of excitotoxic amino acids, cytosolic calcium, activation of phospholipases, generation of free radicals, and a series of related metabolic events that lead to cell death (see later discussion). The crucial mitochondrial disturbance that precipitates this cascade of deleterious events *persists* into the period *following* the termination of the insult (see later discussion). Indeed, these deleterious events occur to a major extent during this post-insult period. The particular vulnerability of the mitochondrion during and following ischemia is supported by biochemical and morphological data.[5,7,8,10,86,92]

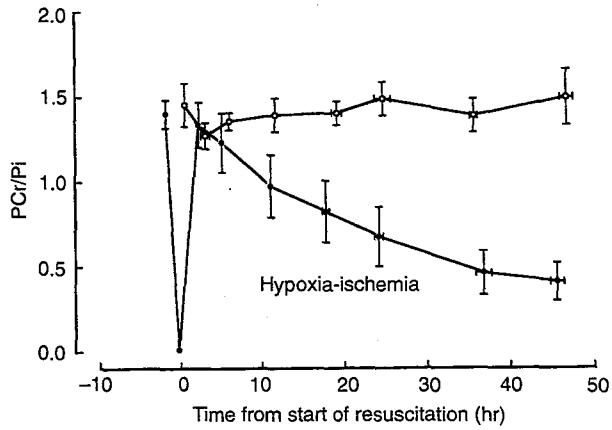


**Figure 6-10** High-energy phosphate levels in hypoxia-ischemia in brain of neonatal piglets. Note the sharp decline with the insult, followed by a recovery to baseline in 2 to 3 hours. *PCr,* Phosphocreatine; *Pi,* inorganic phosphate. (From Lorek A, Takel Y, Cady EB, Wyatt JS: *Pediatr Res* 36:699-706, 1994.)

## Effects of Asphyxia on Carbohydrate and Energy Metabolism

Asphyxia, rather than hypoxemia or ischemia or both, is the most common *clinical* insult in the perinatal period that results in the brain injury under discussion. Although hypoxemia and ischemia usually occur concurrently or in sequence with perinatal asphyxia, certain additional metabolic effects, particularly hypercapnia, are prominent. Most experimental studies of perinatal asphyxia have involved lambs and monkeys and have been concerned with changes in cerebral blood flow and with the neuropathology (see later sections on cerebral blood flow and Chapter 8 on neuropathology). Some work has provided useful information regarding the biochemical (as well as the physiological) effects in brain with neonatal asphyxia and is reviewed next.[91,93-97]

### *Major Changes*

Striking changes in biochemical, cardiovascular, cerebrovascular, and electrophysiological parameters were observed in neonatal dogs subjected to ventilatory standstill after paralysis with succinylcholine or curare.[98] *Survival* occurred in all animals after 10 minutes of asphyxia, in two thirds after 15 minutes of asphyxia, but in only one fourth after 20 minutes of asphyxia. Changes in *arterial blood gas levels and acid-base status* were dramatic. Thus after 2½ minutes of respiratory arrest, $Pa_{O_2}$ had fallen to 4 mm Hg, $Pa_{CO_2}$ had risen to 51 mm Hg (from control value of 35), and pH had fallen to 7.18 (from control value of 7.38). After 10 minutes, $Pa_{CO_2}$ was 100 mm Hg and pH was 6.79. *Cardiovascular* effects were also marked (Fig. 6-11); mean arterial blood pressure declined gradually to a low of 10 mm Hg after 14 minutes, and bradycardia was marked after only 4 minutes. *Cerebral perfusion,* assessed qualitatively by carbon black infusion, overall appeared to decline *pari passu* with mean arterial blood pressure, although diminutions were greatest in cerebral cortex and least in brain stem. This more severe affection of cerebral blood flow has been reproduced in other neonatal models of asphyxia (see later discussion). *EEG* demonstrated rapid deterioration (Fig. 6-11); between 1 and 2 minutes after the onset of asphyxia there was a distinct reduction in the amplitude and frequency, and by 2½ minutes the EEG was isoelectric. The occurrence of the isoelectric EEG did *not* correlate with any marked change in

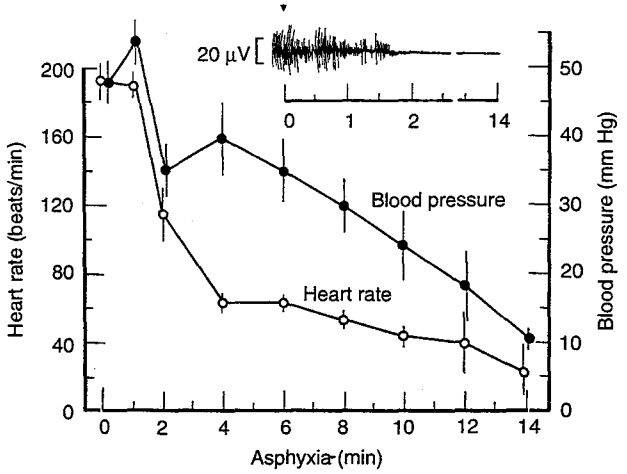


**Figure 6-11** Cardiovascular and EEG effects of asphyxia (respiratory arrest) in newborn dogs. A representative EEG during 14 minutes of asphyxia is shown in the upper right-hand portion of the figure; the arrow indicates the onset of respiratory arrest. (From Vannucci RC, Duffy TE: *Ann Neurol* 1:528, 1977.)

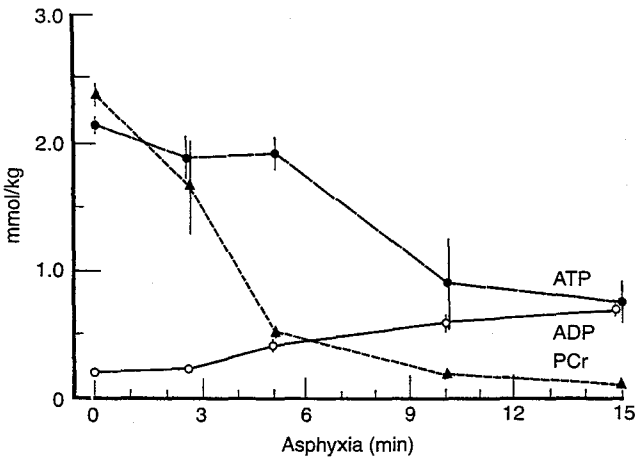


**Figure 6-13** Biochemical effects of asphyxia. Concentrations of adenosine triphosphate *(ATP)*, adenosine diphosphate *(ADP)*, and phosphocreatine *(PCr)* in brain of newborn dogs as a function of duration of asphyxia (respiratory arrest). (From Vannucci RC, Duffy TE: *Ann Neurol* 1:528, 1977.)

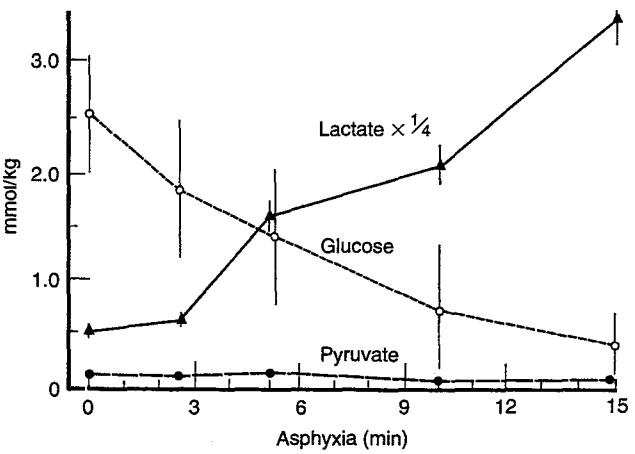


**Figure 6-12** Biochemical effects of asphyxia. Concentrations of glucose, pyruvate, and lactate in brain of newborn dogs as a function of duration of asphyxia (respiratory arrest). (From Vannucci RC, Duffy TE: *Ann Neurol* 1:528, 1977.)

cerebral perfusion nor with any measurable change in brain lactate or ATP levels.

*Biochemical effects* were qualitatively similar to those observed with hypoxemia or ischemia or both (Figs. 6-12 and 6-13). Thus brain glucose level declined rapidly (despite normal blood glucose level), lactate concentration rose (after a 2½-minute delay), and PCr concentration decreased markedly (to values approximately 20% of control within 5 minutes). However, ATP levels were maintained for 6 minutes of asphyxia but then declined by 10 minutes. The changes in high-energy phosphates have been documented in the living animal by MR spectroscopy.[93] Thus after 5 minutes of asphyxia, in which electrocerebral silence occurred after 3 minutes, a 40% decrease in the PCr/Pi ratio and a 30% decrease in the ATP/Pi ratio occurred. Despite these changes, upon reinstitution of ventilatory support, cerebral metabolism returned to normal within 20 to 30 minutes. However, studies in neonatal piglets have shown that during a similar recovery period after an even less severe asphyxial insult (2 to 3 minutes), evidence for lipid peroxidation and altered membrane function (depressed $Na^+$, $K^+$-ATPase activity) could be demonstrated.[95] Production of *intrauterine* asphyxia by impairment of placental blood flow also results in decreases in cerebral high-energy phosphate levels, measured by MR spectroscopy in the living animal.[99]

### *Additional Effects of Asphyxia (Versus Solely Hypoxemia or Ischemia or Both)*

At least four major factors are added to the constellation of biochemical features controlling outcome when asphyxia, with its attendant increase in arterial $CO_2$ tension, occurs. The first three of these factors appear to be beneficial, at least initially, and the fourth of these, deleterious. First, the hypercapnia acts to maintain or even augment cerebral blood flow via an increase in perivascular hydrogen ion concentration in brain, which may be of beneficial importance early in asphyxia. Second, the hypercapnia may be associated with a diminution in cerebral metabolic rate. Moderate hypercapnia has been shown to cause a diminution in cerebral metabolic rate in adult rat brain, adult monkey brain, and developing rat brain.[15,100-102] Third, an increase in arterial $CO_2$ tension leads to acidemia, which is accompanied by a shift in the oxygen-hemoglobin dissociation curve such that the affinity of hemoglobin for oxygen is decreased. The

result is an increase in the delivery of oxygen to cells. The operation of one or more of these three factors could underlie the protective effect of moderate hypercapnia in immature rats subjected to hypoxia-ischemia.[11,103] The fourth important factor relative to hypercapnia and the outcome with asphyxia may be a deleterious one; intracellular pH falls more drastically for a given amount of lactate formed when the effect of elevated $CO_2$ tension is added by asphyxia.[21,94] Thus extreme acidosis and consequent tissue injury could result. Future studies directed at defining the relative roles of these four factors in the genesis of the biochemical and physiological derangements associated with asphyxia in the perinatal animal will be of great interest.

## Influence of Carbohydrate Status on Hypoxic-Ischemic Brain Injury

### *Deleterious Role of Low Brain Glucose in Perinatal Animals*

A series of older studies with immature animals suggests a beneficial effect of prior administration of glucose and a deleterious effect of hypoglycemia on the survival response to anoxic insult (i.e., nitrogen breathing).[104-107] The effects of glucose appeared to be exerted on the central nervous system rather than the heart, because time to last gasp was altered before cardiac function. This observation is compatible with data indicating the particular resistance of immature heart to combined hypoxia and hypoglycemia, presumably because of rich carbohydrate stores and high glycogenolytic and glycolytic capacities.[108-110] Later work on the survival and neuropathological response to hypoxia and ischemia of neonatal animals also has demonstrated a beneficial effect of pretreatment with glucose and a deleterious effect of hypoglycemia (Fig. 6-14) (see also Chapter 12).[7,8,39,111-114]

**Importance of endogenous brain glucose reserves.** The biochemical mechanisms for the relation between carbohydrate status and resistance to hypoxic-ischemic insult relate to glycolytic capacity. Thus with hypoxic-ischemic states, replenishment of brain high-energy phosphate levels is dependent on anaerobic glycolysis. Because there is a nineteenfold reduction in ATP production per molecule of glucose when the brain is forced to oxidize glucose anaerobically, glycolytic rate must be enhanced greatly. The adaptive mechanisms that come into play for this purpose are summarized in previous sections. The greatly enhanced glycolytic rate leads to a decline of brain glucose levels.[7,8,11,20,22,39,98] If this decline is prevented (e.g., by prior administration of glucose), glycolytic rate and, hence, ATP production are increased, and the biochemical and clinical outcome for animals rendered hypoxic or partially ischemic is improved considerably.[7,8,39,49,111,112,115,116] Indeed, the careful studies of Vannucci and Vannucci[7,8,113] indicate that the major factor accounting for the difference in outcome between normoglycemic and hypoglycemic animals rendered hypoxic is the amount of *endogenous brain glucose reserves* at the time of the insult. In the hypoglycemic animals a tenfold to twentyfold reduction in endogenous brain glucose resulted and correlated

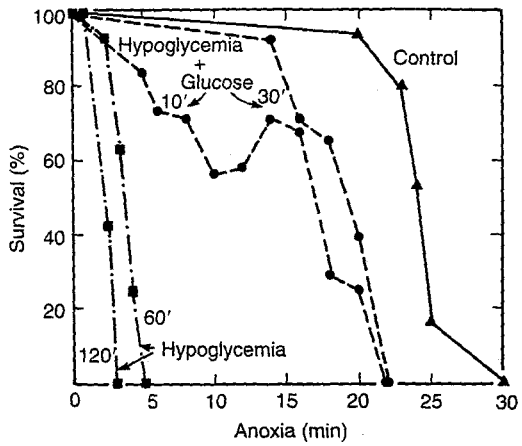


**Figure 6-14** Deleterious effect of hypoglycemia on vulnerability to anoxia ($N_2$ breathing). Percent survival of newborn rats was determined as a function of duration of anoxia. Hypoglycemia was produced by insulin injection 1 to 2 hours prior to onset of anoxia; some hypoglycemic animals were pretreated with glucose (1.8 g/kg, subcutaneous) either 10 or 30 minutes prior to anoxia. (From Vannucci RC, Vannucci SJ: *Ann Neurol* 4:73, 1978.)

best with the impaired glycolytic rate and the decline in high-energy phosphate levels in brain with nitrogen breathing. Brain glycogen levels seemed less important. Thus the capacity for surviving hypoxemia was reduced fivefold in hypoglycemic animals at a time (i.e., 60 minutes after insulin injection) when brain glycogen level was reduced by only 20%, but brain glucose level was reduced by more than tenfold (Fig. 6-15). Similarly, reversal of the vulnerability correlated with a rapid normalization of brain glucose levels but no significant change in brain glycogen levels.

**Summary.** Taken together, these data with immature animals (principally rodents) indicate that *carbohydrate status plays an important role in determining the biochemical and clinical responses to hypoxemic and ischemic insults. Hypoglycemia is deleterious, and pretreatment with glucose is beneficial.* The mechanism of the effect appears to relate to changes in endogenous, readily mobilized brain glucose reserves, which lead to the enhanced glycolytic rate required to slow the decline of, or even maintain the levels of, high-energy phosphate in brain.

#### *Deleterious Role of Abundant Brain Glucose in Adult Animals*

A potentially deleterious role for abundant brain glucose in the clinical, pathological, and biochemical responses to hypoxemia and ischemia was suggested initially by studies with juvenile rhesus monkeys.[117-120] In a series of experiments with animals routinely food deprived for 12 to 24 hours before subjection to circulatory arrest, it was shown that as much as 14 minutes of circulatory arrest was compatible with apparently good neurological recovery and "minimal" neuropathological abnormalities, restricted principally to brain stem nuclei, hippocampus, and Purkinje cells.[117] However, animals that were administered an infusion of 1.5 to 3 g/kg of glucose (5% dextrose in saline) that terminated 10 minutes prior to the 14-minute period of circulatory arrest did very poorly. The

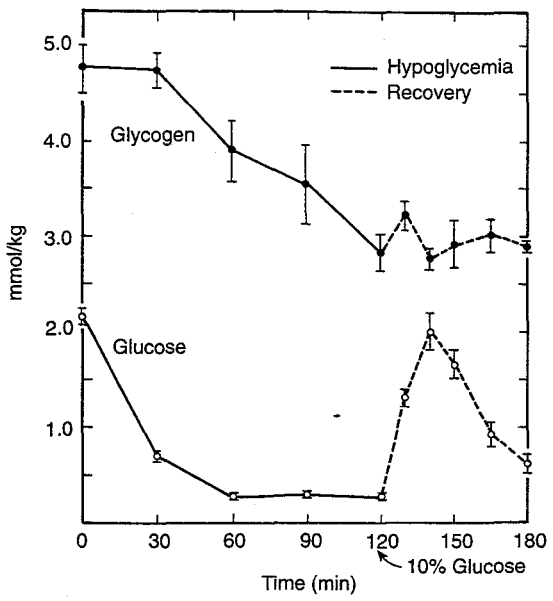


**Figure 6-15** Importance of brain glucose reserves in effects of hypoglycemia on vulnerability to anoxia ($N_2$ breathing). Brain glucose and glycogen levels in newborn rats were determined as a function of duration of anoxia. Hypoglycemia was produced as described in the legend to Fig. 6-14. Arrow indicates subcutaneous administration of 10% glucose (1.8 g/kg). (From Vannucci RC, Vannucci SJ: *Ann Neurol* 4:73, 1978.)

clinical course was characterized by seizures, hypertonia, and ultimately, decerebrate rigidity, evolving over hours. Upon sacrifice, these glucose-pretreated monkeys, in contrast to the food-deprived monkeys, exhibited "changes indicative of widespread injury to tissue . . . and diffuse cytologic injury" with widespread involvement of cerebral cortex. In a subsequent study glucose was administered as a 50% solution in a dose of 2.5 to 5 g/kg 15 minutes prior to circulatory arrest, and similar clinical and neuropathological consequences were observed.[119]

**Importance of severe lactic acidosis in brain.** The biochemical mechanism for the deleterious effect of pretreatment with glucose in the previously mentioned juvenile monkeys may relate to the greater accumulation of lactic acid in the glucose-pretreated than in the food-deprived monkeys (Table 6-6).[118,121] ATP levels declined approximately tenfold in food-deprived animals subjected to circulatory arrest, and only a minimal difference in the magnitude of that decline was observed in animals pretreated with glucose. However, whereas lactate levels increased approximately fourfold in the food-deprived animals subjected to circulatory arrest, the levels increased more than tenfold in those pretreated with glucose. The greater increases in brain lactate levels in the glucose-pretreated animals presumably reflected higher endogenous brain glucose reserves and, as a consequence, enhanced lactate production by anaerobic glycolysis. These experiments and related observations with animals rendered severely hypoxemic[122] led Myers and Yamaguchi to suggest that the accumulation of brain lactate to concentrations of approximately 20 mmol/kg or greater leads to tissue destruction and brain edema. This approximate threshold level is supported by the observations that accumulation of lactate above this level occurs in the brain of monkeys rendered ischemic in

**TABLE 6-6**

**Effect of Carbohydrate Status on Biochemical Response to Circulatory Arrest (10 Minutes) in Juvenile Monkeys**

| | BRAIN CONCENTRATION (μmol/g) | |
|---|---|---|
| EXPERIMENTAL CONDITION | ADENOSINE TRIPHOSPHATE | LACTATE |
| Control | 2.2 | 3.0 |
| Circulatory arrest | 0.2 | 13.0 |
| Circulatory arrest and glucose pretreatment | 0.3 | 33.0 |

Data from Myers and co-workers.[117-120,122]

those regions that have been shown to be particularly vulnerable to neuronal injury.[121]

Considerable support for the concept of a deleterious effect of abundant glucose and resulting lactic acidosis in brain in the pathogenesis of hypoxic-ischemic brain injury in the adult has been provided by further studies in a variety of experimental models in *mature animals*.[63,122-136] A threshold value of lactate of approximately 20 mmol/kg, above which major tissue injury occurs, can be suggested from the data. The apparent mechanism for the principal injury from these high levels of lactate is injury to endothelial cells, and perhaps also to perivascular astrocytes, with resulting disturbance of cerebral perfusion. Direct neuronal injury is likely, but widespread, secondary ischemic injury develops primarily because of the vascular changes.

## *Beneficial(?) Role of Abundant Brain Glucose in Perinatal Animals*

In contrast with the deleterious role for glucose in hypoxic-ischemic injury in adult animals (see previous section), considerable data in the immature rat suggest a beneficial role for abundant glucose administered primarily during or at the termination of the insult.[7,8,13,49,112,114,116,137-142] Hattori and Wasterlain,[141] using a model of bilateral carotid occlusion and ventilation with 8% oxygen for 1 hour, showed marked reduction of neuropathological injury in animals treated with supplemental glucose at the termination of the hypoxic breathing (Fig. 6-16). Supplementation 1 hour after termination of the hypoxia had no beneficial effect. In a neonatal lamb model of asphyxia, glucose supplementation prevented the prolonged postasphyxial impairment in cerebral oxygen consumption observed in control (or hypoglycemic) animals (Fig. 6-17).[138] Moreover, neonatal rats breathing 8% oxygen survived twice as long if treated with 50% glucose; 50% survival was approximately 4 hours in saline-treated animals versus 8 hours in glucose-treated animals.

The *mechanism* for any beneficial effect of glucose in these models of hypoxia-ischemia is not conclusively known but probably relates to preservation of mitochondrial energy production. Thus Yager and coworkers[71] have shown that glucose supply becomes limiting in hypoxia-ischemia (unilateral carotid occlusion and 8% oxygen breathing) in the neonatal rat, a conclusion based on the relatively oxidized state of mitochondrial $NAD^+$/NADH. Brain glucose levels clearly

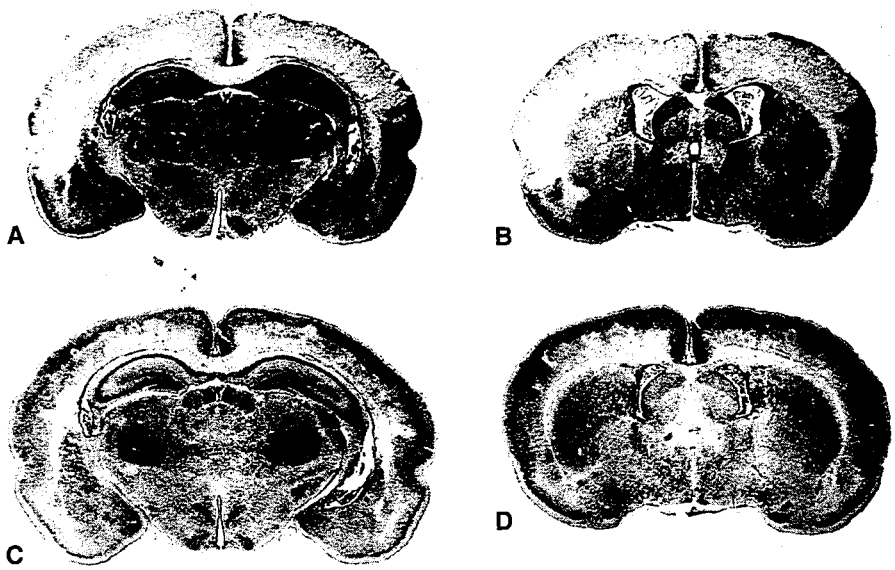


**Figure 6-16** Coronal brain sections of rat pups, which had been subjected to bilateral ligation of the carotid arteries followed by exposure to an 8% oxygen atmosphere for 1 hour at the age of 7 days, and were sacrificed 72 hours later. Note gross infarction in, **A,** neocortex and, **B,** lateral part of the striatum in a saline-injected pup. **C** and **D,** Immediate (0 hour) posthypoxic glucose supplement–reduced neocortical and striatal infarction. (H & E × 2.5 before 52% reduction.) (From Hattori H, Wasterlain CG: *Ann Neurol* 28:122-128, 1990.)

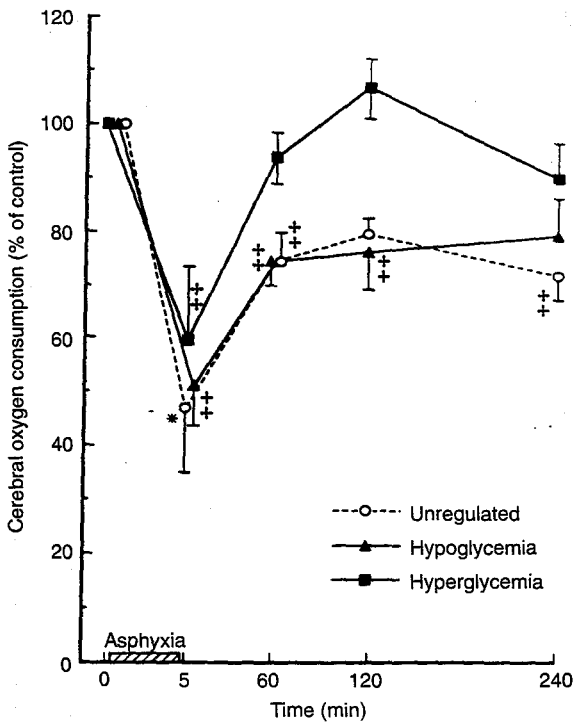


**Figure 6-17** $CMRO_2$ (percentage of control) over time in the unregulated glucose, hyperglycemic, and hypoglycemic groups of newborn lambs during and following asphyxia. Zero (0) time represents the control measurement that is followed by the 75-minute period of asphyxia. Measurements were then made at 5 minutes and at 1, 2, and 4 hours postasphyxia. All values are means ± SEM. Note highest $CMRO_2$ in animals rendered hyperglycemic. *$CMRO_2$,* Cerebral metabolic rate for oxygen; ++, $p < .05$ compared to control; *, $p < .005$ compared to control. (From Rosenberg AA, Murdaugh E: *Pediatr Res* 27:454-459, 1990.)

**TABLE 6-7**

**Effect of Glucose or Saline Treatment on Brain Adenosine Triphosphate (ATP) in Hypoxic-Ischemic Rats (Unilateral Carotid Ligation and Hypoxemia)***

| EXPERIMENTAL CONDITION | PHOSPHOCREATINE* | ATP* | LACTATE* |
|---|---|---|---|
| Control | 3.00 | 2.41 | 1.6 |
| Ligation-hypoxemia† | | | |
| Saline (60 min) | 1.00 | 1.25 | 11.1 |
| Glucose (60 min) | 1.80 | 2.31 | 15.2 |
| Saline (120 min) | 0.35 | 0.43 | 9.4 |
| Glucose (120 min) | 1.00 | 1.80 | 25.5 |

Data from Vannucci RC, Brucklacher RM, Vannucci SJ: *J Cereb Blood Flow Metab* 16:1026-1033, 1996.

*Values are mean concentrations (mmol/kg) in hemisphere ipsilateral to carotid ligation.

†All values for ligated-hypoxemic animals different from controls ($p < .05$), and all values for glucose-treated animals different from saline-treated animals ($p < .05$).

**TABLE 6-8**

**Tentative Conclusions Concerning Effects of Glucose Administration with Perinatal Hypoxic-Ischemic Insults***

Glucose transport into brain and glucose concentration in brain are increased. Lactate levels are increased and intracellular pH values are decreased but recover promptly.

Decrease in cerebral metabolic rate of oxygen is prevented, perhaps reflecting improved mitochondrial function.

Improvement in high-energy phosphate levels is usual but has not reached statistical significance in all studies.

Neuropathological injury may be prevented, ameliorated, or accentuated, according to the model of hypoxia-ischemia and to the species and state of maturation of the animal.

Improved survival occurs and may relate at least partially to improvement in cardiorespiratory function.

Determinations of cerebral lactate and high-energy phosphates, as a function of blood glucose, are needed in asphyxiated human newborns for definitive recommendations concerning glucose supplementation.

*See text for references.

increase after glucose supplementation, in several models of hypoxia-ischemia.[8,116] With the model of carotid occlusion and 8% oxygen breathing, brain levels of high-energy phosphates were clearly higher in glucose-treated versus saline-treated animals (Table 6-7).[116] Also of note is that brain lactate levels in the hypoxic-ischemic neonatal rats were considerably higher in the saline-treated and glucose-treated animals.[116] Indeed, after 2 hours of hyperglycemia, brain lactate levels reached 25.5 mmol/kg. However, there was no evidence for tissue injury caused by the elevated brain lactate levels. Moreover, in other perinatal models (in near-term fetal sheep, newborn lamb, and newborn dog) brain lactate levels did not rise to such levels with hypoxia-ischemia or asphyxia.[40,41,44,143] Increase in brain lactate levels in neonatal brain relative to adult brain is limited by the lower capacity for glucose uptake by the glucose transporter proteins, especially GLUT1 (55 kDA), and by lower hexokinase activity, the rate-limiting enzyme for glucose utilization.[7,8,11,12,16,18] Indeed, the possibility should be considered that any increase in lactate that might occur in the glucose-treated animal is utilized for energy production (by oxidation to pyruvate and entrance into the tricarboxylic acid cycle), since lactate is a preferred fuel in neonatal brain.[11,116,144-146] Moreover, lactate is transported rapidly across the blood-brain barrier in the immature animal, and, at least in the rat, brain pH normalizes by 10 minutes of recovery and tissue lactate levels normalize by 4 hours, unlike the prolonged tissue acidosis that occurs in adult rats subjected to hypoxia-ischemia and glucose treatment.[116] Indeed, the combination of rapid utilization of lactate by brain and rapid efflux from brain may explain the lack of serious tissue injury by levels of lactate that lead to injury in adult brain.

Enthusiasm for supplementation with glucose during or after hypoxic-ischemic insults in the immature brain must be tempered by the results of three other studies of young animals.[147-149] Thus in a model of focal ischemia in the 7-day-old rat, glucose administration *following* hypoxia-ischemia led to more severe neuronal injury (though no increase in infarct size) than did saline administration.[147] Moreover, in a model of global hypoxia-ischemia in 1- to 3-day-old piglets, glucose administration *during* the insult led to accentuated neuronal injury.[148] Glucose administration *following* the insult did not ameliorate the injury, as such therapy accomplished in the immature rat (see earlier.)[149] The reasons for the differences in results obtained in the several perinatal models (see earlier discussion) are unclear but may relate to methodological differences.

**Conclusions.** Current experimental data allow several tentative conclusions to be made about the effects of glucose administration with perinatal hypoxic-ischemic insults (Table 6-8). On balance the findings favor maintenance of blood glucose concentrations in the normal range in infants who have sustained hypoxic-ischemic insults.

## Influence of Maturation on Hypoxic-Ischemic Brain Injury

The influence of the maturational state of the brain on the severity and topography of the brain injury caused by hypoxia-ischemia is complex. It is now clear that the long-held general notion that the perinatal brain is more resistant than the adult brain is too simplistic. There is evidence that cerebral glucose and energy metabolism are *more resistant* to perturbation by hypoxia-ischemia in the immature than in the adult brain.[5,7,8,19,20,23,25,44,111,112,150-157] Some of the mechanisms underlying the resistance of energy metabolism in the immature brain are summarized in Table 6-9. However, neuropathological studies indicate that many critical neuronal groups are *more vulnerable* to hypoxic-ischemic injury in the immature animal.[158-160] This vulnerability of immature neurons relates particularly to

**TABLE 6-9**

**Resistance of High-Energy Phosphate Levels in Perinatal (Versus Adult) Brain to Hypoxic Injury—Probable Mechanisms**

| |
|---|
| Lower rate of energy utilization |
| Lower rate of accumulation of toxic products, i.e., lactate |
| Utilization of lactate and ketone bodies for energy |

enhanced density and function of excitatory amino acid receptors (see later discussion). A particular vulnerability of immature oligodendrocytes to hypoxic-ischemic and excitatory amino acid–induced injury is discussed later but also is relevant in this context. The various influences of maturation on the regional aspects of hypoxic-ischemic brain injury and on the responses to interventions are highlighted in the appropriate subsequent sections of this chapter.

## Birth as an Additive or Potentiating Factor in Hypoxic Injury

Perinatal hypoxic-ischemic injury occurs in the setting of a profound alteration of biochemical and physiological homeostasis, i.e., the process of birth. Transient hypoxemia and hypercapnia, variable in severity and duration, are consistent occurrences.[161-163] Transient disturbances in cerebral blood flow may also occur (see Chapter 4).[164,165] It is appropriate to ask whether the biochemical state of the brain is affected by the major systemic alterations that take place at birth.

Careful studies of perinatal rat brain indicate that *spontaneous vaginal delivery* is associated with the *signs of hypoxemic or ischemic insult to brain.*[166,167] Alterations in glycogen, certain glycolytic intermediates, and high-energy compounds after spontaneous vaginal delivery are shown in Table 6-10. Evidence for glycogenolysis, enhanced lactate production, PCr conversion to ATP, and a decline in ATP concentrations is apparent in the first minute after birth. Simultaneous elevation of the concentrations of glucose-6-phosphate and glucose-1-phosphate and decline of the concentration of glycogen are consistent with the occurrence of glycogenolysis.[167] The elevation of lactate level and of the lactate/pyruvate ratio indicates enhanced anaerobic glycolysis. The sharp decline of PCr is not adequate in the first minute to preserve ATP concentrations, and in fact the small persisting deficit in ATP levels 10 minutes after birth is statistically significant.[166] Not shown, but accompanying these changes, is a decline in brain glucose concentrations relative to blood glucose, reflecting further the enhanced glycolysis. By 1 hour after delivery, high-energy phosphate concentrations were no longer depressed, and the lactate/pyruvate ratio was considerably improved. The latter was normal by 8 hours after delivery. Determinations of identical parameters after cesarean section showed very transient and much smaller changes; indeed, no significant change in ATP levels was observed at any time after delivery by cesarean section.[166]

**TABLE 6-10**

**Effect of Spontaneous Vaginal Delivery on Glycogen, Glycolytic Metabolites, and High-Energy Compounds in Rat Brain**

| | TIME AFTER DELIVERY (min) | | |
|---|---|---|---|
| METABOLIC COMPOUND | 1 | 10 | 60 |
| | (Percent of Term Fetal Values) | | |
| Glycogen | 88 | 74 | 90 |
| Lactate | 367 | 408 | 230 |
| Lactate-pyruvate | 425 | 181 | 157 |
| Phosphocreatine | 38 | 105 | 170 |
| Adenosine triphosphate | 67 | 92 | 96 |

Data from Vannucci and co-workers.[166,167]

These data indicate that the process of birth via the vaginal route is associated with the biochemical signs of hypoxic insult to brain. The influence of this phenomenon on the impact of hypoxic-ischemic insults occurring prior to or immediately following birth is not known. An intuitive conclusion would be that the insult at birth might be additive. However, it is possible that the insult at birth may play a protective role in relation to a subsequent insult. Thus a degree of protection to hypoxic-ischemic brain injury has been shown in the immature rat by prior exposure to hypoxia.[168,169] This "hypoxic preconditioning" appears to be related to induction of genes, still undefined, that blunt the adverse effects of hypoxia-ischemia.

## Biochemical Mechanisms of Neuronal Death with Hypoxia-Ischemia—Beyond Glucose and Energy Metabolism

The principal biochemical mechanisms of cell death with hypoxemia, ischemia, and asphyxia are presumably very similar, if not identical, and are initiated by oxygen deprivation. Of course, the differences among these three insults in the nature of certain metabolic accompaniments do have a bearing on the rapidity and probably the mode of cell death, but I ignore these issues in this discussion. Because all the principal, currently considered mechanisms for cell death with oxygen deprivation at least begin with the disturbances of brain glucose and energy metabolism, it is appropriate to synthesize current concepts concerning the mechanisms for cell death immediately following the preceding sections, which emphasized these disturbances. Nevertheless, it is now clear that the mechanisms of cell death with oxygen deprivation are not simply the result of energy failure and, indeed, extend beyond glucose and energy metabolism. An enormous amount of literature attests to the complexity of the mechanisms, and in the following section I attempt to synthesize the essential data and isolate the most critical mechanisms. The emphasis is on mechanisms of *neuronal* death. Although mechanisms of white matter injury, especially oligodendroglial death, bear many similarities, sufficient differ-

ences warrant separate consideration in the next major section.

### General Themes—Importance of the Reperfusion Period and Mode of Cell Death (Necrosis-Apoptosis)

**Importance of the reperfusion period.** As discussed in the next section, the cascade of deleterious events that lead to cell death after insults that result in oxygen deprivation and energy failure appears to occur primarily following termination of the insult. Careful studies in animal models and in human patients provide strong support for this notion.[62,170-178] The phenomena are initiated particularly by energy depletion, accumulation of extracellular excitatory amino acids (particularly glutamate), increase in cytosolic calcium, and generation of free radicals. Disturbances of cerebral blood flow also are important (see later discussion of cerebral blood flow). The importance of this "delayed" death of brain in the hours following termination of the insult is related in largest part to the possibility that intervention during the post-insult period could be beneficial. Data to support this possibility are now available, as discussed later.

**Importance of the mode of cell death (necrosis-apoptosis).** Two fundamental modes of cell death in the nervous system, as in other tissues, are distinguished, i.e., *necrosis* and *apoptosis*. It is now clear that hypoxic-ischemic insults may lead to necrosis or apoptosis or both, dependent on the severity of the insult, its temporal characteristics, the local environment of the affected region, and the maturational state of the cell. Certain characteristics readily distinguish these two forms of cell death (Table 6-11).[179-190] Thus necrotic cell death is characterized by cell swelling, membrane disintegration, cell rupture, release of intracellular contents, and as a consequence, inflammation and phagocytosis. By contrast, apoptosis is characterized by condensation and margination of chromatin, cell shrinkage, relative preservation of cellular membranes, and death without inflammation. Apoptotic cell death is difficult to detect in tissue because of the lack of inflammation and the rapid removal of the cell debris. Apoptotic cell death requires activation of specific death genes and new protein synthesis, which result particularly in a series of biochemical changes that include cleavage of DNA at specific sites to result in the characteristic oligonucleosomal fragmentation. Necrotic cell death occurs typically after intense, relatively brief insults, whereas apoptotic cell death occurs typically after less intense, longer acting insults. Apoptotic cell death may be the dominant form of so-called delayed cell death, observable after several days in various experimental models and human brain. Important factors in determining whether the mode of cell death is necrotic or apoptotic also include still undefined differences in the local environment of target cells.[185] Important intrinsic properties of the cell itself in the determination of the mode of cell death also relate to the developmental stage of the cell. Thus in certain paradigms the susceptibility to apoptosis is enhanced in immature versus mature neurons.[184] Apoptotic cell death was noted to be common in a study of infants who died after intrauterine hypoxic-ischemic insult.[189] Moreover, careful studies in the neonatal piglet subjected to hypoxia-ischemia have demonstrated in the same paradigm exclusively necrotic cell death in certain neuronal populations, both necrosis and apoptosis in other neuronal populations, but exclusively apoptotic cell death in immature cerebral white matter.[183]

In the following sections I outline the major biochemical mechanisms, beyond glucose and energy metabolism, involved in the cascade to cell death with hypoxic-ischemic insults. Where possible, I indicate whether the mode of cell death has been determined, although in most studies this difficult determination has not been accomplished.

### Initiating Role of Energy Failure

In previous years, cell death with oxygen deprivation was explained by reference to the sharply decreased production of high-energy phosphate from anaerobic glycolysis (Fig. 6-2). The mechanism cited was deficiency of high-energy phosphates that are necessary for synthesis of macromolecules and lipids and, thus, maintenance of structural integrity. It is now clear that this explanation is too simple and that cell death does not require energy depletion severe enough to eliminate synthesis of structural components. However, it is likely that the initial decrease in high-energy phosphates and perhaps particularly the secondary energy failure in the postinsult period, as discussed earlier, are capable of *triggering a series of additional mechanisms* that probably

**TABLE 6-11**

**Necrosis and Apoptosis: Distinguishing Characteristics***

| DISTINGUISHED FEATURE | NECROSIS | APOPTOSIS |
|---|---|---|
| Morphology | Cell swelling; membrane fragmentation; inflammatory responses | Cell shrinkage; intact membranes; no inflammation |
| DNA fragmentation | Nonspecific | Specific oligonucleosomal cleavage |
| Intracellular $Ca^{2+}$ | Early increase | No early increase |
| Protein synthesis | Not required | Required |
| Involvement of specific death genes (e.g., *ced-3, bax, bad, JNK, p38*) | Not required | Required |
| Temporal characteristics | Usually rapid (minutes to hours) | Slow (hours to days) |

*See text for references.

begin with membrane depolarization, accumulation of extracellular glutamate, and increase in cytosolic calcium ($Ca^{2+}$) (Fig. 6-18). The membrane depolarization results because of failure of the ATP-dependent $Na^+$, $K^+$–pump and because of the activation of glutamate receptors (see later discussion). The increase in cytosolic calcium is a consequence of (1) failure of energy-dependent $Ca^{2+}$-pumping mechanisms, (2) opening of voltage-dependent $Ca^{2+}$ channels (secondary to membrane depolarization), and (3) activation of specific glutamate receptors (see later discussion). The increase in extracellular glutamate results from (1) excessive glutamate release (secondary to membrane depolarization and to increased cytosolic calcium) and (2) failure of energy-dependent glutamate uptake mechanisms in astrocytes and presynaptic nerve endings. The subsequent deleterious events leading to cell death after these initial events are described next.

### Role of Accumulation of Cytosolic Calcium

A large body of information indicates a major role for accumulation of cytosolic calcium during and following hypoxia-ischemia in the mediation of cell death.[5,10,157,191-202] In perinatal models of hypoxia-ischemia, increased $Ca^{2+}$ uptake into the insulted brain regions, close correlation within brain regions between increased uptake of calcium and subsequent neuronal

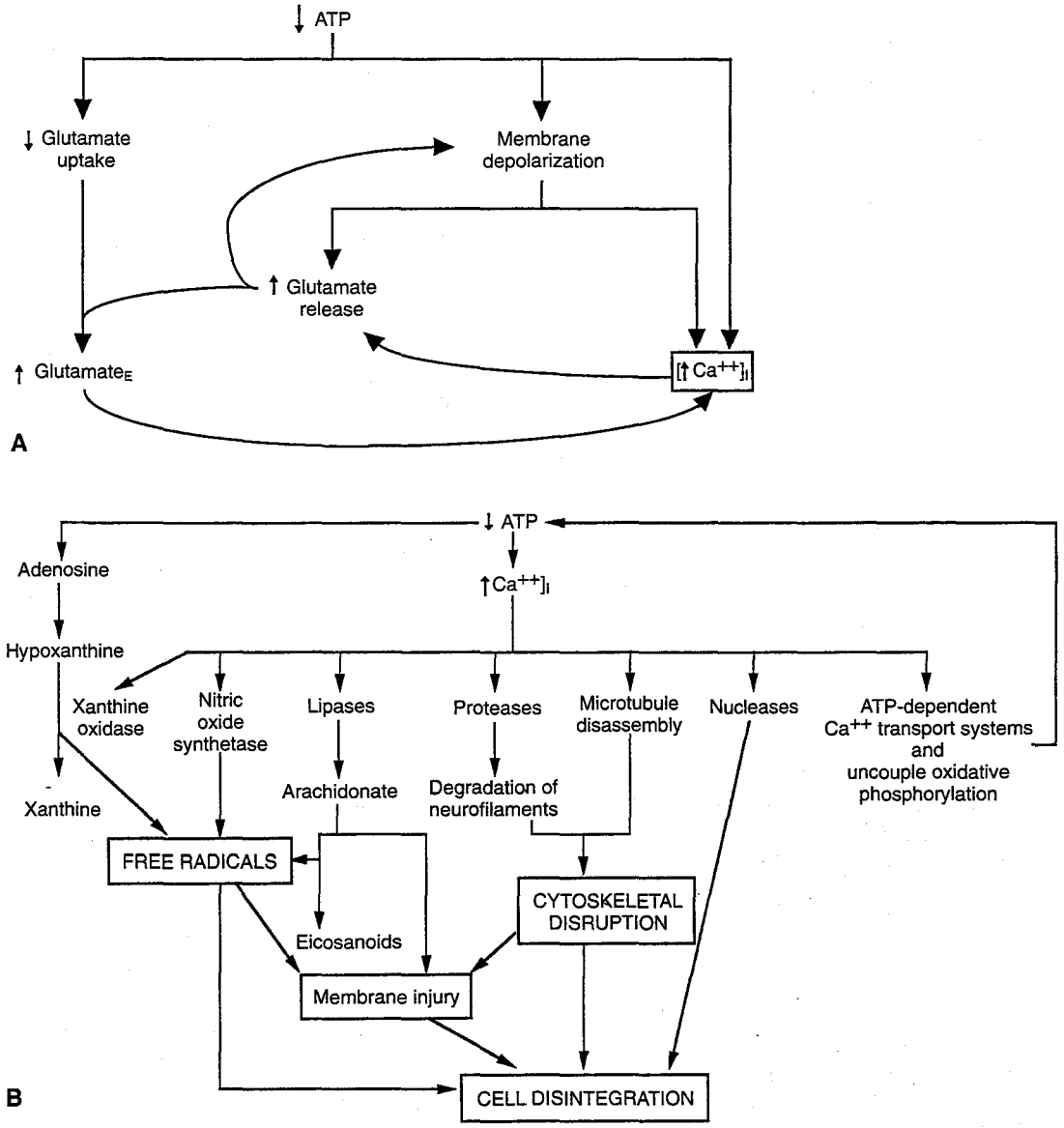


**Figure 6-18** Relations between energy depletion, accumulation of, **A,** intracellular cytosolic $Ca^{++}$ ($[Ca^{++}]_i$), and, **B,** brain injury with hypoxic-ischemic insult. The deleterious effects of, **B,** elevated cytosolic $Ca^{++}$ ($[Ca^{++}]_i$) are multiple and include an important role for free radicals. *ATP,* Adenosine triphosphate.

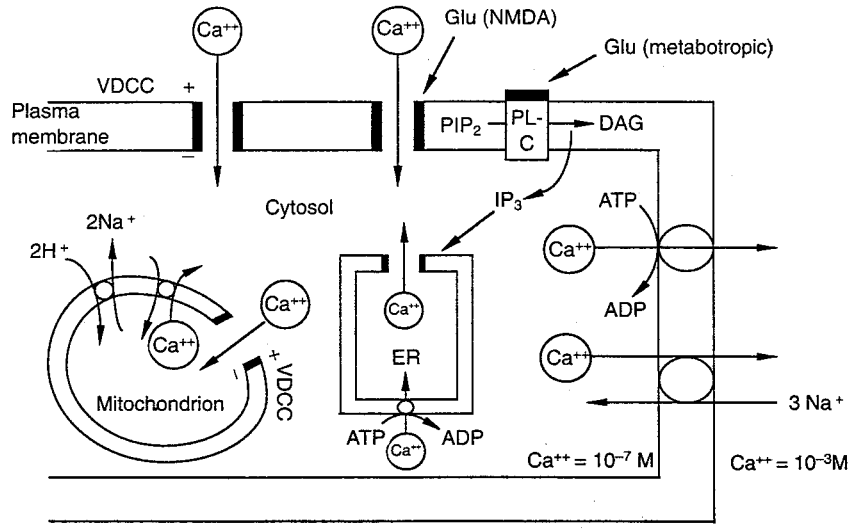


**Figure 6-19** Cellular $Ca^{++}$ homeostasis. *ADP,* Adenosine diphosphate; *ATP;* adenosine triphosphate; *DAG,* diacylglycerol; *ER,* endoplasmic reticulum; *GLU,* glutamate; *NMDA, N*-methyl-D-aspartate; *$PIP_2$,* phosphatidylinositol-4,5-diphosphate; *PL-C,* phospholipase C; *VDCC,* voltage-dependent $Ca^{++}$ channel. (Modified from Siesjö BK: *Eur Neurol* 30:3-9, 1990.)

**TABLE 6-12**

**Mechanisms for Increased Cytosolic Calcium in Neurons with Hypoxia-Ischemia**

| SITE | MECHANISM* |
|---|---|
| Plasma membrane | ↑ $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels *(cell depolarization)* |
| | ↑ $Ca^{2+}$ influx through agonist-dependent $Ca^{2+}$ channels *(glutamate action at N-methyl-D-aspartate receptor)* |
| | Activation of phospholipase C and liberation of inositol triphosphate ($IP_3$) (see below) *(glutamate action at metabotropic receptor)* |
| | ↓ $Ca^{2+}$ efflux via ATP-dependent uniport system *(ATP depletion)* |
| | ↓ $Ca^{2+}$ efflux via $Na^+$-dependent antiport system (↓ *extracellular $Na^+$*) |
| Endoplasmic reticulum | ↑ $Ca^{2+}$ release to cytosol via effect of $IP_3$ (see above) |
| | ↓ $Ca^{2+}$ uptake by ATP-dependent uniport system *(ATP depletion)* |
| Mitochondrion | ↑ $Ca^{2+}$ release to cytosol via $Na^+$,$H^+$–dependent antiport system (↑ *cytosolic $Na^+$,$H^+$ ions)* |

*ATP,* Adenosine triphosphate.
*The primary effect of ischemia to cause the indicated change in $Ca^{2+}$ homeostasis is shown in parentheses.

injury, and protection from subsequent brain injury by pretreatment with voltage-dependent calcium-channel antagonists have been documented.[10,75,76,196,197,201,203-207] Moreover, because an important mechanism of $Ca^{2+}$ influx into the cytosol is via the NMDA type of glutamate receptor-channel complex, the protection from brain injury afforded by antagonists of this complex (see later discussion) may be mediated primarily by decreasing accumulation of cytosolic calcium.

**Mechanisms.** The mechanisms by which increased cytosolic $Ca^{2+}$ leads to cell death are multiple but are best discussed in the context of normal $Ca^{2+}$ homeostasis (Fig. 6-19).[5,10,191-197,201,208] The cellular mechanisms for maintenance of low cytosolic $Ca^{2+}$ concentrations ($10^{-7}$ M) relative to high extracellular $Ca^{2+}$ concentrations ($10^{-3}$ M) are located in the *plasma membrane* (voltage-dependent channels; two agonist-dependent, i.e., glutamate-dependent, channels, the NMDA and metabotropic receptor-activated channels [see later discussion]; an ATP-dependent uniport system and a $Na^+$-dependent antiport system), *endoplasmic reticulum* (an ATP-dependent import system and a release mechanism activated by inositol triphosphate [IP3]), and the *mitochondrion* (a voltage-dependent channel and an $Na^+$, $H^+$–dependent antiport system) (Fig. 6-19).[195-197] The mechanisms for the increased cytosolic calcium in neurons subjected to hypoxia-ischemia, and the metabolic and ionic changes caused by ischemia that underlie these mechanisms, are summarized in Table 6-12. The central roles for ATP depletion, membrane depolarization, and voltage-dependent and glutamate-activated $Ca^{2+}$ channels are apparent.

The deleterious effects of increased cytosolic calcium are multiple and affect the cell in a variety of ways (Table 6-13).[5,10,191-197,201,205,209-214] These effects include degradation of cellular lipids by activation of phospholipases, of cellular proteins (especially cytoskeletal elements) by activation of proteases, and of cellular DNA

**TABLE 6-13**

**Deleterious Effects of Calcium in Hypoxia-Ischemia**

| CALCIUM ACTION | DELETERIOUS EFFECT |
|---|---|
| Activate phospholipases | Phospholipid hydrolysis and membrane injury<br>Generation of arachidonic acid and ultimately free radicals via cyclo-oxygenase and lipo-oxygenase pathways |
| Activate proteases, disassembly of microtubules | Cytoskeletal disruption (caused by microtubular disruption and proteolysis of neurofilaments)<br>Proteolysis of other cellular proteins |
| Activate nucleases | Nuclear injury |
| Activate calcium-ATPase and other energy-dependent $Ca^{2+}$ extrusion mechanisms | Consume ATP at a time of deficient ATP |
| Enter mitochondrion and uncouple oxidative phosphorylation | Decrease ATP production |
| Increase neurotransmitter release (e.g., glutamate, catecholamines) | Activate glutamate receptors—$Ca^{2+}$ influx, etc.<br>Auto-oxidation of catecholamines with production of free radicals |
| Activate a protease for transformation of xanthine dehydrogenase to xanthine oxidase | Oxidation of hypoxanthine to xanthine and of xanthine to uric acid with production of free radicals |
| Activate nitric oxide synthetase | Generation of nitric oxide with toxic effect on neurons |

*ATP,* Adenosine triphosphate.

**TABLE 6-14**

**Free Radicals in Hypoxia-Ischemia**

**SOURCES**

Mitochondrial electron transport system
Action of cyclo-oxygenase and lipo-oxygenase on arachidonic acid
Action of xanthine oxidase on hypoxanthine and xanthine
Auto-oxidation of catecholamines
Infiltrating neutrophils and microglia
Action of nitric oxide synthetase

**ENDOGENOUS DEFENSES**

Major—superoxide dismutase (generates $H_2O_2$), catalase (degrades $H_2O_2$), and glutathione peroxidase (degrades $H_2O_2$)
Free radical scavengers—vitamin E (alpha-tocopherol), other sterols (21-aminosteroids), vitamin C (ascorbic acid), glutathione, other thiol compounds

**MAJOR DELETERIOUS EFFECTS**

Peroxidation of polyunsaturated fatty acids (PUFA) of membrane phospholipids (PUFA especially abundant in brain membranes)
Damage to DNA and to proteins containing unsaturated or sulfhydryl groups
Activation of proapoptotic genes

by activation of nucleases, as well as crucial indirect mechanisms of destruction mediated by generation of free radicals and nitric oxide (Fig. 6-18). The utilization of ATP by ATP-dependent $Ca^{2+}$-transport systems, attempting to correct the cytosolic $Ca^{2+}$-accumulation, and the $Ca^{2+}$-mediated uncoupling of oxidative phosphorylation serve to perpetuate the process (Fig. 6-18).

### *Role of Free Radicals, Including Nitric Oxide*

**Free radicals.** The crucial role of free radicals, generated in considerable part by $Ca^{2+}$-activated processes just described, in the mediation of cell death with hypoxia-ischemia has been established by study of a variety of models in vivo, in culture, and in vitro.[5,82,85,190,215-252b] Free radicals are highly reactive compounds with an uneven number of electrons in the outermost orbital. These compounds can react with certain normal cellular components, e.g., unsaturated fatty acids of membrane lipids, and can generate a new free radical and thereby a chain reaction, which results in irreversible biochemical injury, e.g., peroxidation of the unsaturated fatty acids, membrane injury, and cell necrosis. With less intense insults free radicals can lead to apoptotic cell death by activation of specific death genes.[240,253,254]

The principal *sources* of free radicals with hypoxia-ischemia, the endogenous *defenses* against such radicals, and their *major deleterious effects* are summarized in Table 6-14. Of the sources of free radicals with hypoxia-ischemia, the electron transport system is important when oxygen deprivation prevents the complete passage of electrons to cytochrome-*c* oxidase. Free radicals, specifically superoxide anion, then are generated proximal to this terminal enzyme in the electron transport system. The next four sources are directly or indirectly related to cytosolic calcium (Fig. 6-18 and Table 6-13). Arachidonic acid is generated by $Ca^{2+}$-activated phospholipase $A_2$, xanthine oxidase is activated by $Ca^{2+}$, catecholamine release is stimulated by an increase in cytosolic $Ca^{2+}$, and nitric oxide synthetase (see next section) is activated by $Ca^{2+}$.[5,197,211-231,237,238,240,252,255,256a] Finally, recent data indicate that early reactive cells at the site of initial insult, e.g., microglia and neutrophils, are potent sources of free radicals (see later discussion).[234,238,240,257,258]

An important role for free radicals in perinatal models of fetal and neonatal hypoxemic, ischemic, hypoxic-ischemic, and asphyxial insults now seems established.[82,190,232,233,235-239,251-252b,259-268] Following the insults, generation of free radicals or elevations of compounds known to lead to generation of free radicals has been found. Moreover, studies principally of asphyxia in the newborn lamb and in the neonatal piglet and of hypoxia-ischemia (carotid ligation and low oxygen breathing) in the immature (7-day-old) rat have shown elevations of free radicals, deleterious effects of free radical attack, or a neuroprotective effect of treatment (pretreatment, treatment during the insult or after termina-

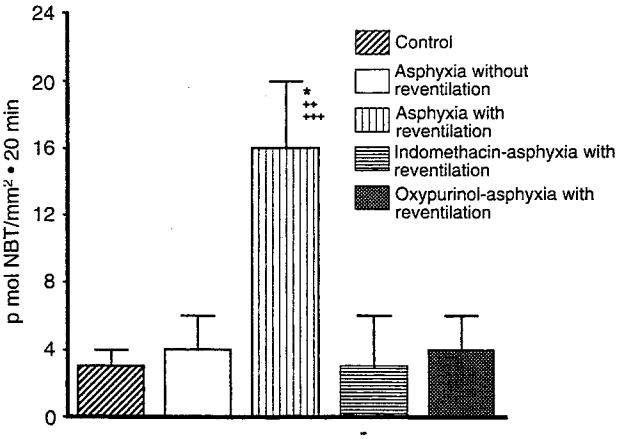


**Figure 6-20** Free radical (superoxide anion) production, determined as superoxide dismutase-inhibitable NBT reduction in control (n = 7), asphyxia without reventilation (n = 9), asphyxia-reventilation (n = 11), asphyxia-reventilation after indomethacin 0.2 mg/kg (n = 4), and asphyxia-reventilation after oxypurinol 50.0 mg/kg (n = 10) pretreated piglets. Values are mean ± SEM. *, $p < .05$ compared with control; ++, $p < .05$ compared with asphyxia without reventilation group; +++, $p < .05$ compared with indomethacin and oxypurinol pretreatment groups. Note the production of superoxide anion after but not during asphyxia and the prevention by administration of indomethacin or oxypurinol. (From Pourcyrous M, Leffler CW, Bada HS, et al: *Pediatr Res* 34:366-369, 1993.)

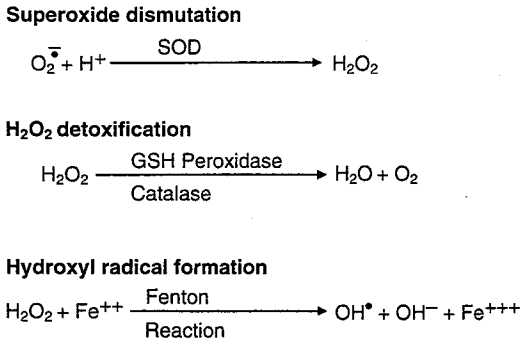


**Figure 6-21** Free radical metabolism. The upper two reactions, catalyzed respectively by SOD and by GSH peroxidase and catalase, are the major antioxidant defense mechanisms. The Fenton reaction generates the highly toxic hydroxyl radical. *GSH*, Glutathione; *SOD*, superoxide dismutase.

tion of the insult) with free radical scavengers or drugs that inhibit free radical formation, e.g., allopurinol, oxypurinol, indomethacin, superoxide dismutase, catalase, iron chelators.[82,85,137,190,233,235,236,241-250,252-252b,261,268-271] The importance of the reperfusion period, rather than the time of the hypoxic-ischemic insult per se, in the generation of the reactive oxygen species also has been emphasized (Fig. 6-20).

The *major endogenous antioxidant defense system* is illustrated in Fig. 6-21. Thus the most commonly generated initial oxygen free radical, the superoxide anion, is converted to hydrogen peroxide by the enzyme, superoxide dismutase (SOD) (the three different forms of this enzyme are cytosolic Cu-Zn SOD, extracellular Cu-Zn SOD, and mitochondrial Mn SOD). The hydrogen peroxide generated is detoxified by catalase and glutathione peroxidase. If this step fails or is overloaded, and if $Fe^{++}$ is available, the Fenton reaction and the production of the deadly hydroxyl radical occur. Studies of animal models and recent studies of asphyxiated human infants indicate that after hypoxia-ischemia, iron is released and therefore relatively abundant.[11,239,249,251,272-275] Recent studies of hypoxia-ischemia in the neonatal mouse indicate that the detoxification of hydrogen peroxide is deficient in the immature brain. Thus mice made transgenic for copper-zinc SOD and therefore overexpressing this enzyme, when subjected to hypoxia-ischemia, exhibit decreased brain injury in the adult but *increased* brain injury in the perinatal period.[250,276] This *exacerbation* of brain injury in the *immature* brain was associated with an accumulation of hydrogen peroxide, because catalase and glutathione peroxidase did not increase in activity in response to the insult (catalase increases after similar hypoxia-ischemia in the adult.)[250] Indeed, the levels of glutathione peroxidase decreased after hypoxia-ischemia, and importantly, in the normal animal levels already are developmentally low in the perinatal period. Thus the data suggest that the normal immature brain has a limited capacity to detoxify hydrogen peroxide and with hypoxia-ischemia accumulates hydrogen peroxide because of this developmental lack and because of failure to respond with an adaptive increase in the antioxidant defense enzymes. With the hypoxia-ischemia–induced increase in iron, generation of the hydroxyl radical and brain injury is the result.

**Nitric oxide.** Particular importance for the synthesis of nitric oxide via a $Ca^{2+}$-activated synthase both in normal brain and under conditions of hypoxia-ischemia is now well established.[256a,277-283] At least three forms of nitric oxide synthase (NOS) are recognized, i.e., a constitutive neuronal form (nNOS), a constitutive endothelial form (eNOS), and an inducible form (iNOS) found in astrocytes and microglia. The constitutive forms are activated by $Ca^{2+}$, whereas iNOS stimulation appears to be $Ca^{2+}$ independent and is activated especially well by cytokines. Because nitric oxide is a diffusible gas, both its normal functions, i.e., cell signaling and neurotransmission, and its deleterious actions, neurotoxicity, appear to be mediated by synthesis and then diffusion to adjacent cells.

Particular importance for the synthesis of nitric oxide, especially by nNOS, in the mediation of neuronal death with hypoxia-ischemia or after exposure to excitatory amino acids has been shown by many studies although not by all.[277-282,284-285a] The discrepant findings appear to relate to the capacity of derivatives of nitrogen monoxide (NO) to exist in several redox forms, including a neurotoxic, free radical–generating form, nitric oxide ($NO^{\bullet}$), and the apparently neuroprotective form, nitrosonium ion ($NO^+$). Work by Lipton and co-

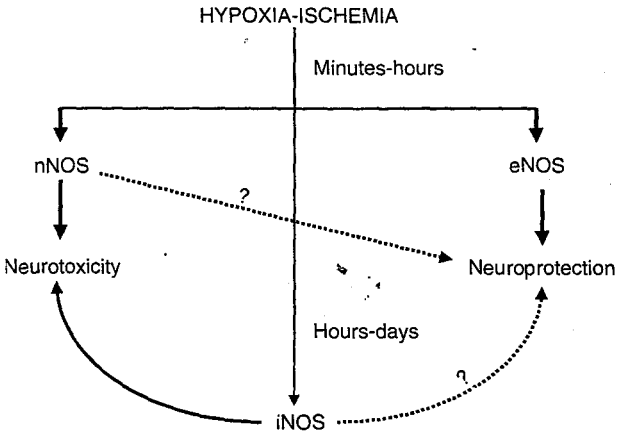


**Figure 6-22** Major effects of the various forms of nitric oxide synthase *(NOS)* after hypoxia-ischemia. Effects in the first minutes-hours and in the hours-days after the insult can be distinguished, although these effects overlap. Dotted lines indicate neuroprotective effects that appear plausible but require more study. See text for details. *eNOS*, Endothelial NOS; *iNOS*, inducible NOS; *nNOS*, neuronal NOS.

workers[285] has demonstrated in cultured neurons that the redox state of NO is crucial in determining whether neurotoxicity or neuroprotection occurs. $NO^{\bullet}$, the toxic form of nitrogen monoxide, can be generated by *N*-methyl-D-aspartate (NMDA) receptor activation. Toxicity occurs by combination of $NO^{\bullet}$ with superoxide anion and generation of a highly toxic free radical species, peroxynitrite ($ONOO^-$). However, $NO^+$, which can be generated by sodium nitroprusside, is neuroprotective because of the occurrence of S-nitrosylation at critical thiol(s) on the NMDA receptor's redox modulatory site to downregulate channel activity. These findings concerning the importance of the redox state of NO not only help explain the discrepant results obtained in various models *in vivo* and in culture but suggest therapeutic possibilities in hypoxic-ischemic and excitatory amino acid–induced neuronal death. In vivo an additional reason for a beneficial effect of nitric oxide synthesis in hypoxia-ischemia is activation of eNOS, which causes vasodilation and preservation of cerebral perfusion.

In *perinatal models of asphyxia or hypoxia-ischemia*, evidence for both the neurotoxic effects and the beneficial vascular effects of nitric oxide synthesis have been obtained, although not all results are consistent.[256a,273,285a,286-302] Evidence for neurotoxic effects of NOS activation has consisted particularly of demonstration of neuroprotection by specific inhibitors of the synthase (e.g., nitrosoarginine derivatives). Although the data are not completely consistent, on balance the scheme shown in Fig. 6-22 depicts the major effects mediated by stimulation of the several forms of NOS. Thus, in the first minutes to hours after hypoxia-ischemia, nNOS and eNOS are activated. The former activation primarily results ultimately in generation of peroxynitrite and neuronal toxicity. Perhaps less likely is the possibility of generation of the neuroprotective nitrosonium ion. The activation of eNOS results in nitric oxide–mediated vasodilation, preserved cerebral perfusion, and the possibility of neuroprotection. The response of iNOS, stimulated for example in astrocytes and microglia by cytokines, occurs later, i.e., after many hours or a day or more, and the principal effect is neurotoxicity. (Whether a neuroprotective effect is possible after stimulation of iNOS is unclear, but it is noteworthy in this regard that nitric oxide donors *protect* oligodendrocytes from free radical attack in cultures that contain small amounts of astrocytes but no neurons or endothelial cells [see later discussion].) Overall, however, from the perspective of neuronal injury, the balance of data suggests that inhibition of nNOS is likely to be neuroprotective, whereas inhibition of eNOS could accentuate neuronal toxicity.

### Role of Excitatory Amino Acids

A remarkable series of studies primarily from the past 15 to 20 years has revolutionized understanding of the role of excitatory amino acids, particularly glutamate, as the mediators of neuronal death under conditions of hypoxia-ischemia. Before discussion of these studies, the normal aspects of glutamate biology at the excitatory synapse are reviewed.

**Normal features.** The relationships at the *glutamate synapse* among the presynaptic nerve ending, the postsynaptic dendrite, and the associated astrocyte are shown in Fig. 6-23.[195,196,303-310] Only the ionotropic receptors are shown in Fig. 6-23 (see next paragraph). Glutamate release is provoked by influx of $Ca^{2+}$ into the presynaptic nerve ending. Depolarization of the postsynaptic dendrite is related to $Na^+$ entry. The action of glutamate is terminated by potent, energy-dependent reuptake mechanisms in both astrocytes and presynaptic nerve endings. In the astrocyte the ATP-dependent enzyme, glutamine synthetase, utilizes $NH_3$ to form glutamine, which diffuses to the presynaptic nerve ending to regenerate glutamate upon removal of this second amino group. ATP depletion clearly results in failure of these reuptake and removal mechanisms and leads to accumulation of extracellular glutamate and to excitotoxicity (see the following discussion).

These mechanisms of reuptake and removal must be highly efficient, because although the intracellular concentration of glutamate is extraordinarily high, i.e., 5 to 10 mmol/kg, the extracellular concentration is approximately 1000-fold less, i.e., in the low micromolar range or perhaps lower.[304,305,310] The high concentration of glutamate in neurons implies a large release of glutamate into the extracellular space when cell death occurs, an occurrence that is relevant not only to amplification of primary excitotoxic cell death, as occurs with hypoxia-ischemia, but also to the development of secondary excitotoxic cell death from other types of injury to neurons, e.g., trauma. Moreover, recent data suggest that release of glutamate from axons injured by ischemia could contribute to the oligodendroglial cell death in periventricular leukomalacia (see later discussion). In addition, release of glutamate from astrocytes may be even more marked than from neurons, under ischemic conditions.[311]

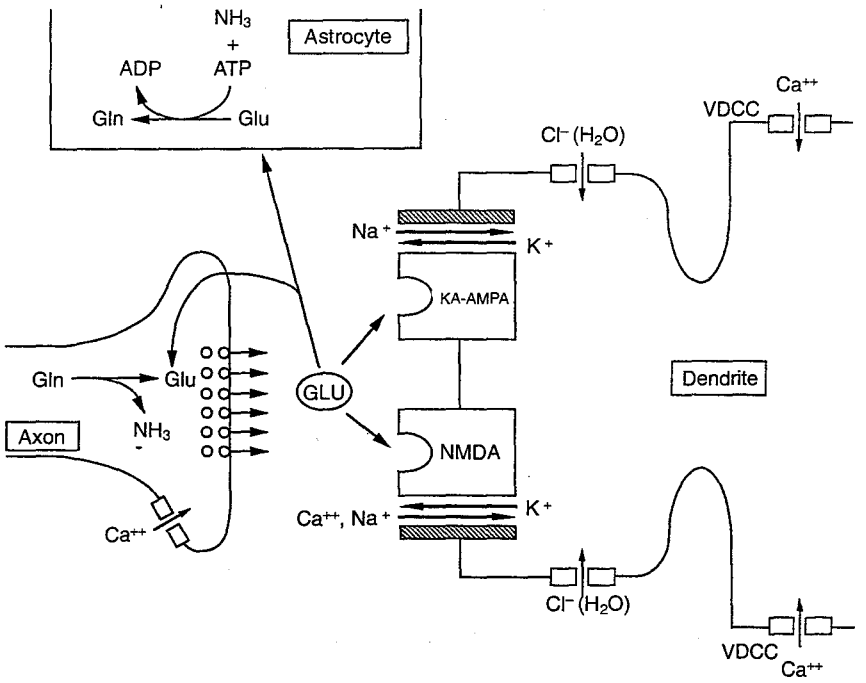


**Figure 6-23** Relationships at the glutamate synapse among the presynaptic axonal terminal, the postsynaptic dendrite, and the associated astrocyte. See text for details. *ADP,* Adenosine diphosphate; *ATP,* adenosine triphosphate; *GLU,* glutamate; *GLN,* glutamine; *KA-AMPA,* kainate–α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; *NMDA, N*-methyl-D-aspartate; *VDCC,* voltage-dependent calcium channel. (Modified from Siesjö BK: *Eur Neurol* 30:3-9, 1990.)

**TABLE 6-15**

**Glutamate Receptors***

| TYPE | FUNCTION |
|---|---|
| **IONOTROPIC** | |
| NMDA | $Ca^{2+}$ entry, $Na^+$ entry |
| AMPA | $Na^+$ entry, $Ca^{2+}$ entry (immature neurons) |
| Kainate | $Na^+$ entry |
| **METABOTROPIC** | |
| ACPD or ibotenate | Phosphoinositide hydrolysis—protein kinase C activation—$Ca^{2+}$ mobilization from endoplasmic reticulum |

*ACPD,* 1-Amino-cyclopentane-15,3R-dicarboxylic acid; *AMPA,* alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; *NMDA, N*-methyl-D-aspartate.
*The receptors are named according to the glutamate analogue most potent in activation of the individual receptor.

Glutamate acts at both *ionotropic and metabotropic receptors* (Table 6-15).[305,307,309,310,312-326] Three of these are *ionotropic,* i.e., are linked to ion channels. The NMDA receptor is linked to an ion channel for $Ca^{2+}$, the AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor, for $Na^+$ entry, and the kainate receptor, for $Na^+$ entry. The AMPA receptor is rendered $Ca^{2+}$ impermeable by the presence of one of its four subunits, namely the GluR2 subunit. Notably this subunit is relatively low in amount during early development, and this feature appears to render the AMPA receptor in immature neurons $Ca^{2+}$ permeable.[309,310] This feature may underlie the involvement of AMPA receptors in hypoxic-ischemic or glutamate-induced death of immature neurons (see later discussion). In general, the NMDA receptor is considered the most crucial for the excitotoxic effects of glutamate. Agents that increase or decrease glutamate activation at the synapse mediated by the NMDA receptor-channel complex are shown in Table 6-16. A fourth glutamate receptor type is *metabotropic,* i.e., is coupled through a guanosine triphosphate (GTP)–binding protein (G protein) to an enzyme producing a second messenger, phospholipase C for phosphoinositide hydrolysis. The resulting products, diacylglycerol and inositol triphosphate, function as second messengers, the former activating protein kinase C, which has many cellular effects, and the latter promoting $Ca^{2+}$ mobilization from the endoplasmic reticulum (see Fig. 6-19).

The normal *ontogeny of glutamate receptors* is relevant to normal brain development and to the vulnerability of immature brain regions to excitotoxic cell death with hypoxia-ischemia. Detailed studies of the development of binding sites for NMDA and non-NMDA receptor agonists in the rat have shown a striking increase in the early phases of brain development.[307-309,326-333] Peak values usually exceed values observed in adults. In addition, several other properties of immature neurons ac-

**TABLE 6-16**

**Excitation at Glutamate Synapse of *N*-methyl-D-aspartate Type**

| SITE | EFFECT ON EXCITATION | |
|---|---|---|
| | INCREASE | DECREASE |
| Presynaptic (glutamate release) | $Ca^{2+}$ | |
| | Theophylline | Adenosine |
| Receptor | Glutamate | APV/CPP |
| | Glycine | Kynurenate |
| Channel | | PCP/MK-801/ketamine/ dextromethorphan |
| | | $Mg^{2+}$ |

*APV,* 2-Amino-5-phosphonovalerate; *CPP,* 3-(2-carboxypiperazine-4-yl)-propyl-1-phosphoric acid; *PCP,* phencyclidine.

**TABLE 6-17**

**Major Evidence Supporting a Critical Role for Glutamate in Hypoxic-Ischemic Neuronal Death in Developing Brain***

- In developing neurons in cell culture, synaptic activity is necessary for hypoxia to lead to neuronal death
- Nonspecific blockade of synaptic activity prevents hypoxic neuronal death in culture
- Specific glutamate receptor-channel blockers also prevent hypoxic neuronal death in culture and in brain slices
- Specific glutamate receptor-channel blockers prevent glutamate-induced cell death in vivo as well as in culture and in brain slices
- Glutamate accumulates extracellularly in vivo with hypoxic-ischemic insult
- Topography of hypoxic-ischemic neuronal death in vivo is similar to the topography of glutamate synapses
- Increased vulnerability of certain brain structures to hypoxic-ischemic injury during early development correlates with transiently increased concentration of glutamate receptors in those structures
- Ontogeny of hypoxic-ischemic neuronal death in vivo is similar to the ontogeny of glutamate-induced neuronal death
- *Delayed* neuronal death after glutamate exposure in cell culture has a correlate in delayed neuronal death after hypoxia-ischemia in vivo, and both can be prevented by specific glutamate receptor-channel blockers, some administered after termination of the insult

*See text for references.

centuate the functional capabilities of these receptors. These properties include a greater electrical excitability, a reduced ability of magnesium to block the channel, and a greater sensitivity to glycine enhancement of the receptor.[307] These receptors, particularly the NMDA receptor, appear to be involved, like other neurotransmitters in development, in such processes as regulation of neurite outgrowth, synapse formation, cell death, selective elimination of neuronal processes, and functional organization of neuronal systems.[307-309,321,329,334] However, in addition, these transient dense expressions of glutamate receptors of enhanced functional capabilities may become the unintended mediators of neuronal death with hypoxia-ischemia (see later discussion). Moreover, the likelihood that these principles apply to developing human brain is supported by the demonstration of early overexpression of glutamate receptors in human hippocampus and deep nuclear structures in basal ganglia and thalamus, regions vulnerable to hypoxic-ischemic injury in the newborn.[307-309,330,335-337]

**Role of glutamate in hypoxic-ischemic cell death in cultured neurons.** The critical role for glutamate in the mediation of hypoxic-ischemic neuronal death is established by a large body of experimental information, as summarized in Table 6-17.[141,201,303-310,323,325,329,330,332,338-344a] The essential neurotoxicity of glutamate was shown initially in cultured neurons and subsequently in other in vitro and in vivo models.

The crucial initial observation was that cultured hippocampal neurons, obtained from the fetal rat, were *resistant to prolonged anoxia before synapse formation* occurred in the cultures, but were very sensitive to the same anoxic insult after synaptogenesis was well developed. Thus such mature cultures markedly deteriorated in the absence of oxygen.[345] However, when synaptic activity in these mature cultures was blocked by addition of high concentrations of magnesium, no effect of anoxia on the cultured neurons occurred (Fig. 6-24). Thus the data demonstrated that *synaptic activity resulted in neuronal death* with oxygen deprivation. This protection by synaptic blockage with magnesium was shown later in a hippocampal slice preparation in which neuronal death could be produced in the CA1 region under anoxic conditions.[346]

Because glutamate (as in hippocampus in vivo) was presumed to be the neurotransmitter mediating the synaptic activity in the experiments with the cultured hippocampal neurons and the slice preparation, a nonspecific postsynaptic blocker of glutamate was investigated to prevent the hypoxic neuronal death in culture. This agent protected neurons dramatically from anoxia (Fig. 6-25).[347] The particular role of glutamate synapses in hippocampal neuronal death was supported further shortly thereafter by the demonstration that hypoxic-ischemic neuronal injury could be prevented in vivo by prior section of glutamatergic afferents to the CA1 region.[348]

The *mechanisms of glutamate-induced neuronal death* in cultured neurons were elucidated next.[201,303,304,306,308-310,321,323,325,329,330,332,338-342] Two basic mechanisms have been identified. One of these is a rapid cell death that occurs in minutes and is initiated by glutamate receptor activation, $Na^+$ entry through all three ionotropic receptors, passive influx of $Cl^-$ down its electrochemical gradient with $H_2O$ following, and ultimately cell swelling and lysis. A second variety, so-called delayed cell death, occurring over many hours, is initiated principally by activation of the NMDA receptor, with influx of $Ca^{2+}$ (as well as $Na^+$) and a series of $Ca^{2+}$-mediated events to cell death (Table 6-13). *Delayed cell death* appears to be the crucial form of neuronal death in vivo,

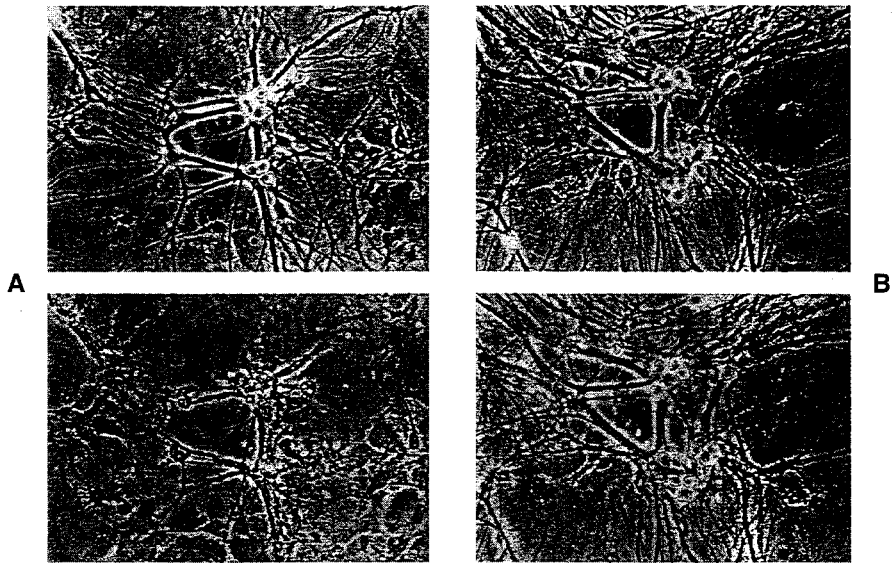


**Figure 6-24** Effect of blockade of synaptic activity on anoxic cell death in hippocampal neuronal cultures. **A,** Phase contrast micrograph *(top)* shows normoxic culture with abundant neurons; *bottom,* cultures rendered anoxic for 24 hours show extensive neuronal destruction provoked by anoxia. **B,** Micrograph shows cultures treated with $MgCl_2$ to block synaptic activity before *(top)* and after *(bottom)* anoxia. Note lack of neuronal destruction. (From Rothman SM: *Science* 20:536, 1983.)

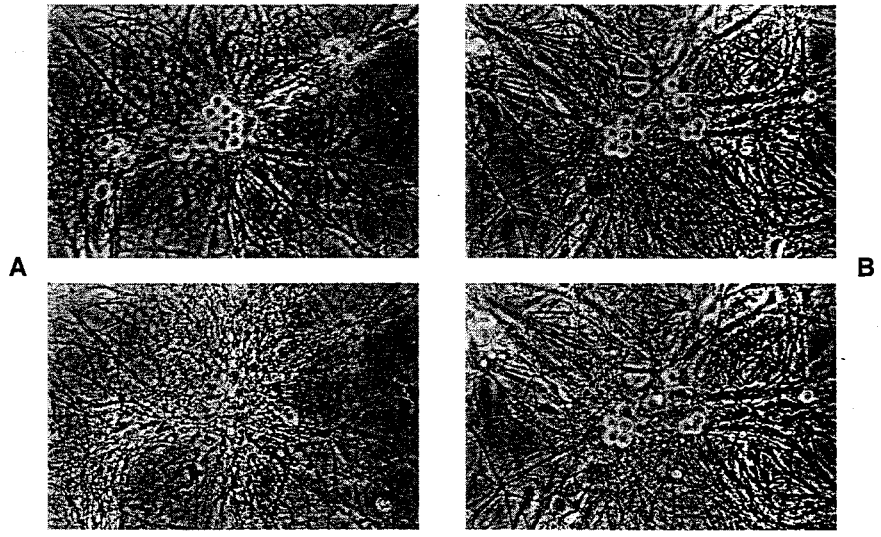


**Figure 6-25** Effect of a blocker of glutamic acid on cell death in hippocampal neuronal cultures. **A,** Phase contrast micrographs show cultures before *(top)* and 8 hours after *(bottom)* anoxia. Note neuronal destruction after anoxia. **B,** Micrographs show cultures treated with the glutamate blocker, gamma-D-glutamylglycine. The appearance before *(top)* and 8 hours after *(bottom)* anoxia are shown. Note prevention of neuronal destruction in the presence of the blocker. (From Rothman SM: *J Neurosci* 4:1884, 1984.)

and the importance of the NMDA receptor and $Ca^{2+}$ influx is well established by studies of specific blockers of the NMDA receptor-channel complex in cultured cells, brain slices, and in vivo models (see later discussion).[141,201,288,303,304,323,325,329,330,338-340,342,349-367] The data support the scheme shown in Fig. 6-26. Note the apparent involvement of all glutamate receptors, although the NMDA receptor is the most crucial. The AMPA receptor may contribute to the $Ca^{2+}$ influx in the immature neuron, as discussed earlier. The deleterious effect of cytosolic $Ca^{2+}$ is presumably mediated by the mechanisms described earlier (Table 6-13). Of importance is the cyclical internal amplification of the mechanisms, with multiple vicious cycles possible in the scheme.

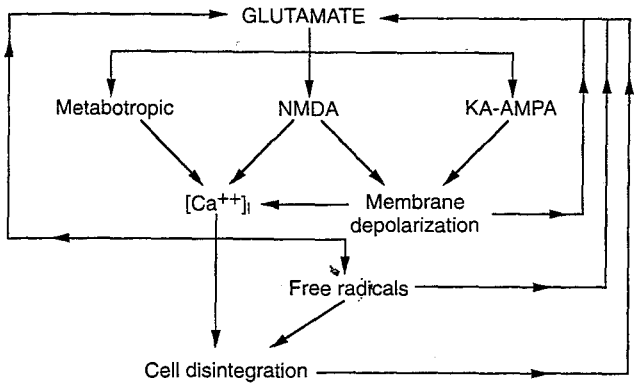


**Figure 6-26** Mechanisms of glutamate-induced neuronal death. Note the potential involvement of both ionotropic (NMDA and KA-AMPA) and metabotropic receptors, although the NMDA receptor appears to be most important. The cyclical amplification mechanisms are shown. *KA-AMPA,* kainate–alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; *NMDA, N*-methyl-D-aspartate.

**Relevance of glutamate-induced excitotoxicity to hypoxic-ischemic injury in vivo.** Relevance of the *glutamate excitotoxic mechanisms* to the in vivo situation is now clear.[201,288,303,304,321,323,325,329,330,332,344,357-368] The first body of evidence establishing this relevance has shown that *extracellular glutamate concentrations in vivo increase* manyfold with hypoxic-ischemic insults.[304] Such increases have been documented in *perinatal* animal models as well as in adults, although glutamate increases tend to be somewhat less in the former models than in the latter.[329,361,363,364,366,369-373] However, studies utilizing microdialysis have documented accumulation of extracellular glutamate in brain of asphyxiated fetal sheep and of hypoxic-ischemic immature rats to concentrations of approximately 500 μmol/L,[369,372,374] concentrations easily sufficient to cause neuronal death in cultured cells. Moreover, glutamate concentrations in the cerebrospinal fluid of asphyxiated human newborns are approximately fivefold greater than concentrations in normal newborns.[375] The reasons for the increase of extracellular glutamate with hypoxic-ischemic insults relate to impaired uptake of glutamate and to excessive release. The impaired uptake is related to defective operation of the energy-dependent glutamate transport system in astrocytes, the reversal of the $Na^+$-dependent glutamate transporter in neurons (because of the depolarization and elevated intracellular sodium levels), and defective function of the glutamine synthetase reaction in astrocytes.[310,376-379] The excessive release of glutamate relates to at least four factors. The first of these is the persistent membrane depolarization resulting from failure of the Na,$K^+$-ATP–dependent pump.[380] Destruction of GABA neurons by hypoxia also may contribute to the excessive release of glutamate.[381] A third factor is the rapid blockade of inhibitory synaptic transmission with relative preservation of excitatory synaptic transition with anoxia in the immature versus adult animal.[382] A fourth factor promoting excessive release of glutamate is the acute development of epileptic phenomena after hypoxia in the immature (but not mature) animal.[30]

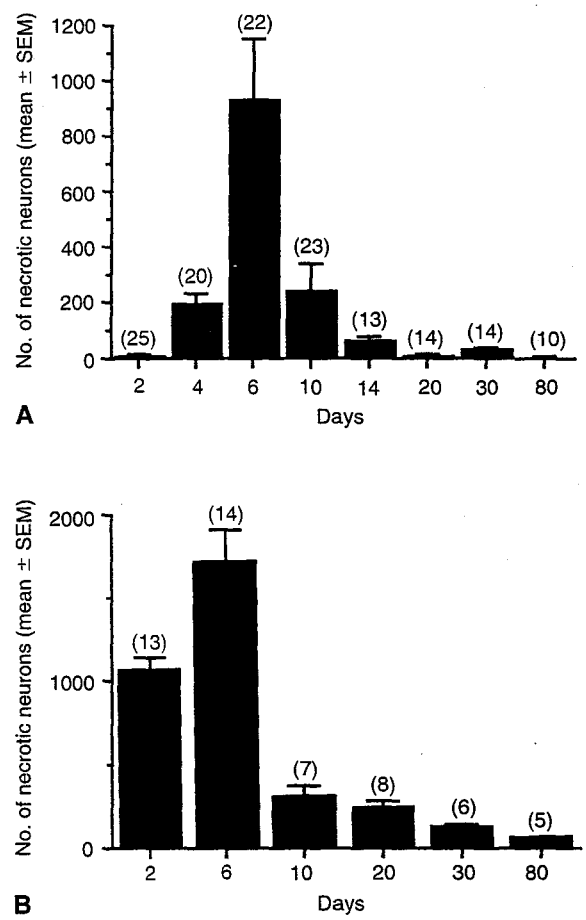


**Figure 6-27** Similar developmental profiles of hypoxic-ischemic and NMDA-mediated neuronal death. Mean number of neurons destroyed in rat brain under conditions of, **A,** hypoxia-ischemia and, **B,** intrastriatal injection of NMDA (9 nmol). Pups at the age of 6 days show the highest number of necrotic neurons under either condition. The numbers on top of each column represent the number of animals studied for each group. *NMDA, N*-methyl-D-aspartate; *SEM,* standard error of the mean. (From Ikonomidou C, Mosinger JL, Salles KS, et al: *J Neurosci* 9:2809-2818, 1989.)

The second body of evidence delineating the relevance of glutamate to the in vivo situation is the demonstration in a wide variety of *perinatal models* of hypoxia-ischemia that *glutamate is toxic to neurons* and that this *toxicity is particularly marked* in the *immature* versus the mature animal.[28,306,308,309,329,342,344a,383] In general, in the immature animal the most toxic glutamate analogue is NMDA, with AMPA less toxic and kainate least toxic.[288,306-309,329,330,342,384-386] The time of peak sensitivity in the rat is 6 days to NMDA and 10 days to AMPA. The especial vulnerability of the brain of the immature animal to hypoxia-ischemia and the importance of the NMDA receptor in this ontogeny of vulnerability is illustrated by the similar developmental profiles of hypoxic-ischemic neuronal death and NMDA-mediated neuronal death (Fig. 6-27). Presumably, this particular vulnerability of the immature animal relates at least in part to the transient dense expres-

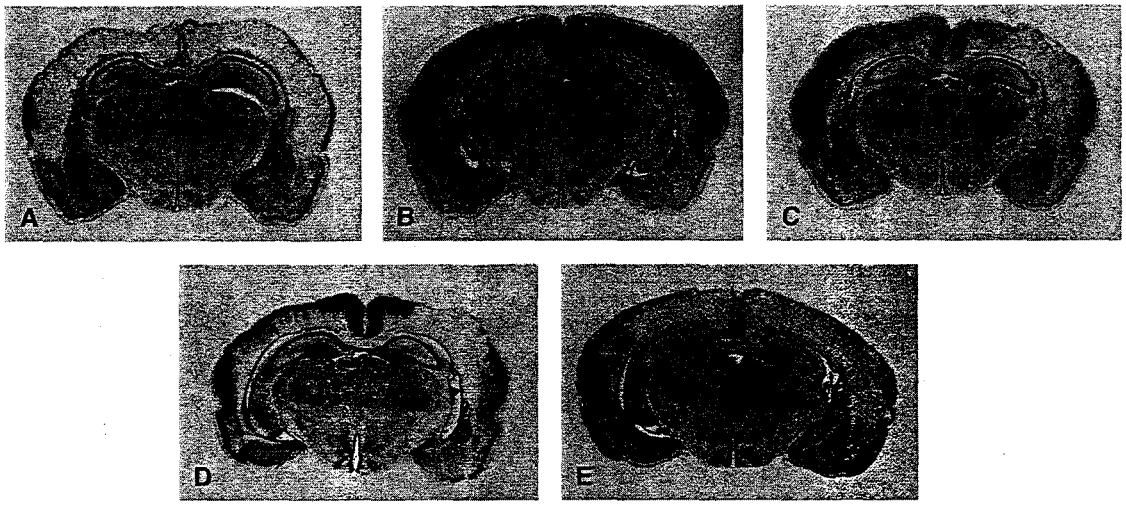


**Figure 6-28** Coronal brain sections showing the middle of the dorsal hippocampus. **A** is from a saline-injected control, and **B** through **E** are from rat pups that were given MK-801 intraperitoneally, **B,** 0.5 hour before and, **C,** immediately, **D,** 1 hour, and, **E,** 4 hours after the hypoxic-ischemic insult. Note sharply demarcated hypoxic-ischemic infarction in **A.** Neuroprotective effects of MK-801 are seen in **B, C,** and **D** in a time-dependent fashion. (H & E stain, original magnification × 2.5 before 31% reduction.) (From Hattori H, Morin AM, Schwartz PH, et al: *Neurology* 39:713-718, 1989.)

sion of NMDA receptors during brain development (see earlier discussion).

The third body of evidence linking glutamate to hypoxic-ischemic injury relates to the fact that the topography of glutamate receptors, particularly NMDA receptors, corresponds closely to the topography of hypoxic-ischemic neuronal injury observed in vivo.[288,306-309,326,329,332,333,387] Although more data are needed concerning the perinatal human and the correspondence is not perfect, the overwhelming balance of evidence indicates a close relation between regional neuronal vulnerability to hypoxia-ischemia and regional distribution of glutamate receptors (see Chapter 8).

Finally, perhaps the strongest evidence of the relevance of the glutamate excitotoxic mechanism to the in vivo situation has been the demonstration of *protection from neuronal death in a variety of perinatal hypoxic-ischemic models by treatment with glutamate receptor-channel blockers.*[337,357,358,362,365,367,368,386,388-400] Nearly all experiments have utilized compounds with effects on the NMDA receptor-channel complex, and in nearly all benefit was achieved. AMPA antagonists provide partial protection. Benefit was manifested as prevention of morphological or biochemical evidence of injury. Although in most studies the antagonist was administered at the onset or during the insult, most striking has been the marked, though not complete, protection in experiments in which the antagonist was administered after termination of the insult (Fig. 6-28). Available data suggest that treatment within 1 to 2 hours is highly effective. This response is compatible with the concepts that delayed cell death is the operative mechanism and that treatment in the clinical situation after termination of the insult ultimately may be beneficial.

### Role of Inflammation-Cytokines

A series of studies, especially in adult models of hypoxic-ischemic brain injury, suggest that inflammatory mechanisms, particularly involving certain cytokines, are important in the final common biochemical pathway to hypoxic-ischemic cell death.[401-412] The principal sequence of events is activation of microglia in the first hours after the insult, with release of a variety of neurotoxic products, including excitatory amino acid agonists, reactive oxygen species, nitric oxide, proteases, and certain cytokines. Most important among the latter appear to be IL-1-beta and tumor necrosis factor-alpha (TNF-alpha). IL-1-beta is particularly important in the activation of endothelial-leukocyte adhesion molecules, especially intercellular adhesion molecule-1 (ICAM-1). The leukocytes involved include not only polymorphonuclear cells but also mononuclear cells, especially of the monocytic-phagocytic series. The leukocytes are important in release of deleterious compounds, especially reactive oxygen species and cytokines. Certain of the cytokines, e.g., TNF-alpha, may have direct toxic effects. IL-1-beta may lead to apoptotic cell death when acted upon by a family of enzymes, IL-1-beta–converting enzyme (ICE), and ICE-like proteases.[413]

That the sequence of events just explained appears to be operative in *perinatal hypoxia-ischemia* is supported by both perinatal experimental models (Table 6-18) and human epidemiological data.[408-415d] Thus studies in perinatal rats have shown *activation of microglia* after hypoxia-ischemia that proceeds more rapidly than in adult animals.[410] Activated microglia begin to accumulate in the first 4 hours after reperfusion and continue to increase over the next 48 hours. *Neutrophil accumulation in brain blood vessels* has been documented upon reperfu-

**TABLE 6-18**

**Inflammation and Cytokines in Experimental Models of Perinatal Hypoxic-Ischemic Brain Injury***

| |
|---|
| Microglial activation occurs promptly and briskly after hypoxia-ischemia |
| Neutropenia is protective |
| Cytokines (especially IL-1-beta and TNF-alpha) increase promptly in brain after hypoxia-ischemia |
| IL-1 receptor antagonist ameliorates hypoxic-ischemic brain injury when administered either before or at the termination of hypoxia-ischemia |

*See text for references.

sion after hypoxia-ischemia in the neonatal rat and piglet.[411,414] In the rat model the accumulation peaked at 4 to 8 hours after reperfusion, although much less infiltration of brain parenchyma was apparent than that occurring in adult animals.[411] However, the neutrophilic accumulation in blood vessels was shown to be important in the genesis of the brain injury by the marked reduction in cerebral edema in animals made neutropenic before the hypoxic-ischemic insult. Whether the deleterious effect of the neutrophils is related to adherence to endothelium and resulting vascular injury or obstruction or both remains to be established. Finally, a burst of *cytokine expression* has been documented in the first 6 hours after cerebral hypoxic-ischemic insult in the immature rat.[408,409,412] Both IL-1-beta and TNF-alpha have been shown to increase markedly in brain in the first 4 to 6 hours after the insult, and notably intracerebral injection of IL-1 receptor antagonist has been shown to ameliorate the brain injury.[409,415]

Additionally supportive of a role for cytokine expression in the genesis of hypoxic-ischemic brain injury are data obtained from studies of levels of *cytokines in human infants*.[415c-418] Thus in asphyxiated infants CSF IL-6 levels correlated directly with the severity of the brain injury.[416] IL-6 is a pleiotropic cytokine with both proinflammatory and antiinflammatory properties. In a careful series of studies of a selected group of children with cerebral palsy of unclear etiology, analysis of archived neonatal blood specimens revealed markedly elevated levels of several proinflammatory cytokines, including IL-1, IL-6, and alpha-, beta-, and gamma-interferons.[417,418] These findings are consistent with the finding in a similar cohort (of term infants) of a relation between unexplained cerebral palsy and evidence of maternal infection.[419] Further studies, especially of infants with clearly documented perinatal hypoxic-ischemic insults, will be of great interest.

## Biochemical Mechanisms of Oligodendroglial Death with Hypoxia-Ischemia

The mechanisms of oligodendroglial death caused by hypoxia-ischemia bear important similarities to those just discussed concerning neuronal death. Thus energy failure, $Ca^{2+}$ accumulation, free radical attack, cytokine-mediated events, and glutamate toxicity are involved. However, the relative importance and the specific operation of these mechanisms are somewhat different for oligodendroglial versus neuronal death. Moreover, certain additional factors appear to be uniquely involved in oligodendroglial death. These various distinctive features concerning oligodendroglial injury are critical for the rational formulation of preventative and ameliorative interventions for such injury, as I discuss later in this chapter.

### *Intrinsic Vulnerability of Oligodendroglia to Hypoxic-Ischemic Injury*

Both human neuropathological studies and experimental work indicate that oligodendroglial cells are particularly vulnerable to hypoxic-ischemic injury. As I discuss in Chapter 8 in relation to periventricular leukomalacia, diffuse injury to oligodendroglia, presumably early differentiating cells in the oligodendroglial lineage, is a prominent feature of this critical form of neonatal white matter injury. Moreover, personal studies of early differentiating oligodendroglia in culture indicate a particular vulnerability to combined glucose and oxygen deprivation but not to either insult alone. A similar vulnerability to the combined insult has been documented by others.[420,420a] A particular vulnerability of differentiating oligodendroglia or immature white matter or both to hypoxic-ischemic injury has been documented in mid-gestation fetal sheep, late-gestation fetal sheep, near-term fetal sheep, 1-day-old piglet, and 5- and 7-day-old rat pups.[97,183,421-425] Our studies of the immature rat indicate that the likely target of the hypoxic-ischemic injury is either the preoligodendrocyte ($O4^+$) or the immature oligodendrocyte ($O1^+$) or both.[426] (Recall from Chapter 2 that the sequence of oligodendroglial development is $A2B5^+$ oligodendrocyte precursor cell, $O4^+$ preoligodendrocyte, $O1^+$ immature oligodendrocyte, and mature, myelin basic protein [$MBP^+$] oligodendrocyte.)

### *Vulnerability of Early Differentiating Oligodendroglia to Free Radical Attack*

To investigate the mechanisms underlying the intrinsic vulnerability of early differentiating oligodendroglia to hypoxic-ischemic injury, we have studied this cell lineage in a defined system in culture. Before undertaking those studies in depth we set out to determine the specific stage in the oligodendroglial lineage present in the cerebral white matter of the human premature infant and the presumed target, therefore, of the diffuse oligodendroglial injury in periventricular leukomalacia. Our initial studies indicated that the dominant form of oligodendrocyte in the white matter of the human premature infant and perhaps therefore the key cellular target in periventricular leukomalacia is an early differentiating oligodendrocyte at the $O4^+$ preoligodendrocyte stage or the $O1^+$ immature oligodendrocyte stage or at both stages.[427-429]

With this information concerning human brain we then set out to develop a chemically defined culture system to study, in nearly pure form, the different stages of the oligodendroglial lineage, i.e., $A2B5^+$, $O4^+$,

O1$^+$, and MBP$^+$, as identified by their specific immunocytochemical markers (see Chapter 2). Utilizing a modification of several previously published methods, we have succeeded in obtaining, in culture, oligodendrocytes conforming to these stages. With this highly developed system of nearly pure oligodendroglial cultures, we have thus far addressed four important questions, as follows. First, are oligodendrocytes particularly vulnerable to free radical attack? Second, what is the mode of cell death caused by such free radical attack? Third, is any such vulnerability to free radical attack maturation dependent? Fourth, what are the mechanisms underlying the maturation-dependence of this vulnerability?

We asked first the question of the *vulnerability of oligodendrocytes to free radical attack,* because periventricular white matter injury has been considered to be an ischemic lesion (see Chapter 8) and as discussed earlier regarding neuronal death, an elevation in a variety of reactive oxygen species is a well-established sequela of ischemia-reperfusion.[238,430-432] Direct and indirect evidence for increases in oxygen free radicals in brain during reperfusion after hypoxia-ischemia has been obtained in studies of neonatal and fetal animals.[137,190,233,235,236,244,245,251-252b,261,269,433-435] This evidence includes direct determinations of elevated free radicals as well as of amelioration of deleterious neural effects by the use of free radical scavengers. In two model systems of free radical accumulation, we have shown that early differentiating oligodendrocytes in culture indeed are exquisitely vulnerable to free radical attack.[436-439] Moreover, clinically safe free radical scavengers, e.g., vitamin E, totally prevented the oligodendroglial death caused by free radical attack (Fig. 6-29).

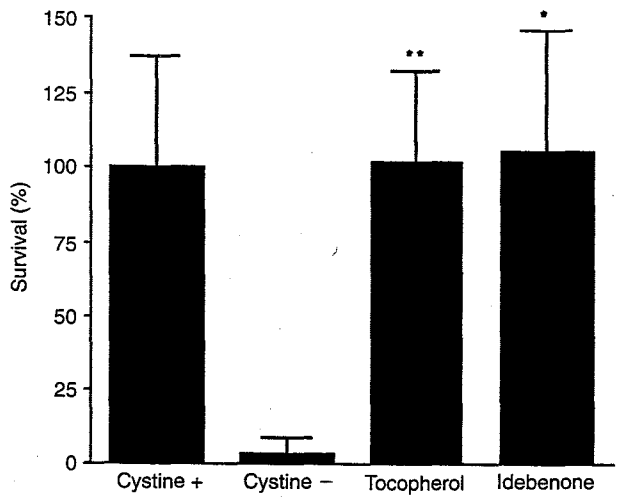


**Figure 6-29** Free radical scavengers, alpha-tocopherol or idebenone, protect developing oligodendrocytes in culture from free radical attack. Free radical attack was produced by the use of cystine-depleted medium, which leads to oxidative stress by provoking glutathione depletion. Note the minimal survival in cystine-depleted medium. When alpha-tocopherol or idebenone was added to the cystine-depleted medium, total protection from free radical–mediated death was observed. (From Back SA, Gan X, Li Y, et al: *J Neurosci* 18[16]:6241-6253, 1998.)

### *Mode of Oligodendroglial Death with Free Radical Death*

Having demonstrated that early differentiating oligodendrocytes are exquisitely vulnerable to free radical–mediated cell death, we next set out to determine the mode of cell death, because the specific form of cell death may provide valuable insights into the molecular mechanisms. The determination of specific mode of death is relevant to periventricular white matter injury, in part because findings obtained in several model systems suggest that a moderate insult leads to neuronal death by apoptosis and a severe insult, to death by necrosis.[182,440] As discussed in Chapter 8 the diffuse oligodendroglial injury in periventricular leukomalacia is likely to be related to moderate ischemia, as contrasted with severe ischemia in deep periventricular white matter that results in necrosis of all cellular elements. Properties intrinsic to the cell also appear important in determining mode of cell death. Thus as discussed earlier, recent data show that ischemia may lead to necrosis or apoptosis in different neuronal populations in vivo because of still-to-be-defined differences

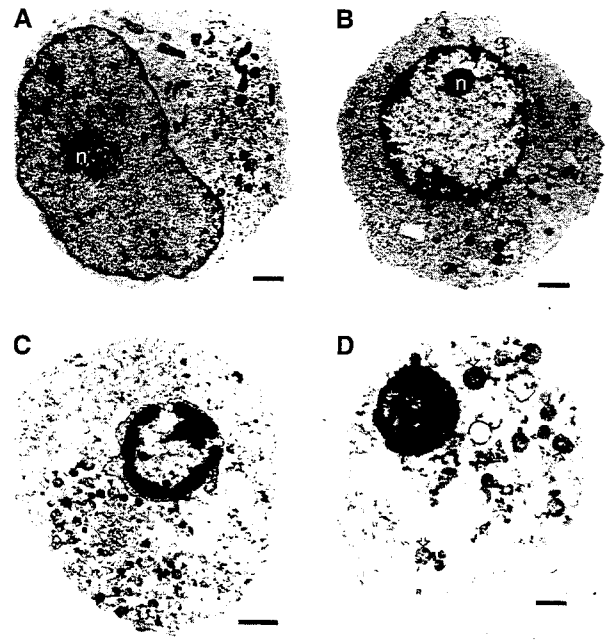


**Figure 6-30** Free radical attack causes apoptotic cell death in developing oligodendrocytes. The ultrastructural characteristics of, **A,** a control cell and, **B** through **D,** cells undergoing progressive free radical attack in cystine-depleted medium over 14 hours are shown. Note in **B** the margination and clumping of chromatin; in **C,** the condensed marginated chromatin but intact nuclear and plasma membranes; and in **D,** the shrunken nucleus with very condensed chromatin but still-intact nuclear and plasma membranes. *n,* Nucleolus. (From Back SA, Gan X, Li Y, et al: *J Neurosci* 18[16]:6241-6253, 1998.)

in the intrinsic properties of the neurons or in their local environment.[185] Important intrinsic properties in the determination of mode of cell death may relate to the developmental stage of the cell. Thus in certain paradigms the susceptibility to apoptosis is enhanced in immature (versus mature) cortical neurons.[184] Moreover, as noted earlier, recent studies in the neonatal piglet subjected to hypoxia-ischemia have demonstrated exclusively necrotic cell death in certain neuronal populations, both necrosis and apoptosis in other neuronal populations, but exclusively apoptotic cell death in immature cerebral white matter.[183] Similarly, the chromatin clumping and nuclear condensation so characteristic of the "acutely damaged glia" of the diffuse component of human periventricular leukomalacia (see Chapter 8) also suggest the possibility of apoptotic cell death. Consistent with all of these data, our studies of oligodendrocytes subjected to free radical attack in culture in fact show features consistent with apoptosis as the mechanism of cell death (Fig. 6-30).[439]

### Maturation-Dependence of Vulnerability of Oligodendroglia to Free Radical Attack

We asked *whether the exquisite vulnerability of oligodendrocytes to free radical attack and cell death is maturation dependent.* Utilizing our stage-specific system of oligodendrocytes in culture and the cystine deprivation model of free radical attack, we have shown that the early differentiating oligodendrocyte is vulnerable to free radical attack, whereas the mature oligodendrocyte is resistant (Fig. 6-31).[439] This maturation-dependent vulnerability thus may be critical for the predilection of this lesion for the human brain early in life and the absence of the lesion in similar form after oligodendroglial maturation and myelination occur.

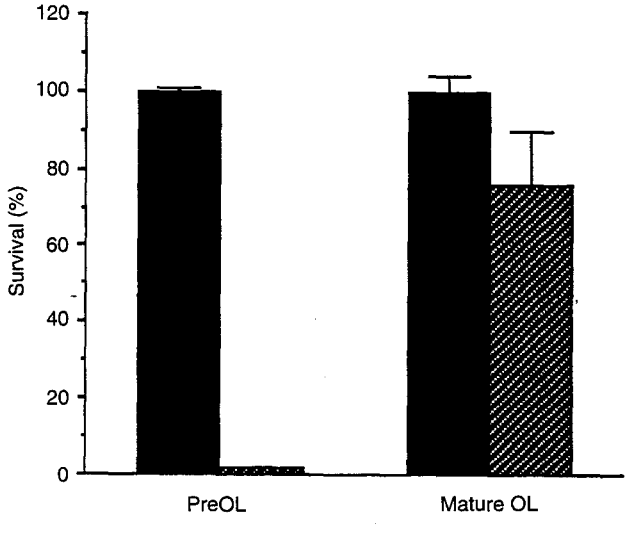


**Figure 6-31** Developing preoligodendrocytes *(PreOL)* are exquisitely vulnerable to free radical attack (cystine-depleted medium), whereas mature oligodendrocytes *(Mature OL)* are resistant. Solid bars are control cells in cystine-containing medium, and hatched bars are cells undergoing free radical attack in cystine-depleted medium. (From Back SA, Gan X, Li Y, et al: *J Neurosci* 18[16]:6241-6253, 1998.)

### Mechanisms Underlying Maturation Dependence of Oligodendroglia to Free Radical Attack

The last of the four questions raised earlier, i.e., *the mechanisms underlying the maturation dependence of the vulnerability of early differentiating oligodendrocytes to free radical attack,* is perhaps the most important. Our initial data, coupled with information derived from studies of experimental models[441-447] and limited analyses of autopsied human brain,[448-450] suggest a maturation-dependent window of vulnerability to free radical attack during oligodendroglial development. This window of vulnerability appears to relate to a combination of a delay in development of antioxidant defenses, especially involving detoxification of hydrogen peroxide, and the accumulation of iron. The antioxidant defenses involved are particularly glutathione and catalase. As shown in Fig. 6-21, when these defenses fail or are overwhelmed, hydrogen peroxide accumulates, and in the presence of $Fe^{2+}$ the Fenton reaction produces the deadly hydroxyl radical. Early in differentiation oligodendroglia are likely to accumulate excessive amounts of iron because of the active acquisition of iron required for oligodendroglial differentiation and probably also because of the accumulation of non-protein-bound iron as a consequence of hypoxic-ischemic insult (see earlier discussion). Interestingly, studies of plasma of human premature infants suggest both a propensity to generate free radicals, including the hydroxyl radical, and impaired antioxidant defenses.[451-458a]

Thus the proposed relationship between ischemia-reperfusion and cell death in the oligodendroglial lineage is provided in Fig. 6-32. The central role of reactive oxygen species and free radical attack is shown. Iron may play a critical role in the biochemical processes leading to the generation of the hydroxyl radical by the Fenton reaction.

### Potential Role of Hemorrhage in Oligodendroglial Death

The particular propensity for the occurrence of hemorrhage in the premature brain (see Chapter 11) may accentuate this maturation-dependent vulnerability to free radical attack. Thus the incidence of periventricular leukomalacia is higher in infants who sustain intraventricular hemorrhage versus those who do not, whether studied postmortem[459,460] or in the living infant.[461-463] Although several reasons for this relationship seem possible, including similarities of the pathogenesis of the two lesions,[461] an excellent possibility is that the hemorrhage provides a rich source of iron for the generation of reactive oxygen species. Of particular importance in this context is the recent observation that the expression of heme oxygenase is especially high in immature cerebral white matter in the rat.[464] Because heme oxygenase is the rate-limiting enzyme that metabolizes heme to biliverdin and $Fe^{2+}$, the abundance in the white matter might provide a large source of $Fe^{2+}$ when hemorrhage is present.

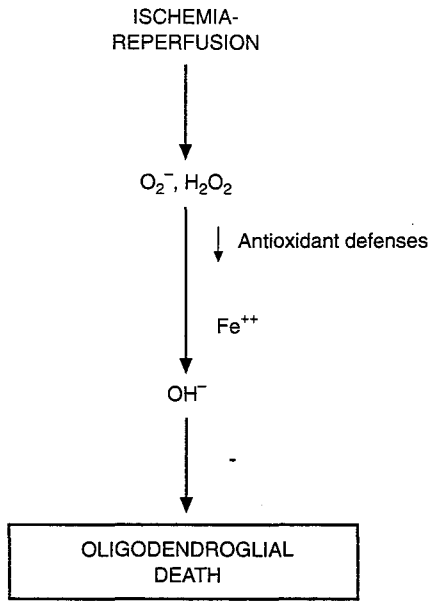


**Figure 6-32** Proposed core relationship between ischemia-reperfusion, free radical attack, and oligodendroglial death. See text for details.

## *Intrauterine Infection-Inflammation and Cytokine Release in Oligodendroglial Death*

An important series of clinical, epidemiological, neuropathological, and experimental studies suggest that maternal intrauterine infection-inflammation and/or cytokines are involved in the pathogenesis of a proportion of cases of periventricular leukomalacia. Thus a role for maternal-fetal infection, endotoxin, and presumably endotoxin-mediated cytokine release in the pathogenesis of periventricular white matter injury was suggested initially by neuropathological-epidemiological studies of infant brain and related experimental studies of Gilles and co-workers[465-467] approximately 20 years ago. A recent demonstration of cerebral white matter lesions in fetal rabbits after the induction of maternal intrauterine infection is consistent with the earlier observations.[468] Several recent human studies lend further support to a contributory role for such factors in the pathogenesis of periventricular leukomalacia. Thus the incidence of periventricular leukomalacia and/or cerebral palsy in premature infants is increased in the presence of (1) evidence for maternal-placental-fetal infection,[469-472] (2) elevated levels of interleukin-6 in cord blood,[473] (3) elevated levels of interleukin-6 and interleukin-1-beta in amniotic fluid,[474] and (4) elevated levels of all interferons, IL-1 and IL-6 among other cytokines, in neonatal blood.[417-419] Moreover, although potentially a secondary effect of ischemia (see later discussion), also possibly supportive of a relation to intrauterine infection and cytokines is the demonstration within lesions of periventricular leukomalacia of interleukin-6 and TNF-alpha.[475,476] Some studies, although not all, suggest that TNF-alpha is toxic to oligodendrocytes.[477-482] Our preliminary data with developing oligodendrocytes are consistent with studies showing that interferon-gamma exhibits high toxicity to oligodendrocytes.[480] Moreover, it has been shown that immature oligodendrocytes in culture are more vulnerable to the cytotoxicity of interferon-gamma than are mature oligodrendrocytes.[483,484] In addition, TNF-alpha potentiates the toxicity of interferon-gamma to developing oligodendrocyte.[485]

A role for cytokines and inflammatory cells in the pathogenesis of cerebral oligodendroglial injury in the absence of infection also must be considered. Thus, as discussed earlier, it is well established in animal models that ischemia-reperfusion is accompanied rapidly by activation of microglia, secretion of cytokines, and mobilization, adhesion, and migration of macrophages and inflammatory cells. Multiple cytokines and white blood cells are involved. Because production of reactive oxygen species is one mechanism for the cytotoxicity caused by these factors,[238,402,486,487] the oligodendroglial vulnerability to such species is relevant in this context.

Finally, infection and cytokines, individually or in combination, may lead to ischemia-reperfusion and thereby oligodendroglial injury. Thus a distinct disturbance of vascular endothelium can be produced by endotoxin and was observed in brain in newborn kittens who developed periventricular leukomalacia after endotoxin injection.[466,488,489] In addition, endotoxin has been shown to cause arterial hypotension in newborn dogs, in sublethal doses, and to produce in the same animals periventricular white matter injury.[490] Moreover, the deficits in blood flow and metabolism produced in cerebral white matter by hemorrhage-induced hypotension resulted to a similar degree with endotoxin-induced hypotension. Later work in immature rabbits also showed a particular propensity for cerebral white matter to develop both decreased cerebral blood flow 1 to 2 hours after endotoxin administration and histological evidence for necrosis subsequently.[491] Finally, because of the pronounced vasoactive effects of certain cytokines (e.g., TNF-alpha) and of other compounds (e.g., nitric oxide) released as part of the inflammatory cascade, an impairment of cerebrovascular regulation and thereby risk for ischemic injury also could become operative. Further data in developing animals will be of great interest.

## *Potential Role for Glutamate in Oligodendroglial Death*

A potential role for excess extracellular glutamate in the pathogenesis of periventricular leukomalacia is suggested by four basic observations. First, the earliest and especially prominent neuropathological feature of focal periventricular leukomalacia is coagulation necrosis and disruption of axons.[492,493] Second, in both human and experimental models of periventricular leukomalacia, immunocytochemical studies suggest that axonal injury can occur without overt focal necrosis.[494,495] Third, neurons, and presumably axons, contain millimolar concen-

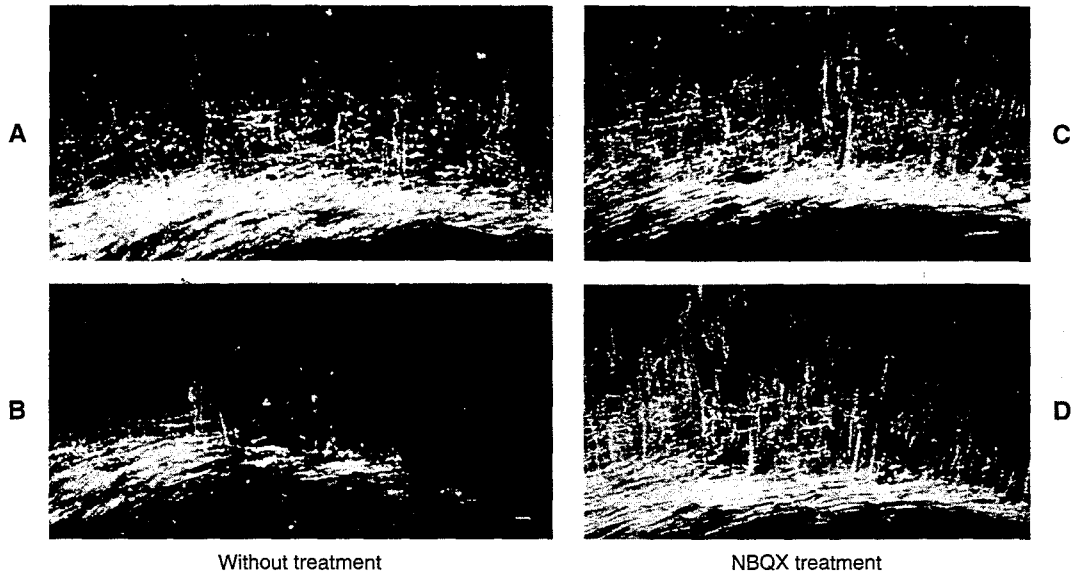


**Figure 6-33** Protection from hypoxic-ischemic cerebral white matter injury in the immature (P7) rat by systemic administration of the non-*N*-methyl-D-aspartate (NMDA) receptor blocker, NBQX, after the termination of the insult. The four panels show myelin basic protein (MBP) staining in cerebral white matter at P11, 4 days after cerebral hypoxia-ischemia produced by unilateral carotid ligation and hypoxemia at P7. Developing oligodendrocytes at P7 normally differentiate into MBP-positive oligodendrocytes by P11. At the termination of the insult on P7, animals received either injection of, **A** and **B,** vehicle or, **C** and **D,** NBQX, the non-NMDA receptor blocker. **A** and **C** show MBP staining contralateral to the ligation, and **B** and **D,** staining ipsilateral to the ligation. Note the marked disturbance in the hypoxic-ischemic (ipsilateral) hemisphere in the nontreated animal **(B** *compared with* **A)** and the protection afforded by NBQX in the hypoxic-ischemic hemisphere in the treated animal **(D** *compared with* **C).** (From Follett P, Rosenberg P, Volpe JJ, Jensen F: manuscript submitted for publication, 2000.)

trations of glutamate,[304,496,497] which could leak into the extracellular space upon disruption. (Other potential sources of glutamate with brain ischemia-reperfusion include failure of glutamate uptake in astrocytes and neurons, reversal of glutamate transporter function in astrocytes and in oligodendroglia,[420a] and cytokine effects on astrocytes, as discussed earlier.) Fourth, glutamate causes glutathione depletion in oligodendrocytes and thereby free radical–mediated cell death.[436] This effect is mediated by activation of a glutamate-cystine exchange transporter, such that glutamate uptake results in cystine efflux, cystine depletion, and thereby impaired glutathione synthesis.[436] The result is free radical–mediated death, which can be prevented totally by such free radical scavengers as vitamin E. Fifth, activation of the AMPA-kainate type of glutamate receptor can lead to oligodendroglial death in culture and in vivo.[426,498-502b] Moreover, our data indicate that this AMPA-kainate form of oligodendroglial death occurs only in the developing oligodendrocyte and not in the mature oligodendrocyte. The relevance of this phenomenon to hypoxic-ischemic cerebral oligodendroglial injury was shown by the demonstration that in the immature rat such injury is prevented by the *systemic* administration of the non-NMDA receptor antagonist NBQX *after termination of the insult* (Fig. 6-33).[426] Thus taken together these findings indicate that glutamate leads to toxicity of immature oligodendroglia by both receptor- and non-receptor-mediated mechanisms. Both mechanisms can be counteracted, the latter by free radical scavengers and the former by use of specific receptor antagonists.

### *Conclusions—Mechanisms of Oligodendroglial Cell Death*

The data support the formulation shown in Fig. 6-34 concerning the mechanisms of death of early differentiating oligodendroglia with ischemia-reperfusion. The fundamental, core mechanism is the vulnerability of early differentiating oligodendroglia to free radical attack. Interacting with that fundamental mechanism is the generation of reactive oxygen species by the excess $Fe^{2+}$ from hemorrhage and by the actions of cytokines and leukocytes, coupled with the adverse effect of glutamate on glutathione levels and thereby a critical antioxidant defense. In addition, glutamate and cytokines may exert independent cytotoxic effects on the differentiating oligodendrocyte.

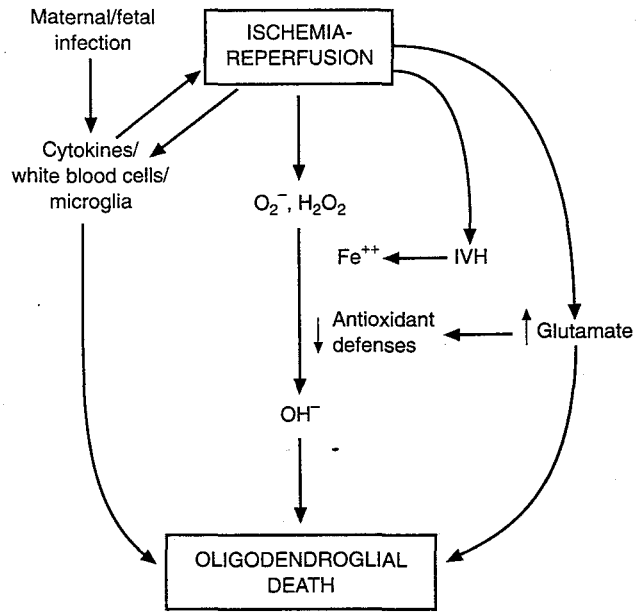


**Figure 6-34** Proposed mechanisms of oligodendroglial death following ischemia-reperfusion. Engrafted on the core relationship between free radical attack and oligodendroglial death are likely interacting effects of IVH, glutamate released by axonal injury and other factors, and maternal-fetal infection with actions of cytokines and inflammatory cells. *IVH*, Intraventricular hemorrhage.

## Interventions Based on Biochemical Mechanisms of Hypoxic-Ischemic Brain Injury

The insights into the biochemical mechanisms of neuronal and glial injury with perinatal ischemic-reperfusion insults, as just discussed, provide a rational basis for formulation of interventions to interrupt those mechanisms and thereby prevent or ameliorate the injury. The general sequence of operation of these mechanisms provides the framework for this discussion of such interventions as outlined in Table 6-19. The following information is devoted almost exclusively to data obtained in *perinatal* models of hypoxia-ischemia.

### *Decrease in Energy Depletion*

Depletion of high-energy phosphates, not necessarily severe, almost certainly initiates the cascade of events leading to neuronal and glial death. The importance of maintenance of glucose at physiological levels was discussed earlier, and data in this regard are summarized in Table 6-8. Barbiturates administered in high doses can lead to decreased cerebral metabolic rate and thereby energy preservation (see Chapter 9).[10] The apparent protective effect of mild hypercapnia, at least in part, may act by decreasing energy utilization.[103]

The most potent and promising intervention to prevent energy depletion is *mild hypothermia* (Fig. 6-35).[503-506a] This effect correlates with the neuroprotective benefit of this approach. Moreover, mild hypothermia ameliorates the secondary energy failure that follows many hours of reperfusion.[507] A preventative-ameliorative effect of mild hypothermia has been documented in a wide variety of perinatal animal models of hypoxia-ischemia.[503,504,506-519] In nearly all studies, hypothermia has been instituted during hypoxia-ischemia or immediately upon reperfusion or both. However, in one informative study of ischemic fetal sheep, mild cooling of the cranium *instituted 5.5 hours after the insult* resulted in reduction of injury assessed electrophysiologically and neuropsychologically (Fig. 6-36). (This beneficial effect did not occur in the fetal sheep if hypothermia was instituted at 8.5 hours, just

**TABLE 6-19**

**Potentially Valuable Interventions in Prevention or Amelioration of Perinatal Hypoxic-Ischemic Brain Injury***

**DECREASE ENERGY DEPLETION**
Glucose
Hypothermia
Barbiturates
Hypercapnia (mild)

**INHIBITION OF GLUTAMATE RELEASE**
Calcium channel blockers
Magnesium
Adenosine or adenosine agonists
Hypothermia
Free radical scavengers
Lamotrigine
Phenytoin

**AMELIORATION OF IMPAIRMENT IN GLUTAMATE UPTAKE**
Hypothermia

**BLOCKADE OF GLUTAMATE RECEPTORS**
NMDA receptor antagonists (MK-801, magnesium, ketamine, dextrorphan)
Non-NMDA receptor antagonists (NBQX, CNQX)

**INHIBITION OF LEUKOCYTE-MICROGLIAL-CYTOKINE EFFECTS**
Neutropenia
Platelet activation factor antagonists
IL-1 receptor antagonists
Anti–intercellular adhesion molecule–1 (ICAM) antibody
Anticytokine antibodies or drugs

**BLOCKADE OF DOWNSTREAM INTRACELLULAR EVENTS**
Hypothermia
Free radical synthesis inhibitors (allopurinol, indomethacin, iron chelators, magnesium)
Free radical scavengers (vitamin E, spin traps, 21-aminosteroids)
NOS inhibitors-scavengers (nitroarginine derivatives)
Antiapoptotic agents, including growth factors (insulin-like growth factor–1, brain-derived neurotrophic factor, nerve growth factor, growth hormone)
Monosialogangliosides (GM-1)

*NMDA, N*-Methyl-D-aspartate.
*See text for references.

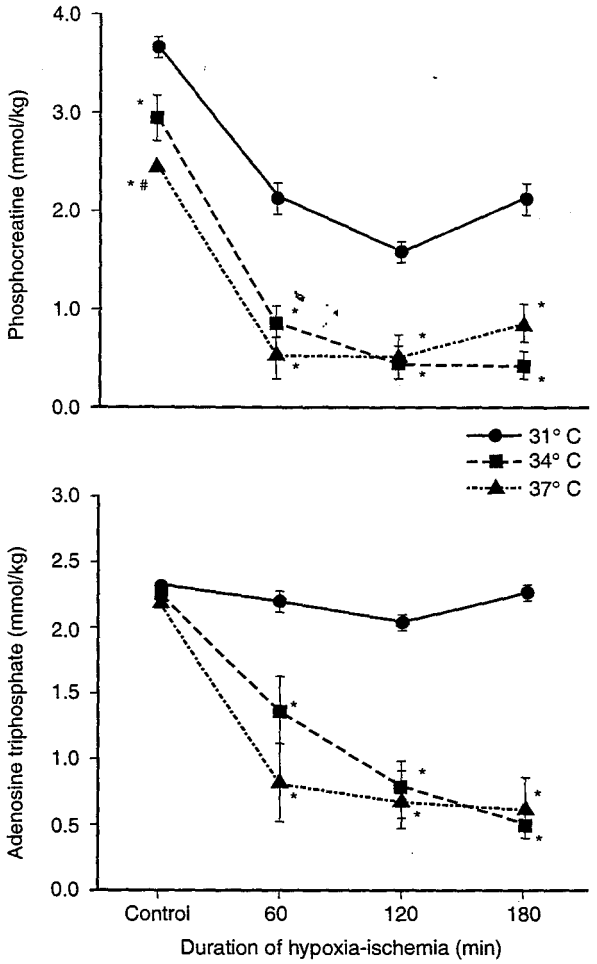


**Figure 6-35** Preservation of high-energy phosphate levels in brain of the immature (P7) rat subjected to hypoxia-ischemia at the temperatures shown. Note that with mild hypothermia, 31° C, there was a partial prevention of the decline in brain phosphocreatine level and a complete prevention of the decline in ATP levels. (From Yager JY, Asselin J: *Stroke* 27:919-926, 1996.)

following the occurrence of seizures at 6 to 8 hours.[520]) *Of all the interventions to be discussed in this section, partial hypothermia shows the greatest potential, and indeed preliminary data to support its value in human infants are available (see Chapter 9).*

### Inhibition of Glutamate Release

Because glutamate appears to be important in both neuronal and oligodendroglial death, inhibition of the enhanced glutamate release with hypoxia-ischemia is important (Table 6-19). Because calcium influx is necessary for glutamate release and because magnesium blocks this process, part of the beneficial effect of calcium channel blockers or of magnesium could occur at this step.[206,521] However, calcium channel blockers have adverse cardiovascular effects, and magnesium administration in a variety of hypoxic-ischemic models has not been clearly beneficial.[522-524]

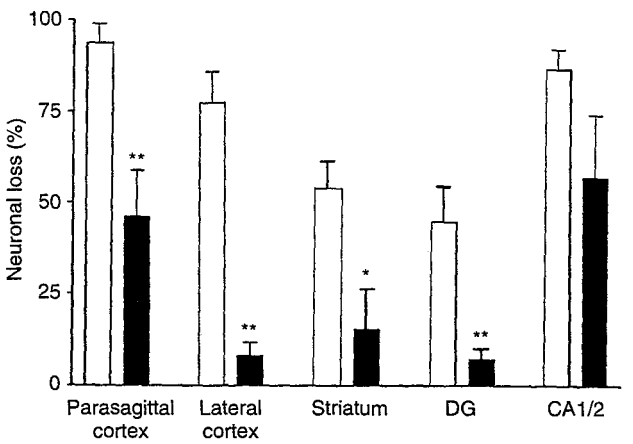


**Figure 6-36** Effect of selective cerebral cooling (extradural temperature, 30.4° C) from 6 to 72 hours after ischemia in near-term fetal sheep on neuronal loss in different brain regions 5 days after the ischemia. A significant overall reduction ($p < .001$) in neuronal loss was observed in fetuses treated with selective cerebral cooling *(black bars)*, compared with sham-cooled fetuses *(white bars)*, except in the most severely affected field of the hippocampus *(CA1/2)*. *, $p < .05$; **, $p < .01$; mean ± SEM. (From Gunn AJ, Gunn TR, Gunning MI, et al: *Pediatrics* 102:1098-1106, 1998.)

Adenosine, adenosine agonists, and adenosine antagonists have been studied because activation of the adenosine receptor inhibits glutamate release. Although in some perinatal models adenosine agonists have been beneficial, the available data do not show clearly consistent effects.[525-528]

Studies in cultured neurons and in newborn piglets suggest that mild hypothermia may exert some of its neuroprotective effects by inhibiting glutamate release from synaptic nerve endings.[529,530]

The neuroprotective effect of free radical scavengers may be exerted partially at the level of glutamate release because free radicals increase neuronal glutamate release in some models.[531] Moreover, phenytoin has partial neuroprotective effects in cultured neurons and in hypoxic-ischemic models in neonatal rats and fetal guinea pigs, perhaps by blocking sodium channels and thereby action potential–induced glutamate release.[532-535] Finally, the beneficial effect of lamotrigine in an adult model of ischemic neuronal injury probably relates to this anticonvulsant's inhibition of glutamate release.[536]

### Amelioration of Impairment in Glutamate Uptake

Hypothermia may exert some of its neuroprotective effect by decreasing the impairment in astrocytic energy-dependent, high-affinity glutamate uptake related to ischemia. This conclusion is based primarily on studies of neonatal piglets and cultured astrocytes.[530,537]

### Blockade of Glutamate Receptors

The neuroprotective effect of NMDA receptor antagonists, especially MK-801 but also magnesium, keta-

mine, and dextrophan, in various models of ischemic neuronal injury, both in culture and in vivo, was discussed earlier concerning glutamate neurotoxicity (Table 6-19). Similarly, in some models a beneficial additional effect is apparent with non-NMDA (AMPA-kainate) antagonists (e.g., NBQX, CNQX). Most often, neuroprotection is apparent when the agents are administered at the termination of, or from up to a few hours following, the insult.

Because of the beneficial role of non-NMDA antagonists in protection of differentiating oligodendrocytes from hypoxic-ischemic injury (see earlier discussion), these agents may be useful versus both neuronal and white matter injury. Oligodendrocytes do not express NMDA receptors; thus NMDA antagonists are not likely to be important in oligodendroglial protection.

### Inhibition of Leukocyte-Microglial-Cytokine Effects

Although the roles of leukocyte adherence, microglial activation, and cytokine action now appear to be important in the genesis of hypoxic-ischemic brain injury (see earlier discussion), relatively little is known about neuroprotection exerted at this level in perinatal models. The beneficial effect of induced neutropenia prior to hypoxia-ischemia was discussed earlier.[411] Platelet activating factor (PAF), the levels of which increase with ischemia-reperfusion, is important in induction of leukocyte adhesion molecules and thereby subsequent events in the inflammatory cascade. In the immature rat, administration of a PAF antagonist has been shown to decrease infarct size with both pretreatment and posttreatment, i.e., begun upon reperfusion.[415a,538] Studies in adult animal models of cerebral ischemia suggest that the use of specific antibodies or drugs directed at leukocytic adhesion molecules or various cytokines may contribute importantly to neuroprotection.[539-541] Finally, a critical product of microglia-macrophages is IL-1-beta, which in turn induces formation of other proinflammatory cytokines, including TNF-alpha. Notably, use of an antagonist of the IL-1 receptor both preinsult and upon reperfusion has had protective effects in hypoxic-ischemic brain injury in the neonatal rat.[409]

### Blockade of Downstream Intracellular Events

The downstream intracellular biochemical events leading to cell death include the large series of $Ca^{2+}$-activated processes, free radical production, nitric oxide synthesis, and apoptotic and necrotic cell death (Table 6-19). Hypothermia probably acts at multiple levels in this cascade, but prominent among these effects are reductions in free radical production and nitric oxide synthesis.[288,506,506a,542,543] Inhibitors of free radical production of demonstrated neuroprotective value in various models of hypoxia-ischemia include allopurinol (inhibits xanthine oxidase step), indomethacin (inhibits cyclo-oxygenase), iron chelation (diminishes hydroxyl radical production by the Fenton reaction), and magnesium (inhibits lipid peroxidation) (see earlier discussions and references cited herein).[10,238,239,252a,544] Free radical scavengers (vitamin E, spin traps [compounds that react with free radicals to produce a product detectable by electron spin resonance], 21-aminosteroids, idebenone) have been shown to have neuroprotective properties (see earlier discussion), and the models include both neuronal and oligodendroglial injury. Thus free radical scavengers hold considerable promise for broad protection.

Inhibitors of nitric oxide synthesis, generally nitroarginine derivatives, have been shown to be beneficial in a variety of perinatal models (see earlier discussion). Although generally beneficial in neuronal models, the effects of inhibition of nitric oxide synthesis on oligodendroglia are unclear. Thus in cultured oligodendrocytes, nitric oxide is toxic under normal conditions but is *protective* under conditions of free radical attack caused by glutathione depletion.[545]

Because a substantial proportion of injury to developing neurons and oligodendroglia with hypoxia-ischemia, especially with moderate or slowly evolving insults, is apoptotic, inhibitors of this form of cell death have been studied for neuroprotection effects. Because growth factors and other neurotrophic substances generally prevent apoptotic cell death, many have been studied for their potential neuroprotective effects. Those with demonstrated value in various neonatal models of hypoxia-ischemia have included insulinlike growth factor–1 (IGF-1), nerve growth factor, brain-derived neurotrophic factor (BDNF), and growth hormone.[546-550d] However, because in neuronal cultures, both BDNF and IGF-1 potentiate necrotic cell death with free radical attack, there is concern that at least under certain circumstances growth factors may prevent apoptosis but accentuate necrosis.[179] More data are needed.

The monosialoganglioside, GM-1, that crosses the blood-brain barrier has been shown in the near-term sheep fetus to provide protection from hypoxic-ischemic injury.[551,552] The beneficial effect was observed in one model in which the infusion was carried out over the 6 hours following reperfusion. The mechanism of the effect of GM-1 is unknown but is likely to be at multiple sites in the downstream intracellular events, since the compound is incorporated into cellular membranes.

### Conclusions Concerning Neuroprotective Interventions

Although many of the interventions described in the preceding discussion exhibit benefit, even when begun in the first hours of reperfusion, only relatively few appear to be relatively safe clinically and likely to be useful for protection of both neurons and oligodendroglia. My view is that the leading candidates for application to the human infant in the relative short term are mild hypothermia, inhibitors of free radical production, and free radical scavengers. Of these, greatest current promise lies with the use of mild hypothermia (see Chapter 9).

# PHYSIOLOGICAL ASPECTS

## Importance of Cerebral Blood Flow and Regulation Thereof

A large amount of clinical and neuropathological data emphasizes the major role ischemia plays in the genesis of brain injury associated with adverse perinatal events (see earlier discussion and Chapter 8). Thus alterations in cerebral blood flow are of prime importance for understanding the neuropathological and neurological consequences of all varieties of perinatal asphyxial and hypoxic-ischemic insults, as well as the pathogenesis, prevention and treatment of these consequences. In the following discussion I review cerebral blood flow, its regulation, and the changes associated with asphyxia and related hypoxic-ischemic insults. By necessity, the discussion involves studies with experimental animals. However, a growing experience with the human newborn, described in the final sections, indicates that the lessons learned from the animal research are largely relevant to the perinatal human.

## Cerebral Blood Flow—Knowledge from Experimental Studies

### *Fetal Circulation*

The essential features of the fetal circulation, based principally on work with large animals, e.g., sheep, goats, and nonhuman primates, begin with events at the placenta.[553-578] Gas exchange occurs efficiently at the placenta, although oxygen diffusion is somewhat restricted, and fetal arterial oxygen tension values are considerably lower than maternal values. Compensatory responses to this lower oxygen tension in the fetus include hemoglobin F, with its favorable oxygen affinity curve, polycythemia, and a relatively high cardiac output. Oxygenated blood from the placenta is carried through the umbilical vein, which empties into the inferior vena cava. This well-oxygenated blood enters the right atrium and is preferentially shunted through the patent foramen ovale ultimately to the aortic arch, and then to the coronary and cerebral circulations.[559] Poorly oxygenated blood from the superior vena cava is preferentially shunted into the right ventricle and the pulmonary artery. Because of the high pulmonary vascular resistance, this blood primarily enters the ductus arteriosus and the descending aorta and returns to the placenta via the umbilical arteries.

### *Regulation of Cerebral Flow—General Principles*

Cerebral blood flow in experimental animals has been measured principally by techniques based upon the clearance of an inert gas (e.g., xenon and nitrous oxide), carotid artery flow determinations, [$^{14}$C]antipyrine infusion with autoradiography, and infusion of radioactive microspheres with subsequent tissue sampling. Because considerable variability in absolute values of cerebral blood flow is observed with different techniques, species, modes of anesthesia, preparation of animals, and so forth, I place most emphasis in this discussion on the major conclusions of the many studies rather than on the absolute values of cerebral blood flow recorded. The focus of this section is on general principles of cerebral hemodynamics, primarily in mature animals; immature animals are discussed in a separate subsequent section.

**Autoregulation.** Autoregulation of cerebral blood flow refers to the maintenance of a constant cerebral blood flow over a broad range of perfusion pressures.[560,561,579,580] This constancy of cerebral blood flow is due to arteriolar vasoconstriction with increased perfusion pressure and vasodilation with decreased perfusion pressure.[562,563,579] Autoregulation is operative in brain of the fetal and neonatal lamb as well as the neonatal puppy and piglet (discussed later). The mechanisms underlying autoregulation are not entirely understood. Currently, the balance of data suggests that autoregulation is mediated primarily by an interplay between endothelial-derived constricting and relaxing factors (see discussion later of perinatal cerebral blood flow).[581-583] Autoregulation in the adult human is operative over a range of mean blood pressure between approximately 60 and 150 mm Hg,[579] and the response time is approximately 3 to 15 seconds.[580,584]

**Coupling of cerebral function, metabolism, and blood flow.** Tight coupling of cerebral function, metabolism, and blood flow is well established and can be demonstrated by a variety of correlative physiological, biochemical, and even clinical studies.[58,560,572,579,585] This coupling appears to be mediated by regulation of cerebral blood flow by one or more local chemical factors that are vasoactive. Vasoactive factors of importance in brain include hydrogen ions, potassium ions, adenosine, prostaglandins, osmolarity, and calcium (Table 6-20). (Nitric oxide is an induced vasoactive factor discussed earlier.) Increase in the perivascular *hydrogen ion* concentration (i.e., decrease in local pH) is associated with arteriolar vasodilation. Greater neuronal metabolic activity can decrease local pH and therefore increase substrate supply. The effect of perivascular hydrogen ion concentration mediates the vasodilating action of arteriolar $CO_2$[573-575,579,586] and is important under a variety of other physiological and pathological conditions (see later discussion). This vascular response is well established in perinatal brain (see later section). *Potassium ion* has a vasoactive effect.[576,577,579,587-589] Vasodilation increases linearly with extracellular potassium levels to 10 mmol/kg (levels above 20 mmol/kg induce vasoconstriction). The vasodilation is mediated by a calcium-activated potassium channel on vascular smooth muscle. Because potassium is released from nerve cells with electrical activity or a variety of insults, including oxygen deprivation (see earlier discussion), this ion may play a role in the regulation of cerebral blood flow under certain pathological conditions. *Adenosine,* administered on the perivascular side of pial arteries, results in a concentration-dependent vasodilation.[579,585,589,590] Changes in adenosine also accompany certain pathological states with changes in cerebral blood flow, including oxygen deprivation (see later section). Adenosine may be important in regulation of cerebral blood flow in perinatal animals (see later discussion). *Prostaglandins,* particularly prostaglandins E, $F_2$, lead to cerebral vasodilation. The concentrations of these compounds increase in response to cerebral ischemia, and agents that inhibit prostaglandin biosynthesis, e.g., indomethacin, have cerebral vasoconstrictive effects. Prostaglandins appear to be of cerebral hemodynamic importance in immature animals (see later discussion). Increases in perivascular *osmolarity* have a vasodilating effect, and decreases, a vasoconstricting effect.[591] These data may bear upon such effects on cerebral blood flow as the vasodilation associated with infusion of hypertonic solutions.[592] *Calcium ion* may also play a role in the control of cerebral blood flow, e.g., high perivascular concentrations of calcium ion lead to vasoconstriction, and low concentrations lead to dilation of cerebral vessels.[579,593] The ability of calcium-channel blockers to lead to increases in cerebral blood flow relates to the prevention of the vasoconstricting effects of calcium ion.[594] Extracellular calcium concentrations decline with hypoxia (see earlier discussion) and status epilepticus.[56,58] Other chemical factors, e.g., renin-angiotensin, vasopressin, endogenous opioids, other neuropeptides, adrenergic compounds, acetylcholine, and endothelium-derived relaxing and

**TABLE 6-20**

**Local Chemical Factors—Change in Brain Extracellular Fluid (ECF) and Effect on Cerebral Blood Vessels***

| LOCAL CHEMICAL FACTOR | CHANGE IN BRAIN ECF | | EFFECT ON CEREBRAL VESSELS | |
|---|---|---|---|---|
| | HYPOXIA | CORTICAL ACTIVITY† | INCREASE | DECREASE |
| Hydrogen ion | Increase | Increase | Dilate | Constrict |
| Potassium ion | Increase | Increase | Dilate | Constrict |
| Adenosine | Increase | Increase (?) | Dilate | Constrict |
| Prostaglandins | Increase | Increase | Dilate | Constrict |
| Osmolarity | ? | ? | Dilate | Constrict |
| Calcium ion | Decrease | Decrease | Constrict | Dilate |

*Data from Kuschinsky and Wahl,[560] Siesjö,[58] Paulson, Strandgaard, and Edvinsson,[579] and Armstead and Leffler.[585]
†Includes seizures.

constricting factors, may play important roles in regulation of cerebral blood flow, but more data are needed on these issues.[581-583,585,595,596] Limited data are available on these agents in immature animals.

## Cerebral Blood Flow in the Perinatal Period

**Ontogenetic effects.** Total and regional cerebral blood flow change significantly with maturation. In general, cerebral blood flow overall increases with postnatal age,[38,556,597-600] and this increase correlates well with similar increases in cerebral metabolic rates and energy demands and with neuroanatomical development (see Chapter 4). Changes in regional cerebral blood flow with maturation also reflect *coupling with metabolic and anatomical development.* The most dramatic short-term ontogenetic change in cerebral blood flow occurs around the time of birth. In the lamb, cerebral blood flow decreases by approximately threefold in the first 24 hours after birth.[601] This decrease correlates well with the postnatal increase in oxygen content (Fig. 6-37), consistent with the importance of oxygen delivery in regulation of cerebral blood flow.[600,602]

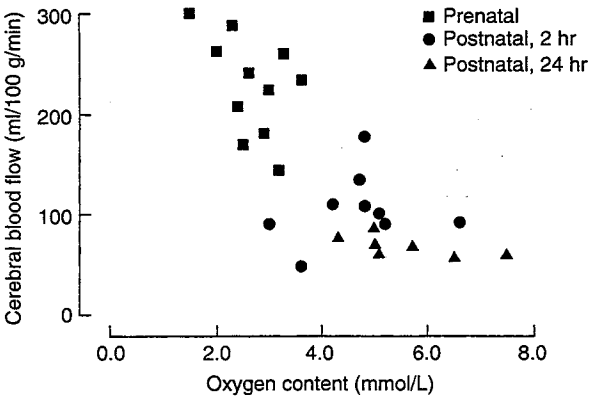


**Figure 6-37** Correlation of the perinatal decrease in cerebral blood flow with the increase in arterial oxygen content in the lamb. (From Richardson BS, Carmichael L, Homan J, et al: *Am J Obstet Gynecol* 160:919-925, 1989.)

**Regional effects.** Impressive regional differences in cerebral blood flow are apparent in the perinatal animal, and these differences relate in considerable part to regional differences in metabolic activity. Utilizing the microsphere technique to study regional cerebral blood flow in term fetal sheep, Ashwal and co-workers[603] initially noted (1) higher flows in brain stem than in cerebrum and (2) higher values in cortical gray matter than in subcortical white matter.[603] Utilizing the 4-iodo-[$^{14}$C]antipyrine technique, Cavazutti and Duffy[33] amplified these findings in a study of blood flow to 32 brain regions in the newborn dog. Blood flows were highest in cerebral gray matter, nuclear structures of brain stem, and diencephalon, and lowest in cerebral white matter. Blood flows to cerebral cortex were approximately fivefold to tenfold those to subcortical white matter. These regional differences have been confirmed in studies of the perinatal rabbit, lamb, piglet, and puppy.[599,600,604-610] Parallel studies of regional cerebral blood flow and cerebral glucose metabolism ([2-$^{14}$C]deoxyglucose method) demonstrated a close correlation with cerebral blood flow, thus indicating that *coupling of blood flow and metabolism* is present in the neonatal and adult animal.[32] (The correlation of blood flow and glucose metabolism in the newborn animals was not as strong as it is in adult animals, presumably because a greater proportion of glucose in the newborn animal enters the pentose monophosphate shunt for synthetic purposes.[32,33]) Finally, studies of blood flow to various regions of primate cerebrum indicate that the *parasagittal regions,* especially in the posterior aspects of the cerebral hemispheres, have significantly lower flow than other cerebral regions.[611] This fact may have major implications for the distribution of brain injury with perinatal ischemic insults (see section on parasagittal cerebral injury in Chapter 8).

**Regulation.** Cerebral blood flow in perinatal brain has been shown clearly to be regulated by the major factors summarized in the previous section on general principles of regulation. The major features are described briefly next.

*Autoregulation* appears to be operative over a broad range of arterial blood pressure in the preterm and term fetal lamb, the neonatal lamb, and the neonatal dog.[564-569,585,605,607,612-621] The principal stimulus for the autoregulatory change in vascular diameter appears to be

induced largely by deformation of endothelial cells and generation of endothelial-derived signals that act on the vascular smooth muscle.[618,620-624] With a decrease in transmural pressure, nitric oxide and calcium-activated potassium channels are important in the vasodilation response, and with an increase in transmural pressure, endothelin-1 is critical in mediation of the vasoconstriction response. The autoregulatory range of blood pressures varies slightly among species and experimental conditions. The curve for the preterm lamb at approximately 80% gestation is shown in Fig. 6-38. The curve for the preterm lamb differs from that for the term or neonatal lamb in two respects.[568] First, the *autoregulatory range in the preterm lamb is narrower,* especially at the upper limit of the curve. Second, and perhaps more strikingly, the *normal arterial blood pressure in the preterm lamb is very near or at the lower autoregulatory limit.* Indeed, in the preterm lamb at 80% of gestation, normal arterial blood pressure is only 5 to 10 mm Hg above the lower limit of the curve, in contrast to the situation in older animals. Thus in term neonatal animals, the normal blood pressure is 30 to 40 mm Hg above the lower autoregulatory limit, and in term fetal animals, the margin of normal arterial blood pressure above the lower limit is intermediate, approximately 15 to 25 mm Hg. In a subsequent study that included preterm fetal lambs at approximately 65% gestation, i.e., the onset of the third trimester, the lower autoregulatory limit was *essentially identical* to the normal resting arterial blood pressure.[607] *These data indicate that with decreasing gestational age, resting mean arterial blood pressure values approach the lower limit of the autoregulatory plateau.* Stated in another way, the observations suggest that the margin of safety, at least in the preterm fetus, and to a lesser extent in the term fetus, is small at the lower end of the autoregulatory curve and points to *vulnerability to ischemic brain injury with modest hypotension,* particularly in the preterm animal. *Vulnerability to hypertension* also may result because there is little change in the upper limit of the autoregulatory range during a brief developmental period (third trimester in the lamb and the human) when normal arterial blood pressure increases markedly.[614] Thus normal arterial blood pressure shifts precariously close to the upper autoregulatory limit and renders capillary beds (e.g., germinal matrix) vulnerable to hemorrhage with modest hypertension.

Autoregulation in the term fetal lamb and in the newborn lamb has been shown to be *sensitive to hypoxia.*[567,569,619] Changes in arterial $P_{O_2}$ from 20 to 16 mm Hg in the fetal animal and from approximately 70 to 30 torr in the newborn animal abolished autoregulation.[567,569] These decreases in arterial $P_{O_2}$ result in decreases in arterial oxygen saturation of less than 50%, which can be considered a hypoxic threshold for impairment of cerebrovascular autoregulation. Notably, the impairment of autoregulation required only a 20-minute exposure to hypoxia, and autoregulation *did not recover until 7 hours after restoration of normoxia.*[569] Studies in adult animals have shown that autoregulation is *abolished in the presence of hypercarbia,*[579,625] although this issue has not been explored systematically in the perinatal animal. In a single study of the newborn lamb, *systemic acidosis* also

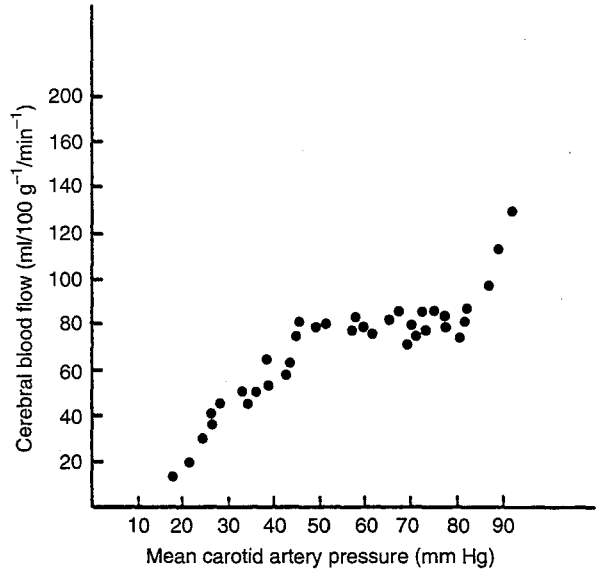


**Figure 6-38** Autoregulation of cerebral blood flow in the preterm lamb. See text for details. (From Papile LA, Rudolph AM, Heymann MA: *Pediatr Res* 19:159-161, 1985.)

was shown to cause a *loss in cerebrovascular autoregulation.*[626]

*Regional variation in the decrease in cerebral blood flow provoked by hypotension* to blood pressure values below the lower limit of the autoregulatory plateau has been described in the neonatal piglet, puppy, and lamb.[607,613,614,627,628] In the neonatal piglet the percent reduction in blood flow was least to the brain stem and greatest to the cerebrum.[627,628] In a more detailed regional study in the newborn puppy, *flow to cerebral white matter was most vulnerable to hypotension.*[613] Similarly, in the preterm lamb, the lower autoregulatory limit with hypotension is lower in brain stem than in cerebrum.[607,614] Perhaps even more importantly, in the preterm lamb at the start of the third trimester, blood flow to cerebral white matter was not only particularly vulnerable to hypotension but did not recover under conditions of reinfusion that restored blood flow to all other brain regions (Figs. 6-39 and 6-40).[607] The latter observations may have implications for the topography of the brain injury with hypoxic-ischemic insults (see following discussion and Chapter 8).

Changes in *arterial* $P_{CO_2}$ have marked effects on cerebral blood flow in perinatal as in adult animals.[10,33,163,604,629-645] In a study of blood flow to 32 brain regions of the newborn dog, a positive linear correlation was obtained in each structure examined.[33] However, the response to carbon dioxide varied widely among brain regions, ranging from an increase of only 0.15 ml/100 g/min/mm Hg in $P_{CO_2}$ in subcortical white matter to an increase of 4.8 in the vestibular and superior olivary nuclei. The *limited vasodilatory response in cerebral white matter* may have implications for the vulnerability of this region to hypoxic-ischemic injury (see later section and Chapter 8). In general, the higher

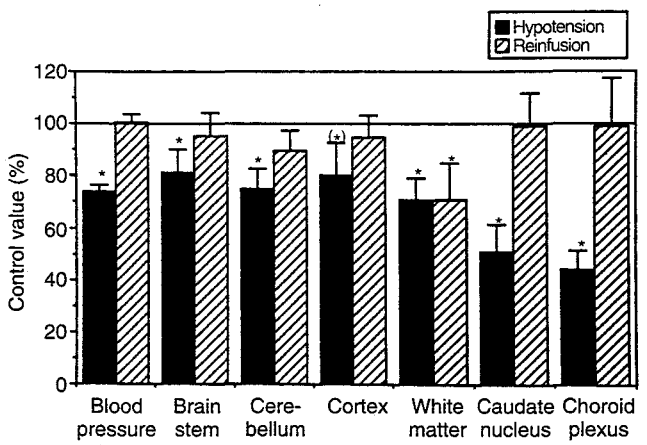


**Figure 6-39** Mean arterial blood pressure and regional cerebral blood flow (CBF) (percent of control values, mean ± SE) after hemorrhagic hypotension and reinfusion in the preterm lamb. Note that the approximately 25% reduction in blood pressure resulted in significant lowering of CBF in all regions. However, only in cerebral white matter did CBF fail to return to baseline levels upon reinfusion. (From Szymonowicz W, Walker AM, Yu VY, et al: *Pediatr Res* 28:361-366, 1990.)

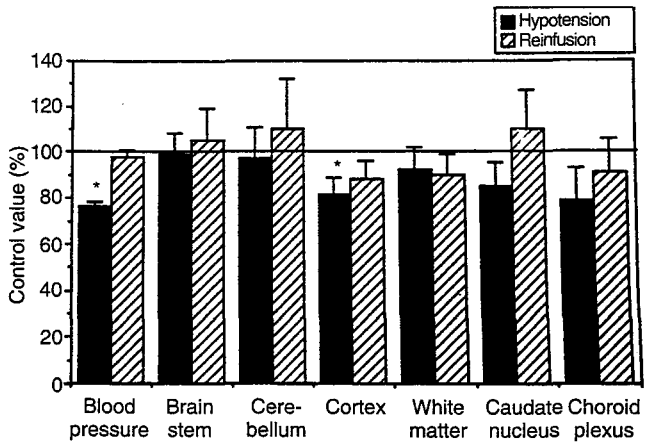


**Figure 6-40** Mean arterial blood pressure and regional cerebral blood flow (CBF) (percent of control values, mean ± SE) after hemorrhagic hypotension and reinfusion in the near-term lamb. In contrast to the effect on CBF of a similar decrease in blood pressure in the preterm lamb (see Fig. 6-39), during hypotension a significant decline occurred only in cerebral cortex, and upon reinfusion all values returned to baseline levels. (From Szymonowicz W, Walker AM, Yu VY, et al: *Pediatr Res* 28:361-366, 1990.)

the blood flow to a particular structure, the greater the vasodilatory response to increasing $P_{CO_2}$ (Fig. 6-41). A similar conclusion can be derived from a less detailed study of regional cerebral blood flow in the newborn piglet.[634]

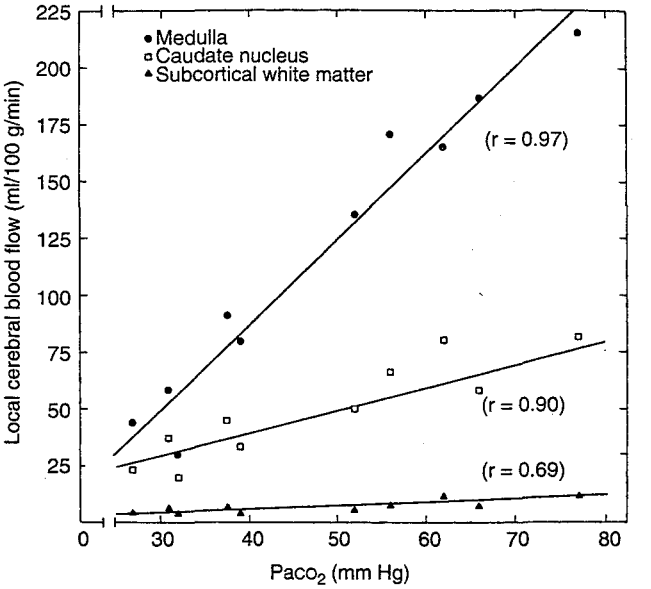


**Figure 6-41** Sensitivity of local cerebral blood flow to $P_{aCO_2}$. Note that regions with the higher normocapnic blood flow exhibit the highest sensitivities to $P_{aCO_2}$. (From Cavazutti M, Duffy TE: *Ann Neurol* 11:247, 1982.)

The effects of profound hypocarbia on cerebral blood flow are of particular importance because hyperventilation is one means of induction of *pulmonary* vasodilation in infants with persistent pulmonary hypertension. Studies in neonatal lambs indicate an abrupt decrease in cerebral blood flow with hypocarbia induced by hyperventilation.[604,643,646] The decrease was nonlinear, such that the vasoconstricting effect of hypocarbia declined at lower arterial $P_{CO_2}$ tensions. Moreover, the decline in cerebral blood flow became less prominent with time, such that cerebral blood flow was no longer statistically different in hypocarbic animals ($P_{aCO_2}$ = 15 mm Hg) compared with control animals ($P_{aCO_2}$ = 36 mm Hg) after 6 hours (Table 6-21). Perhaps most importantly, the declines in cerebral blood flow did *not* cause any change in cerebral metabolic rate for oxygen because cerebral oxygen extraction fraction increased. The attenuation of the decline in cerebral blood flow with increasing duration of hypocarbia probably relates to an increase in perivascular $H^+$ ion concentration, primarily secondary to an increase in lactate levels. This formulation is supported by *the marked increase in cerebral blood flow above baseline levels* upon restoration of normocarbia.[604] This marked cerebral hyperemia could be of pathogenetic importance for development of hemorrhage in the clinical circumstance in which the infant with severe pulmonary hypertension first is hyperventilated and then restored to normocarbia by extracorporeal membrane oxygenation (see Chapter 10).

The effects of *modest hypocarbia*, e.g., $P_{aCO_2}$ of 26 mm Hg, interact adversely with hypoxic-ischemic insults in the 7-day-old.[103,645] Thus animals exposed to hypoxia-ischemia sustained a greater decline in CBF and a greater degree of brain injury when simultaneously rendered modestly hypocarbic and compared with CBF and brain injury in animals who were normocarbic

**TABLE 6-21**

**Effect of Hyperventilation and Abrupt Termination Thereof on Cerebral Blood Flow in Newborn Lamb***

| CONDITION | CEREBRAL BLOOD FLOW*† (% CHANGE) |
|---|---|
| **HYPERVENTILATION** | |
| 30 min | −36 |
| 6 hr | −12‡ |
| **AFTER HYPERVENTILATION** | |
| 30 min | +210 |
| 6 hr | +226 |

Data from Gleason CA, Short BL, Jones MD Jr: *J Pediatr* 115:309-314, 1989.
**No effect on $CMRO_2$ was observed at any time.*
†Blood flow to cerebral hemispheres and midbrain.
‡Not significantly different from zero; all other numbers different from control at $p < .05$ level.

($PaCO_2$ = 39 mm Hg) or rendered mildly hypercarbic ($PaCO_2$ = 54 mm Hg). The latter two groups had better preserved CBF, less severe cerebral metabolic deficits, and less severe brain injury. The hypercarbic animals had the most favorable hemodynamic, biochemical, and neuropathological outcomes.

Alterations in *arterial* $PO_2$ also cause distinct changes in cerebral blood flow.[32,33,163,602,632,647-652] Decreases in oxygen tension result in increases in flow and vice versa. Jones and co-workers[602,648,649] have demonstrated that cerebral oxygen delivery is maintained via this increase in cerebral blood flow over a wide range of arterial oxygen content. In the study of 32 brain regions of the newborn dog, as with arterial $PCO_2$, the magnitude of the vasodilatory response to hypoxia varied.[33] Hypoxia caused the largest percentage increases in regional cerebral flow in brain stem structures, moderate increases in cortical and diencephalic structures, and smallest increases in cerebral white matter. These observations again suggest that *cerebral white matter has limited vasodilatory capacity,* and they have implications for the vulnerability of this region to hypoxic-ischemic injury (see later discussion and Chapter 8). The *mechanisms* of the increase in cerebral blood flow with hypoxia are likely to be related to local metabolic factors and to vascular factors per se.[585,650] Thus with hypoxia, there are rapid local increases in vasodilatory metabolic factors, e.g., perivascular $H^+$ ion, $K^+$ ion, adenosine, and prostaglandins, and decreases in vasoconstricting factors, e.g., $Ca^{2+}$ ion. Additionally, Gilbert et al. have shown strikingly rapid decreases in isometric tension generated by the major cerebral arteries isolated from near-term fetal lambs and studied in vitro as isolated segments with $PO_2$ lowered to 15 mm Hg.[650] The relaxation was much more rapid than that observed in adult cerebral arterial segments. Whether this effect is related to local release of nitric oxide or another factor(s) remains to be determined. However, the findings indicate that the myogenic properties of the major cerebral vessels per se must be considered in the evaluation of mechanisms of changes in cerebral hemodynamics. Importance for the large cerebral vessels in regulation of cerebral blood flow in the preterm human is suggested further by the presence in their vascular wall of a muscularis, in distinct contrast to the absence of a muscularis in the smaller penetrating cerebral arteries and arterioles.[653]

The role of *acidemia* in the regulation of cerebral blood flow in the perinatal animal requires further study. Whether produced by hypoxemia, lactate infusion, or respiratory means, acidemia caused a sharp increase in cerebral blood flow in perinatal goats.[654] However, effects were most impressive when acidemia was induced by elevation in arterial $PCO_2$, a potent effector of cerebral blood flow (as described earlier). Moreover, a subsequent study of the fetal lamb did not report an alteration in regional cerebral blood flow over an arterial pH range from 6.9 to 7.5 produced by infusions of lactate or bicarbonate.[632] Studies of newborn dogs and piglets have shown inconsistent effects of lactate infusions or other changes in arterial pH on cerebral blood flow.[655-657]

A role for *adenosine* in regulation of cerebral blood flow in the immature brain is suggested by observations primarily in the neonatal piglet.[658,659] Studies have correlated cerebral blood flow with parallel measurements of interstitial concentrations of adenosine and have utilized specific agonists and antagonists of the $A_2$ receptor (the adenosine receptor on vascular smooth muscle; the $A_1$ receptor, the adenosine receptor on neurons, is involved in decreasing glutamate release and $Ca^{2+}$ influx). The data suggest that adenosine has a vasodilatory effect and that it is involved in the cerebrovascular response to decreases in blood pressure and, thereby, cerebrovascular autoregulation. Recall that brain adenosine concentrations increase with hypoxia and seizures; both conditions require increases in substrate influx to brain. Finally, in addition to its vasodilatory effect, adenosine may influence cerebral blood flow by inhibitions of platelet aggregation and activation of neutrophils (implicated in endothelial dysfunction), events shown to be of importance in the postischemic impairment of cerebral blood flow in adult models.[659,660]

*Prostaglandins* are important regulators of cerebral blood flow in the perinatal period.[260,585,608,609,620,621,661-674a] Prostanoids may exhibit vasodilator or vasoconstrictor properties, depending on the specific prostanoid, and appear to be important in the setting of both the upper and lower limits of autoregulation. However, in neonatal animals prostanoids exert effects that are different from those observed in the adult. In general, these compounds function as cerebral vasodilators and are important in regulation of cerebral blood flow with decreases in blood pressure within and below the autoregulatory range, with changes in blood $PCO_2$ and perivascular $H^+$ ion concentrations, and following ischemia, asphyxia, and seizures (conditions characterized by increases in cerebral prostaglandin biosynthesis). Prostaglandins also attenuate the vasoconstrictor responses of norepinephrine and are the apparent mediators of the vasodilatory responses of endogenous opiates. As a consequence of these important roles, indomethacin, via its inhibition of cyclooxygenase and thereby prostanoid biosynthe-

sis, may have a variety of important cerebral hemodynamic effects, vasoconstrictor in basic type. Such vasoconstrictor effects may be potentially beneficial, e.g., concerning prevention of intraventricular hemorrhage (see Chapter 11), or potentially deleterious, e.g., under conditions requiring maintenance or increase in cerebral blood flow, as with hypotension, asphyxia, or seizures.

## Cerebral Blood Flow During and Following Perinatal Asphyxia or Other Hypoxic-Ischemic Insults

Important cerebral circulatory effects of perinatal asphyxia and related hypoxic-ischemic insults have been defined by studies of a variety of experimental models, some based on techniques that result in impaired gas exchange between mother and fetus or postnatally and others based on controlled manipulation of only specific blood gases or of blood pressure.[32,33,48,92,163,165,248,269,270,294,297,566,572,602,603,609,610,613,628,632,644,661,675-690] *During asphyxia,* three of these circulatory effects occur initially and two occur with more prolonged episodes. The effects include, initially, (1) an alteration in the fetal circulation such that a larger proportion of the cardiac output is distributed to the brain, (2) an increase in total and regional cerebral blood flow, and (3) a loss of vascular autoregulation; and, later, (4) a diminution in cardiac output with the occurrence of systemic hypotension, and, largely as a consequence, (5) a decrease in cerebral blood flow (Table 6-22). *Following asphyxia* critical additional circulatory effects develop, and, indeed, from the clinical standpoint, these postinsult effects are as important, if not more so, than those occurring during asphyxia (Table 6-23). These phenomena during and following the insult are discussed next.

**Redistribution of fetal circulation.** Promptly after the onset of asphyxia in the term fetal primate or lamb, there is a redistribution of cardiac output such that a significantly larger proportion enters the brain, the coronary circulation, and the adrenals, at the expense of blood flow to other regions.[165,559,603,678,691,692] Approximately twofold increases in the proportion of cardiac output to brain were noted in studies of term fetal primates. This redistribution of blood flow is reminiscent of the diving reflex observed in aquatic animals and appears designed to protect the most critical and vulnerable organs. The response requires an intact sympathoadrenal system.[553] The important afferent components of the response include particularly the oxygen chemoreceptors.[559,692] Moreover, in order to be effective, circulation must be maintained; thus the hypertension noted shortly after the onset of fetal asphyxia is particularly important.[553,676,677]

**Increase in cerebral blood flow.** The major purpose of the circulatory changes as outlined is to maintain cerebral blood flow in the face of impending tissue oxygen debt. In experiments with fetal and neonatal lambs, puppies, and primates, cerebral blood flow in perinatal asphyxia increased generally by 50% to 500%.[32,33,163,165,269,603,632,675,677,678,680-683] In severe and prolonged asphyxia, cerebral blood flow eventually falls as a consequence of decreasing cardiac output (secondary to myocardial failure and hypoxic-induced bradycardia) and the loss of vascular autoregulation (see next section).

The *mechanisms* underlying the initial increase in cerebral blood flow relate in part to cerebral vasodilation, secondary to hypoxemia or hypercapnia, or both, presumably with increased perivascular hydrogen ion concentration.[693,694] Roles for elevated extracellular fluid concentrations of potassium, adenosine, and prostaglandins, all of which increase markedly in brain with hypoxemia and ischemia, are likely.[260,585,658,659,695-699] The particular importance of a rise or at least maintenance of blood pressure in the increase of cerebral blood flow with asphyxia is indicated by several studies.[676-678] In term fetal sheep subjected to asphyxia by cord compression, the initial increase in mean arterial blood pressure persisted for 60 minutes before decreasing to normal values.[676] Carefully controlled experiments with the same animal suggest that fetal blood pressure may be even more critical than local chemical factors, which lead to cerebral vasodilation, in the enhancement of cerebral blood flow.[677]

Although blood flow to various regions of brain increases generally in concert with the increase in total cerebral blood flow, *distinct regional differences* in this increase are apparent. In general, the increase in blood flow is most marked in brain stem structures and least in cerebral white matter. This general pattern has now been documented in the fetal lamb, neonatal lamb, and neonatal puppy.[32,33,138,566,609,632,677,680-682,700] This effect

**TABLE 6-22**

**Major Circulatory Effects During Perinatal Asphyxia**

| |
|---|
| **INITIALLY** |
| Redistribution of cardiac output so that larger proportion enters brain |
| Increase in cerebral blood flow |
| Loss of cerebral vascular autoregulation |
| **LATER** |
| Diminution of cardiac output; hypotension |
| Decrease in cerebral blood flow |

**TABLE 6-23**

**Major Circulatory Effects Following Perinatal Asphyxia**

| |
|---|
| Increase in cerebral blood flow beginning within minutes after the insult and lasting for up to several hours |
| Decline in cerebral blood flow toward baseline or below, with hypotension, following initial hyperemia |
| "Delayed" increase in cerebral blood flow ("delayed" hyperemia) beginning between 12-24 hours and lasting many hours—attenuated by nitric oxide synthase inhibitors |
| Delayed cerebral hyperemia correlates with impaired mitochondrial oxygenation, "secondary" energy failure, and neuropathological injury |

has been interpreted as an attempt to maintain integrity of vital brain stem centers. The mechanism for the heterogeneity in regulation of cerebral blood flow is unknown; an endogenous opioid-mediated mechanism appears likely.[681,701] Thus administration of naloxone results in an increase in telencephalic blood flow and oxygen metabolism, and, as a result, a decrease in the fraction of cerebral blood flow to the brain stem. This decrease in fraction of flow to brain stem may negate the attempt to preserve vital brain stem centers. A likely conclusion from this work is that with hypoxia or asphyxia the role of endogenous opiates is to suppress cerebral rate of oxygen consumption, which results secondarily in a decrease in cerebral blood flow and, thereby, an increase in fraction of total brain blood flow to brain stem. The burst in release of endogenous opiates with hypoxia and asphyxia and the well-known suppression of neural activity and oxygen consumption by endogenous opiates support this notion.[702-704] In this context, administration of naloxone during asphyxia might be deleterious.

**Loss of vascular autoregulation.** A serious impairment of cerebral vascular autoregulation develops with perinatal asphyxia. Utilizing the radioactive microsphere technique and producing asphyxia (pH 6.8 to 7.0) in term fetal sheep by partial occlusion of umbilical vessels, Lou and co-workers[676] demonstrated a striking pressure-passive cerebral blood flow.[676] Marked hyperemia, with cerebral blood flow values up to six times normal, occurred when mean arterial blood pressure was raised to 60 to 70 mm Hg, whereas cerebral blood flow declined to close to zero in large cortical areas when mean arterial blood pressure was lowered to 30 mm Hg. Vascular autoregulation in these term fetal animals appeared to be very sensitive to asphyxia. The likely mechanism relates most probably to the hypoxemia and hypercapnia that are the hallmarks of perinatal asphyxia. The sensitivity of the autoregulatory system in fetal and neonatal brain to these alterations in blood gas levels was described earlier (see section on autoregulation). The implications of these data for ischemic injury to perinatal brain are obvious.

**Hypotension and diminished cerebral blood flow.** Although the initial response to asphyxia is hypertension, this response is followed by hypotension.[553] The rapidity and severity of this occurrence depend upon the duration and severity of the asphyxial insult. In large part this effect is related to a diminution in cardiac output,[38,165] probably secondary to an effect on the myocardium. The consequence for the brain may be devastating, because the impairment of vascular autoregulation leaves cerebral blood flow at the mercy of perfusion pressure. Deficits in cerebral blood flow may be marked with relatively modest changes in mean arterial blood pressure. Impressive deficits in cerebral blood flow (20% to 80%) have been demonstrated, particularly in the parasagittal regions of the cerebral hemispheres and especially posteriorly, in the term fetal monkey subjected to severe and prolonged asphyxia.[611] A similar parasagittal distribution of cerebral cortical injury was demonstrated in near-term fetal sheep subjected to cerebral ischemia.[294,705] Detailed regional study of newborn dogs has demonstrated that cerebral white matter also is particularly likely to exhibit diminished blood flow with hypotension.[613] These observations correlate well with the neuropathological and clinical observations made of asphyxiated human infants (see Chapters 8 and 9).

A graphical summary of the major relationships between perinatal asphyxia and cerebral blood flow *during asphyxia* is shown in Fig. 6-42. The initial effects leading to increased cerebral blood flow are considered best as compensatory, adaptive responses (which could become maladaptive by leading to hemorrhage in vulnerable capillary beds). The later effects represent a decompensation of these responses and a cascade that leads to diminished cerebral blood flow and brain injury.

**Postasphyxial-postischemic effects.** The period *following termination of the asphyxial-ischemic insult* is critical because during this interval progression to brain injury occurs (see earlier discussion) and, in the clinical setting, this time represents a window of opportunity for therapeutic intervention. The principal experimental models utilized have been near-term fetal sheep and neonatal piglets and rat pups, and the insults have primarily consisted of hypoxia-ischemia and, less commonly, asphyxia.[42,43,92,138,248,269,270,294,297,609,610,643,661,662,675,683-686,689,690,705] The major circulatory effects identified are summarized in Table 6-23. A consistent observation has been a marked increase in cerebral blood flow upon reperfusion, a hyperemia that continues for up to

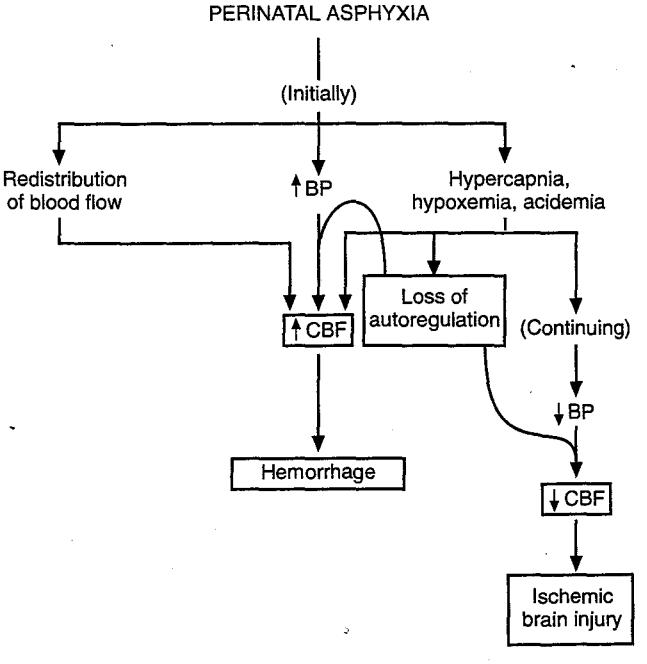


**Figure 6-42** Major relationships between perinatal asphyxia and cerebral blood flow *(CBF)*. The major consequences of the changes in cerebral blood flow, i.e., hemorrhage and ischemic brain injury, are shown. *BP*, Blood pressure.

several hours (Fig. 6-43). This *early increase in cerebral blood flow* is presumably related to the same mechanism operative for the initial increase in cerebral blood flow during asphyxia noted earlier, i.e., the accumulation of such vasodilating factors as $H^+$, $K^+$, adenosine, and prostaglandins. This early increase in cerebral perfusion is followed by a decline toward baseline. In some models, especially if associated with hypotension, cerebral blood flow declines below normal with the threat of cerebral ischemia. This postasphyxial cerebral hypoperfusion has not been a consistent feature in all experimental models. It is likely that hypotension could lead to cerebral ischemia in this period, since autoregulation is not operative (Fig. 6-44).

Importantly, a second, i.e., "delayed," increase in cerebral blood flow develops, with onset generally between 12 to 24 hours and with a duration of many hours or a day or more (Fig. 6-43). This increase is associated with evidence for impaired mitochondrial oxygenation (as assessed by brain levels of oxidized cytochrome-*c*), with the energy failure described earlier, and with neuropathological evidence for neuronal and white matter injury. The delayed hyperemia, its association with energy failure, and its correlation with severity of brain injury have been observed also in the asphyxiated human infant (see later discussion and Chapter 9). The mechanisms underlying the vasodilation and hyperemia are not established but may relate to nitric oxide. Because nitric oxide synthesis in endothelial cells (eNOS) is activated after hypoxic-ischemic insults, an attempt to reduce the delayed cerebral hyperemia with a nitric oxide synthase inhibitor was attempted in the fetal sheep model of ischemia.[294,297] The inhibitor did attenuate the delayed hyperemia, but, surprisingly, inhibition of nitric oxide synthesis *increased* histological injury.[294] This combination of findings suggests that nitric oxide synthesis has a protective effect either because of the vasodilatory effect or because of a biochemical effect. Concerning the latter, as noted in the earlier discussion of nitric oxide, when nitric oxide exists as $NO^+$, the nitrosonium ion, a protective effect on neurons occurs because of S-nitrosylation of the NMDA receptor

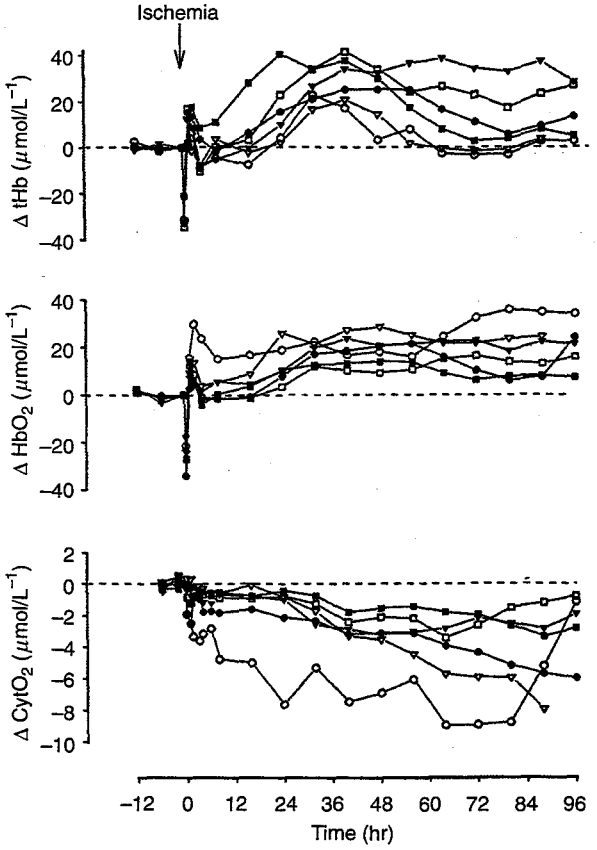


**Figure 6-43** Cerebral hemodynamic changes after transient cerebral ischemia in the late gestation fetal lamb. Changes in total cerebral hemoglobin *(tHb)*, oxygenated hemoglobin *($HbO_2$)*, and cytochrome oxidase *($CytO_2$)* were measured by near-infrared spectroscopy. In each graph different symbols represent data from separate fetuses. Note the two phases of cerebral vasodilation and increased cerebral blood flow, as assessed by the hemoglobin signals; the early increase occurs in the first 2 to 3 hours after ischemia, and the delayed increase, from 12 to 48 hours. The delayed increase in flow is accompanied by a decline in $CytO_2$, consistent with impaired mitochondrial oxygenation. (From Marks KA, Mallard EC, Roberts I, et al: *Pediatr Res* 39:48-54, 1996.)

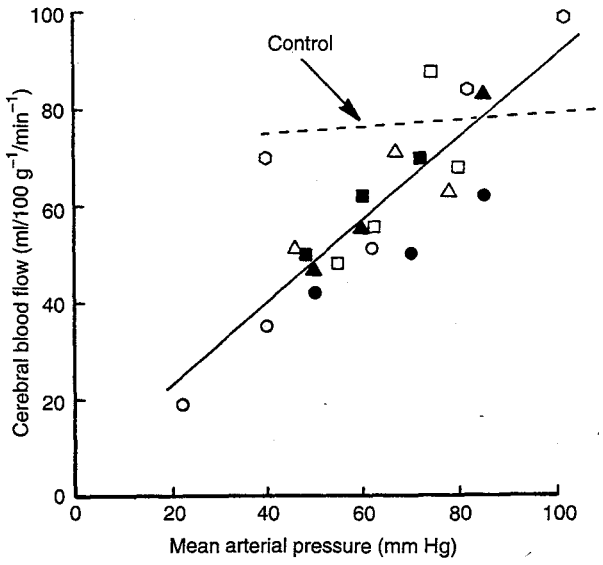


**Figure 6-44** Postasphyxial impairment of cerebrovascular autoregulation. Cerebral blood flow versus mean arterial blood pressure following asphyxia. Symbols represent responses to changes in blood pressure in individual asphyxiated lambs (n = 7). Regression line is derived from pooled data of all lambs. Dashed line represents data from nonasphyxiated (control) lambs. (From Rosenberg AA: *Stroke* 19:239-244, 1988.)

and resulting decline in $Ca^{2+}$ influx. NO also may act as a scavenger of superoxide anion. Moreover, under conditions of free radical attack, nitric oxide donors are protective for differentiating oligodendroglia.[545] Further data will be of great interest.

## Cerebral Blood Flow in the Human Newborn

### *Methodology*

In approximately the past 15 years, considerable insight into cerebral blood flow in the human newborn has been provided by application of one or more of several techniques (Table 6-24). The largest amount of information has been provided by the *xenon-133 clearance technique.*[706-732] These data are emphasized in this section. The technique utilizes administration of xenon 133, either by intraarterial or intravenous injection or by inhalation (preferably intravenous injection), and detection of the brain clearance of xenon 133, specifically the gamma radiation thereof, by external detectors. The detectors are collimated and placed over the skull, usually in the frontotemporal and frontoparietal regions, where the skull is thin and little extracranial muscle contributes to the measured signal. The particular advantage of the xenon-133 clearance techniques is the ability to provide quantitative data with relatively low radiation exposure and portable equipment. *Xenon computed tomography* has the advantage of providing regional data, but the technique requires transport to a specialized suite.

*PET* has been valuable in demonstration in the premature and term newborn of normal values of regional cerebral blood flow, coupling to oxygen consumption, increases of flow with seizure, and characteristic changes in premature infants with periventricular hemorrhagic infarction and intraventricular hemorrhage and in term asphyxiated infants with parasagittal cerebral injury (see Chapters 4, 5, 9, and 11). The particular advantage of PET is the ability to provide not only quantitative data but regional information as well. *Single photon emission tomography (SPECT)* also provides regional data but is nonquantitative. *Near-infrared spectroscopy,* a noninvasive optical technique, has the capability to provide serial quantitative measurements of cerebral blood flow and is discussed in detail in Chapter 4. With *venous occlusion plethysmography,* changes in intracranial volume after brief occlusion of the jugular veins are determined by a strain-gauge instrument placed around the compliant infant skull.[733-737] This technique cannot provide quantitative information and has the disadvantage of causing a transient rise in intracranial pressure. Application of this method is discussed briefly later. The *Doppler ultrasonic technique* for measurement of cerebral blood flow velocity, a noninvasive method, has the capability of providing serial information about cerebrovascular resistance and flow velocity in the insonated cerebral vessels. Determination of changes in cerebral blood flow from the velocity data is complicated by the inability to determine cross-sectional diameter of the insonated vessel; this method is discussed in Chapter 4. *Electrical impedance techniques* are noninvasive but have not proven sufficiently sensitive to be consistently useful.[738-740] *Magnetic resonance* techniques for determination of cerebral blood flow, either after administration of a paramagnetic contrast agent, e.g., gadolinium, or by use of gradients, are under intensive study but have not yet been applied to the newborn.

In the following section, I review information concerning, first, normal values and development of cerebral blood flow in the human newborn; second, regulation of flow; and, finally, alterations in flow following perinatal asphyxia.

**TABLE 6-24**

**Methods for Determination of Cerebral Blood Flow in the Newborn**

| |
|---|
| Xenon-133 clearance techniques (intravenous, intraarterial, or inhalation administration) |
| Xenon computed tomography |
| Positron emission tomography (intravenous administration of $H_2{}^{15}O$) |
| Single photon emission computed tomography |
| Near-infrared spectroscopy (intermittent inhalation of $O_2$ or continuous measurement of HbD [$HbO_2$ – Hb]) |
| Venous occlusion plethysmography |
| Doppler ultrasonic techniques |
| Electrical impedance techniques |
| Magnetic resonance techniques* (utilization of motion-sensitizing gradient pulses or paramagnetic contrast agent, e.g., gadolinium) |

*Not yet applied to the newborn.

### *Development and Normal Values of Cerebral Blood Flow in the Human Newborn*

**Changes in cerebral blood flow immediately after delivery.** A sharp decrease in "apparent" cerebral blood flow ("apparent" because the plethysmographic method is only semiquantitative) occurs in the term infant in the first hours after delivery (Fig. 6-45).[733] The decrease in the first 3 hours is nearly twofold, and, over the ensuring hours, cerebral blood flow is relatively stable. The reason for the relatively higher value shortly after delivery is not known, although a relation to raised arterial $P_{CO_2}$ levels has been suggested.[733] Alternatively, it is possible that a reflex activity, mediated by vagal afferents, is operative because the relatively higher cerebral blood flow near the time of birth requires intact vagus nerves in the sheep.[163] Both factors may be operative in the human newborn, when arterial $P_{CO_2}$ levels may be elevated and vagal activity from lung expansion may be considerable.[733] A third factor may involve arterial oxygen content, since, as noted earlier, a similar sharp decline in cerebral blood flow after birth has been observed in the lamb and correlates well with the increase in arterial oxygen content in the newborn versus fetal state. The relatively enhanced cerebral blood flow in the first hours after delivery may provide a margin of safety for cerebral metabolic needs in the period of adaptation to birth.

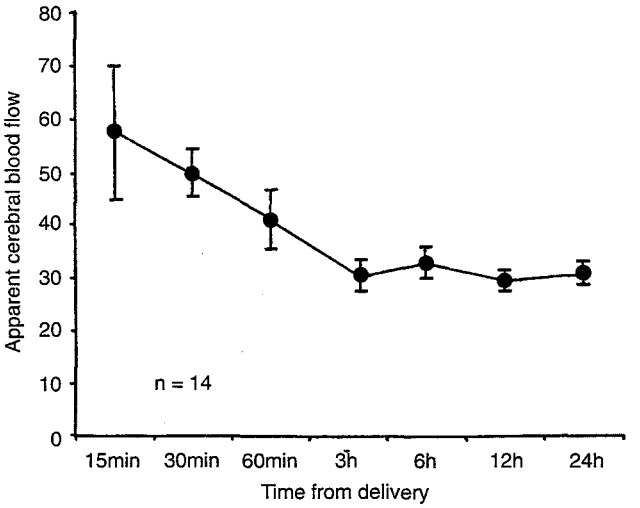


**Figure 6-45** Apparent cerebral blood flow in term infants following delivery, as estimated by the jugular venous occlusion plethysmographic technique. (From Cooke RWI, Rolfe P, Howat P: *Dev Med Child Neurol* 21:154, 1979.)

**Changes in cerebral blood flow beyond the immediate postpartum period.** Detailed ontogenetic data are limited concerning cerebral blood flow in the human newborn. Data obtained by PET suggest that cerebral blood flow is approximately 20% of the adult value in the premature infant of approximately 28 weeks' gestation and approximately 40% of the adult value in the term newborn.[741] These data and their relation to changes in cerebral oxygen consumption are discussed in Chapter 4. Serial studies of cerebral blood flow in normal preterm infants show an approximately twofold increase in flow over the first 3 days of life, perhaps related to an increase in cardiac output.[742]

**Normal values for cerebral blood flow.** Values for cerebral blood flow in the human premature newborn, shown in Table 6-25, are generally between 10 and 20 ml/100 g/min. A similar range is apparent in studies by PET and near-infrared spectroscopy (see Chapter 4). The relation of these relatively low values of cerebral blood flow and of oxygen consumption to the state of immaturity of neuronal and oligodendroglial development is described in Chapter 4. The analogies with findings in developing animals, described earlier, are obvious. Regional values for cerebral blood flow are notable for higher flows in cerebral gray matter structures than in cerebral white matter (see Chapter 4).

**Regulation of cerebral blood flow in the human newborn.** The major established regulatory mechanisms for cerebral blood flow in the human newborn include autoregulation, $PaCO_2$, oxygen delivery, blood glucose, and neuronal activity (e.g., seizure). Certain pharmacological agents also have been shown to exert regulatory effects. These various regulatory factors and their effects on cerebral blood flow are summarized in Table 6-26 and reviewed briefly next.

***Autoregulation.*** Autoregulation appears to be operative in both the normal human preterm and full-term infants.[718,720,726,727,729-731,743,744] Although the actual limits of the autoregulatory plateau cannot be established with certainty, the approximate autoregulatory range appears to be from 25 to 50 mm Hg mean arterial blood pressure. Changes of these limits with varying gestational ages and postnatal ages are not yet clear. Autoregulation in mature animals and adult humans is rendered inoperative by factors that lead to pronounced vasodilation, e.g., hypercarbia, hypoxia, hypoglycemic seizure, and postasphyxial state,[729] and initial data suggest that these factors also impair autoregulation in the human infant (see Chapters 9 and 11 and Table 6-26).[720,724,731,732,744-745a] Indeed, a pressure-passive state of the cerebral circulation was observed both in seriously asphyxiated full-term infants and in mechanically ventilated preterm infants prior to the occurrence of severe intracranial hemorrhage.[720,724,731,746] In a study of preterm infants based on monitoring of the cerebral circulation by measurement of the HbD signal by near-infrared spectroscopy (see Chapter 4), a pressure-passive cerebral circulation appeared to be associated with the subsequent occurrence of intraventricular hemorrhage or periventricular leukomalacia or both.[744] The cause of this vasoparalytic state is unclear, but a similar phenomenon has been documented in the asphyxiated neonatal lamb (see earlier discussion). It is likely that maximal vasodilation is present and related to the action of a variety of vasoactive compounds, e.g., perivascular $H^+$ ion concentration, prostaglandins, adenosine, free radicals, and nitric oxide.

***Carbon dioxide.*** The arterial tension of carbon dioxide is a potent regulator of cerebral blood flow in the human newborn.[707,708,715,720,721,726,727,731-733,735] The marked reactivity of cerebral blood flow to $PaCO_2$ is present in the first hours of life in spontaneously breathing preterm infants but does not appear until the second day in mechanically ventilated preterm infants (Table 6-27).[720,721,727] The reason for the attenuated reactivity of cerebral blood flow to $PaCO_2$ in the first day of life in mechanically ventilated infants is unclear, although the same phenomenon has been observed in the newborn monkey, rat, dog, and lamb.[631,646,729,747] It is noteworthy that a state of attenuated or absent reactivity to $PaCO_2$, as with blood pressure (see earlier discussion), has been observed both in seriously asphyxiated full-term infants and in mechanically ventilated preterm infants prior to severe intracranial hemorrhage (Table 6-28; see Chapters 8, 9, and 11). In general, the loss of reactivity to $PaCO_2$ follows the loss of autoregulation (but precedes loss of reactivity to hypoxemia).[729]

The *mechanism* for the vasodilating effect of carbon dioxide relates to the increase in *perivascular hydrogen ion concentration,* as observed in experimental models (see earlier discussion). The observation of a 50% decrease in cerebral blood flow after sodium bicarbonate administration to seven term and premature infants with acidosis also supports the important role of perivascular $H^+$ ion concentration.[707] The mechanism proposed for a decrease in the latter situation was enhanced movement of bicarbonate across the blood-brain barrier "because of vasodilation caused by the asphyxia" in these infants.[707] The demonstration of a

**TABLE 6-25**

**Cerebral Blood Flow (CBF) in the Newborn as Determined by the Intravenous $^{133}$Xe-Clearance Technique***

| BIRTH WEIGHT/ GESTATIONAL AGE (MEAN OR RANGE) | NO. OF INFANTS | AGE AT STUDY (MEAN OR RANGE) | CONDITIONS | MEAN CBF (ml/100 g/min) | REFERENCE (FIRST AUTHOR/YEAR) |
|---|---|---|---|---|---|
| 33.4 wk | 16 | 5 days | Stable | 29.7 | Griesen, 1984 |
| 29-34 wk | 15 | 15-17 days | Quiet sleep | 17.4 | Griesen, 1985 |
| | | | Active sleep | 17.0 | |
| | | | Wakeful | 21.8 | |
| | | | Unclassified | 16.8 | |
| 1510 g/31 wk | 42 | 0-5 days | Nonventilatory support | 19.8 | Griesen, 1986 |
| | | | Continuous positive airway pressure | 21.3 | |
| | | | Mechanical ventilation (IMV < 20) | 12.4 | |
| | | | Mechanical ventilation (IMV > 20) | 11.0 | |
| | | | Entire group | 15.5 | |
| 1340 g/31 wk | 15 | 3.7 wk | Stable | $F_1$-87.5† $F_2$-17.2 | Younkin, 1987 |
| <33 wk | 25 | 1.6 days | Mechanical ventilation | 12.3 | Griesen, 1987 |
| 1420 g/30.9 wk | 14 | 3 hr | Glucose ≥ 1.7 mmol/L | 11.8 | Pryds, 1988 |
| 1210 g/30.5 wk | 10 | 3 hr | Glucose ≤ 1.7 mmol/L | 26.0 | |
| 1569 g/31.7 wk | 21 | 31 days | Stable | 35.4-41.3† | Younkin, 1988 |
| 1050 g/29.2 wk | 18 | 12.6 hr | Stable | 13.1 | Lipp-Zwahlen, 1989 |
| 1540 g/30.4 wk | 18 | 6.4 hr | Mechanical ventilation | 8.4 | Pryds, 1989 |
| 1380 g/30.4 wk | 8 | 16.9 hr | Mechanical ventilation | 10.2 | |
| 1470 g/30.3 wk | 12 | 34.3 hr | Mechanical ventilation | 11.5 | |
| 27-33 wk | 20 | 48 hr | Mechanical ventilation | 10.0 | Griesen, 1989 |
| 1310 g/29.5 wk | 12 | 2 hr | Glucose ≥ 30 mg/dl | 12.0 | Pryds, 1990 |
| 1500 g/31.2 wk | 13 | 2 hr | Glucose ≤ 30 mg/dl | 18.6 | |
| 1175 g/29 wk | 20 | <12 hr | Mechanical ventilation | 8.7 (total group) 9.2 (nine infants with normal outcome) | Pryds, 1990 |
| 1300 g/28.0 wk | 16 | 4 days | Before aminophylline | 13.2 | Pryds, 1991 |
| | | | After (1 hr) aminophylline | 10.9 | |
| 1060 g/28 wk | 10 | <36 hr | Mechanical ventilation | 10.4 | Muller, 1997 |

*IMV,* Intermittent mandatory ventilation.

*Excludes studies based on administration of xenon 133 by inhalation or intraarterial injection and values obtained from infants with documented major brain lesions.

†Calculation of CBF employed partition coefficients derived from studies of adults, which may result in overestimation of CBF in the newborn.

decrease in cerebral blood flow after sodium bicarbonate administration in acidotic postasphyxial infants may have important implications for management.

***Oxygen.*** Arterial oxygen concentration is an important effector of cerebral blood flow in the human infant, as it is in the perinatal animal.[602,648,718,719,721,748-755] A vivid demonstration of the vasoconstrictive effect of oxygen is the observation that preterm infants administered 80% oxygen during stabilization at birth had a 23% lower cerebral blood than did infants administered room air during stabilization, when measured by xenon-133 clearance at 2 hours of life.[755] This finding may have implications concerning the use of high concentrations of inspired oxygen at the time of birth. Arterial oxygen concentration is related not only to $PaO_2$ but also to hemoglobin concentration and the oxygen affinity of hemoglobin. In preterm infants studied in the first day of life, Pryds and co-workers[721] observed a mean increase in cerebral blood flow of 11.9% per 1 mmol/kg decrease in hemoglobin concentration. In a separate series of preterm infants studied at a mean postnatal age of 3.7 weeks, a mean increase in cerebral blood flow of 5% per percentage point of decrease in hematocrit was documented.[718]

It is important to recognize that oxygen delivery to brain may be affected not only by hemoglobin concentration, but also by the viscosity of blood, at which point the inverse relationship of cerebral blood flow to hemoglobin concentration becomes more pronounced. However, in general, hematocrit does not alter blood viscosity in the newborn below approximately 60% (and perhaps somewhat higher).[731,756,757]

Finally, a direct relationship of cerebral blood flow (determined by xenon-133 clearance) with the relative proportion of fetal hemoglobin has been shown,[751] presumably reflecting the stronger affinity of fetal hemoglobin for oxygen. This conclusion had been suggested by a prior study of cerebral blood flow velocity.[754]

***Glucose.*** A striking observation by Pryds and coworkers[717,722] has established an important role for glu-

**TABLE 6-26**

**Regulation of Cerebral Blood Flow (CBF) in the Human Newborn***

| INCREASE IN REGULATORY FACTOR | CHANGE IN CBF |
|---|---|
| BP—normal preterm or term infant | 0 (autoregulation) |
| BP—severely asphyxiated term infant | ↑ |
| BP—prior to severe intracranial hemorrhage or periventricular leukomalacia or both—preterm infant | ↑ |
| $Paco_2$—normal preterm or term infant | ↑ |
| $Paco_2$—severely asphyxiated term infant | 0 |
| $Paco_2$—prior to severe intracranial hemorrhage in preterm infant | 0 |
| Total hemoglobin concentration | ↓ |
| Proportion of fetal hemoglobin | ↑ |
| Glucose (blood) | ↓ |
| Seizure | ↑ |
| Indomethacin | ↓ |
| Ibuprofen | 0 |
| Aminophylline | ↓ |
| Dopamine | ↑ |

*BP,* Blood pressure.
*See text for references.

**TABLE 6-27**

**Relation of Cerebral Blood Flow (CBF) to Partial Pressure of Carbon Dioxide in Arterial Blood ($Paco_2$) in the Human Preterm Infant in the First 2 Days of Life**

| AGE | VENTILATION | CHANGE IN CBF (%)/CHANGE IN $Paco_2$ (mm Hg) |
|---|---|---|
| 2-3 hr | Spontaneously breathing | 3.85 |
| 2-12 hr | Mechanically ventilated | 1.50 |
| 12-24 hr | Mechanically ventilated | 1.57 |
| 24-48 hr | Mechanically ventilated | 4.35 |

Data for mechanically ventilated infants from Pryds O, Greisen G, Lou H, Friis-Hansen B: *J Pediatr* 115:638-645, 1989, and derived from 38 preterm infants (mean birth weight 1470 g) with persistently normal neonatal ultrasound scans; data for spontaneously breathing infants from Pryds O, Andersen GE, Friis-Hansen B: *Acta Paediatr Scand* 79:391-396, 1990.

cose in regulation of cerebral blood flow in the human newborn (Table 6-26). Although this is discussed in more detail in Chapter 12, it should be noted here that an increase in cerebral blood flow became apparent as blood glucose concentration decreased to less than approximately 30 mg/dl (1.7 mmol/L). Increases in cerebral blood flow of twofold to threefold then occurred in proportion to the decline in blood glucose. The mechanism for this vasodilatory effect of glucose is not clear, but stimulation of beta-receptors by the increased compensatory secretion of epinephrine is suggested by data in human adults.[729] The clinical significance of this effect of glucose could be enormous (see Chapter 12).

***Neuronal activity (seizure).*** The coupling of neuronal activity to cerebral blood flow is apparent in two situations, sleep states and seizure. A decrease in cerebral blood flow during *sleep* has been shown by xenon-133 clearance.[758] The effect is not striking. A striking increase (approximately 50%) in cerebral blood flow with the excessive neuronal activity of *seizure* has been documented in the human newborn by PET (see Chapter 5).[759] This effect had been suggested by earlier studies of cerebral blood flow velocity by Doppler.[760]

***Pharmacological agents.*** Indomethacin and aminophylline are the two pharmacological agents shown to have a clear effect on cerebral blood flow in the human newborn. *Indomethacin* administration in doses utilized for closure of the ductus arteriosus leads to a 20% to 40% decrease in cerebral blood flow in the premature infant as studied by xenon-133 clearance and near-infrared spectroscopy.[674a,716,761] This effect is mediated by the inhibition of synthesis of vasodilatory prostaglandins at the cyclooxygenase step, as shown in experimental models and studies of isolated neonatal human cerebral artery.[762,763] An increase in cerebrovascular resistance has been shown by Doppler studies of very low birth weight infants after indomethacin administration.[764] Interestingly, by contrast with indomethacin, *ibuprofen* does not lead to a decline in cerebral blood flow.[731]

Administration of *aminophylline,* an antagonist of adenosine, leads to only a small decrease (10% to 15%) in cerebral blood flow in the human premature infant within 1 hour of intravenous administration.[728] No alteration of visual evoked potentials accompanied this modest decrease in blood flow. *Dopamine* administered

**TABLE 6-28**

**Relation of Outcome in Term Infants to Mean Cerebral Blood Flow (CBF) in the First 12 Hours After Asphyxia***

| CLINICAL GROUP | NEUROLOGICAL OUTCOME | MEAN CBF (ml/100 g/min) | MABP REACTIVITY† | $CO_2$ REACTIVITY‡ |
|---|---|---|---|---|
| Asphyxiated | Death or severe brain injury | 30.6 | – | – |
| Asphyxiated | Moderate to severe brain injury | 15.1 | – | + |
| Asphyxiated | Normal | 9.2 | + | + |
| Nonasphyxiated | Normal | 11.9 | + | + |

*MABP,* Mean arterial blood pressure.
Data from Pryds O, Greisen G, Lou H, Friis-Hansen B: *J Pediatr* 117:119-125, 1990.
*CBF determined in three groups of asphyxiated infants at mean age of 9 hours and in a group of nonasphyxiated infants at age of 1-5 days.
†MABP reactivity: –, CBF fluctuates directly with MABP, i.e., autoregulation not operating; +, CBF does not change with MABP, i.e., autoregulation normal.
‡$CO_2$ reactivity: –, CBF does not change directly with changes in $Paco_2$ tension, i.e., lack of normal reactivity of CBF; +, CBF changes directly with changes in $Paco_2$ tension, i.e., normal reactivity of CBF.

to treat hypotension has been reported in preliminary data to cause an increase in cerebral blood flow as well as arterial blood pressure.[731] However, a Doppler study of preterm infants showed no increase in cerebral flow velocity despite an increase in blood pressure.[765] More data are needed.

### *Perinatal Asphyxia, Autoregulation of Cerebral Blood Flow, and Cerebral Hyperemia*

**Impaired autoregulation.** A xenon-133 study of cerebral blood flow in a group of 19 term and preterm infants first suggested that autoregulation in the human newborn is very sensitive to perinatal asphyxia.[708] Thus 19 infants were examined with the xenon clearance technique "a few hours after birth."[708] Eleven of the infants weighed less than 2000 g. Although most of the infants were considered "distressed," Apgar scores at 5 minutes were less than 7 in only 4 of the 19 infants. At the time of study, pH was less than 7.20 in only four. For the total group of 19 infants, there was a linear relationship between cerebral blood flow and systolic blood pressure (Fig. 6-46). This pressure-passive relationship suggests inoperative vascular autoregulation and was seen to a similar degree in the infants less than or more than 2000 g body weight. This apparent impairment of vascular autoregulation is directly reminiscent of the data obtained with fetal and neonatal animals after asphyxia (see earlier section).

**Impaired vascular reactivity and cerebral hyperemia.** Subsequent work has clarified the relationship between perinatal asphyxia and impairment of vascular reactivity, particularly autoregulation. In a systematic study of 19 term infants (mean birth weight 3200 g) with perinatal asphyxia defined by a 5-minute Apgar score ≤ 5 and umbilical cord pH < 7.0, or both, a striking relationship between the severity of brain injury, the absolute value of cerebral blood flow, and the reactivity to changes in blood pressure and $PaCO_2$ was defined (Table 6-28).[724] Thus infants with the poorest neurological outcome (isoelectric amplitude-integrated EEG, death) had the highest values for cerebral blood flow and no autoregulation or $CO_2$ reactivity (Table 6-28). Infants with burst-suppression EEG and moderate to severe brain injury had slightly elevated values for cerebral blood flow and impaired autoregulation, but retained reactivity to $PaCO_2$ (Table 6-28). Infants without evidence of brain injury had normal values for cerebral blood flow, intact autoregulation, and reactivity to $PaCO_2$. A later study of 16 term infants with hypoxic-ischemic encephalopathy utilized PET to determine cerebral blood flow primarily at 1 to 4 days of life and found higher flows in those infants with abnormal neurological outcome (35.6 ml/100 g/min) than in those with normal neurological outcome (18.3 ml/100 g/min).[766]

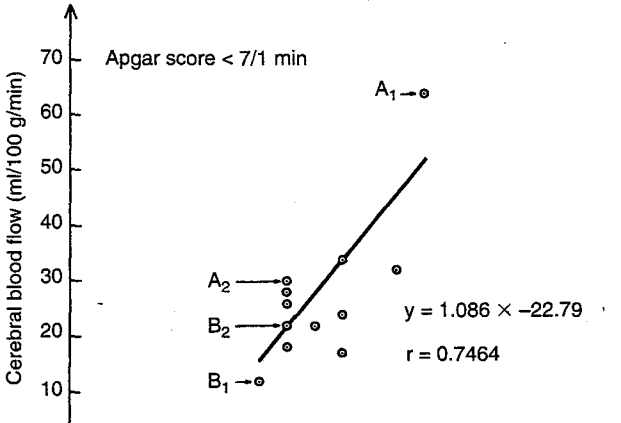


**Figure 6-46** Linear relationship between cerebral blood flow (CBF) and systolic blood pressure *(BP)* in 10 newborns with Apgar scores of < 7 at 1 minute. CBF was measured by the xenon clearance technique. $A_1$ and $A_2$ represent measurements of CBF in one patient before and after a spontaneous decrease in blood pressure. $B_1$ and $B_2$ represent measurements in another patient before and after a spontaneous increase in blood pressure. (From Lou HC, Lassen NA, Friis-Hansen B: *J Pediatr* 94:118, 1979.)

The pronounced, sustained *cerebral hyperemia* observed in the human infant has been shown by less invasive techniques, such as Doppler ultrasound and near-infrared spectroscopy. Thus determinations of cerebral blood flow velocity in term infants with hypoxic-ischemic encephalopathy from approximately 6 to 130 hours after the insult have shown an increase in mean flow velocity with decreased resistance indices, i.e., vasodilation.[767-776] Similarly, studies of asphyxiated human infants on the first day of life by near-infrared spectroscopy are consistent with a loss of vascular reactivity and an increase in cerebral blood volume and cerebral blood flow, with temporal characteristics similar to those observed in fetal sheep, described earlier.[92,777] The mechanism for this hyperemia is unclear. An increase in neuronal excitability, although documented following hypoxic-ischemic insults, seems unlikely in view of the relation of highest flows to isoelectric EEG. It appears more likely that the accumulation of vasodilatory compounds or vascular injury or both occurs and that this accumulation or injury in many ways may be unique to the human newborn relative to the perinatal animal. Delineation of the mechanisms underlying this vasoparalytic state and cerebral hyperemia following perinatal asphyxia in the human infant will be of major importance.

The aforementioned data thus define in the postasphyxial human newborn a state of *vasoparalysis* and *cerebral hyperemia* that is correlated with the degree of brain injury and presumably the severity of the asphyxial insult. The altered vascular reactivity, with autoregulation impaired more readily than $CO_2$ reactivity, is similar to observations made in the postasphyxial state in perinatal animal models (see earlier discussion). Presumably, a state of maximal vasodilation exists, related perhaps to the effect(s) of elevated perivascular $H^+$ ion concentration, prostaglandins, adenosine, free radicals, or nitric oxide or all of these factors. Whether this hyperemic state is caused by the same factors that

lead to the brain injury, is an adaptive mechanism to preserve brain tissue, or in some way causes additional brain injury remains to be clarified. It does appear likely that the loss of vascular reactivity renders the infant vulnerable to systemic hypotension and resulting cerebral ischemia.

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