REVIEW ARTICLE

MEDICAL PROGRESS

Julie R. Ingelfinger, M.D., *Editor*

Bilirubin-Induced Neurologic Damage — Mechanisms and Management Approaches

Jon F. Watchko, M.D., and Claudio Tiribelli, M.D., Ph.D.

From the Division of Newborn Medicine, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh (J.F.W.); and Centro Studi Fegato, Fondazione Italiana Fegato and Department of Medical Sciences, University of Trieste, Trieste, Italy (C.T.). Address reprint requests to Dr. Tiribelli at Centro Studi Fegato, Fondazione Italiana Fegato, Bldg. Q, AREA Science Park, Basovizza Campus, 34149 Trieste, Italy, or at ctliver@csf.units.it.

N Engl J Med 2013;369:2021-30.
DOI: 10.1056/NEJMra1308124

Neonatal unconjugated hyperbilirubinemia and resultant clinical jaundice affect up to approximately 85% of newborns. Although this condition is generally a benign, transitional phenomenon, unconjugated bilirubin levels that can pose a direct threat of serious brain injury develop in a small proportion of neonates. Acute bilirubin encephalopathy may ensue and progress to kernicterus (chronic bilirubin encephalopathy), a permanent disabling neurologic condition that is classically characterized by the extrapyramidal movement disorders of dystonia, choreoathetosis, or both; hearing loss due to auditory neuropathy spectrum disorders; and oculomotor pareses.[1] These central nervous system (CNS) sequelae reflect the regional CNS topography of bilirubin-induced neuropathology, which involves the globus pallidus, subthalamic nucleus, brainstem nuclei, hippocampal CA2 neurons, and cerebellar Purkinje's cells.[1-3]

Kernicterus as a Worldwide Problem

Although kernicterus continues to be reported worldwide,[4-8] major geographic differences exist. In North America and Europe, the estimated incidence of kernicterus ranges from 0.4 to 2.7 cases per 100,000 live births among term and late preterm neonates (those born at ≥35 weeks' gestation).[9] In some developing nations, the incidence of severe neonatal jaundice is approximately 100 times as high as it is in the developed world.[8] In such areas, approximately 3% of neonates admitted to a hospital have signs of acute bilirubin encephalopathy,[10] and kernicterus causes neonatal death as frequently as tetanus does.[8] Hazardous neonatal hyperbilirubinemia and kernicterus are not included in the current World Bank and World Health Organization calculus of the global burden of disease[8,11]; a more comprehensive assessment of these conditions is needed.

Factors that contribute to the incidence of kernicterus in developing nations include inadequate screening for neonatal jaundice; the inability to measure total serum bilirubin levels easily; and a high prevalence of medical conditions that increase the risk of severe hyperbilirubinemia or bilirubin neurotoxicity, such as glucose-6-phosphate dehydrogenase deficiency,[12] Rh isoimmunization,[13] and sepsis.[14] These factors also include delays in referral of neonates with jaundice to treatment facilities; the challenge of implementing phototherapy in settings that often lack effective light sources and electricity; and the limited availability of whole blood, safe blood-banking practices, or both to support exchange transfusion in infants who have critically high bilirubin levels or evident acute bilirubin toxicity.[8] This problem requires a multifactorial response. If, for example, levels of total serum bilirubin could be measured accurately at points of care without the need for a laboratory, toxic bilirubin levels could be identified more quickly. The development of a prom-

ising, low-cost, point-of-care device has been reported recently,[15] though efficacy testing of the device in larger studies is needed.

The complex cascade of molecular and cellular events leading to bilirubin-induced neurotoxicity remains incompletely delineated.[2,3,16] This review describes bilirubin-induced brain damage and recent insights into its pathogenesis and prevention.

WHICH BILIRUBIN IS NEUROTOXIC?

The decision to treat an infant who has marked hyperbilirubinemia with the aim of preventing acute bilirubin toxicity is conventionally based primarily on the total serum bilirubin level. This level alone, however, is of limited value in predicting neurologic impairment and kernicterus in newborns with hyperbilirubinemia[17,18-20]; this conclusion has been confirmed in recent clinical studies.[14,21]

The total serum bilirubin level is the level of albumin-bound bilirubin. The small circulating fraction that is not bound to albumin or other serum proteins is indexed according to the level of unbound, or free, circulating bilirubin.

During the past few years, there has been renewed interest in the measurement of unbound circulating bilirubin and its usefulness in predicting bilirubin-induced neurologic injury. Unbound circulating bilirubin is in dynamic equilibrium with extravascular tissues, including the CNS, and it provides a measure of the relative amount of bilirubin that will exit the vascular space at a given level of total serum bilirubin, the albumin concentration, and the albumin–bilirubin binding constant (or constants)[18,22] (Fig. 1). The latter two values vary among newborns.[23] The bilirubin-binding capacity of albumin is reduced in infants in unstable condition[21,24] and is also reduced by the presence of competing compounds[25-27] and by low serum albumin levels. Although a low albumin concentration increases the bilirubin–albumin binding affinity in vitro,[28,29] this effect is substantial only when albumin levels are very low, which is not characteristically seen in neonates. Accordingly, the level of unbound circulating bilirubin should be a more reliable index of the risk of neurotoxicity than the level of total serum bilirubin.

Even though unbound circulating bilirubin has biologic effects in the brain, the level alone does not dictate the risk of bilirubin encephalopathy. Bilirubin-induced neurotoxicity depends on a complex interaction between the level and duration of CNS exposure to unbound bilirubin and the innate cellular characteristics of the developing CNS that may confer either a predisposition to or protection against bilirubin-induced neuronal injury.[30]

Gauging unbound bilirubin levels in the CNS presents challenges and limitations,[31,32] given the possible effects of bilirubin oxidation within the CNS[33] and carrier-mediated bilirubin efflux across the blood–brain and blood–cerebrospinal fluid barriers (Fig. 1).[34,35]

There is also little agreement about what constitutes the threshold for neurotoxic unbound bilirubin[32,36] (i.e., the concentration of unbound bilirubin producing changes in cellular function that may culminate in permanent cell injury and cell death). In addition, limited data exist on the values of unbound circulating bilirubin that should be used as established thresholds for initiating treatment.[37] Even the large data set for the cohort of infants with extremely low birth weight in the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Network phototherapy trial[38] could not be used to estimate reference levels of plasma unbound circulating bilirubin. In that study, outcome measures were not necessarily specific to bilirubin, coexisting CNS insults were common, and half the infants died or had neurodevelopmental impairment.[38] Gestational age and birth weight were by far the strongest predictors of an adverse outcome[38]; these factors may also have been related to differences in the binding affinity of albumin for bilirubin at various developmental ages.[39]

Clinical management also affects the risk of bilirubin-induced CNS toxicity from bilirubin. For example, photoisomers, which account for up to 25% of the total bilirubin produced during phototherapy,[40] may affect bilirubin–albumin binding, altering the level of unbound circulating bilirubin.[26,41] The presence and extent of the effect of photoisomers are still poorly defined.

THE BILIRUBIN:ALBUMIN RATIO

Clinical laboratory measurement of unbound circulating bilirubin is not generally available, and the most common technique, the peroxidase method, requires sample dilution, which results

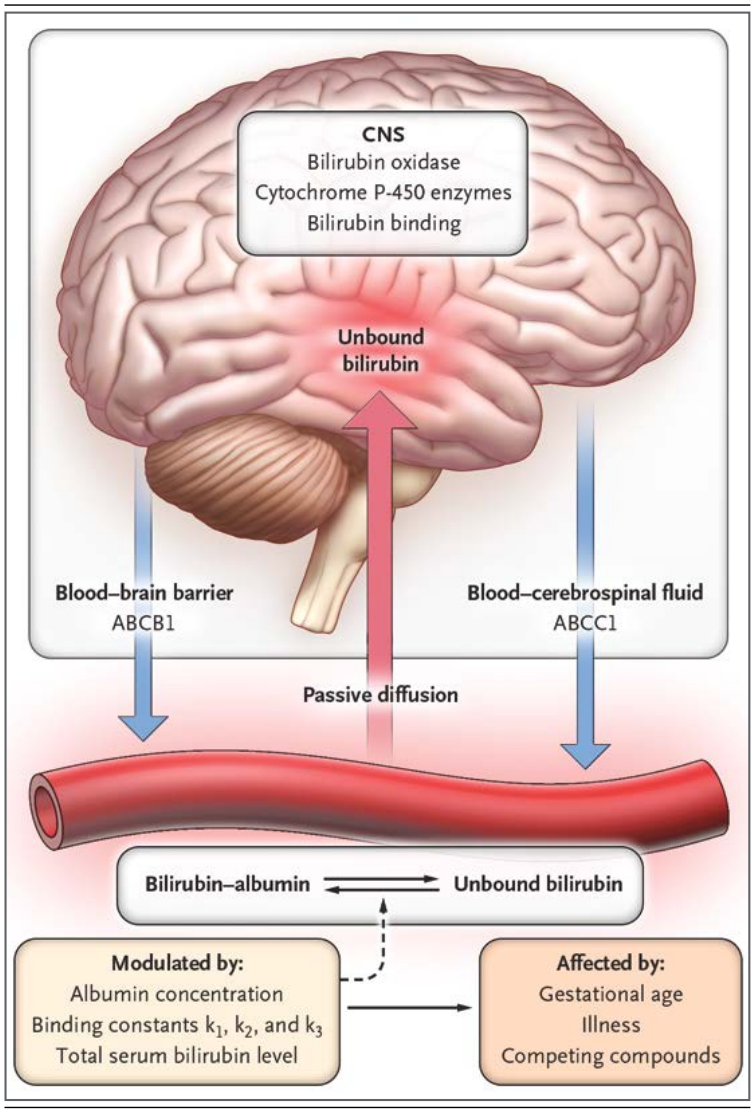

Figure 1. Relationship of Albumin-Bound and Unbound Bilirubin Levels in the Vascular Space to the Entry and Disposition of Unbound Bilirubin and Its Clearance from the Central Nervous System (CNS).

Unbound circulating bilirubin is in dynamic equilibrium with albumin-bound bilirubin as determined by the plasma albumin concentration; the bilirubin–albumin binding constants k_1, k_2, and k_3; and the total serum bilirubin level. These values vary significantly among newborns,[23] and the albumin–bilirubin binding capacity is reduced in infants in unstable condition,[21,24] as well as by the presence of competing compounds[25-27] and low serum albumin levels. Although a low albumin level increases the bilirubin–albumin binding affinity,[28,29] this effect is prominent only at albumin levels not generally seen in the clinical arena. Albumin characteristically binds more than one bilirubin molecule at bilirubin:albumin molar ratios of more than 0.5, and circulating bilirubin levels therefore increase more slowly than predicted by a single-binding-site model.[19,22] Humans also have considerable albumin polymorphism, and the resultant binding constants further complicate this calculus. Circulating unbound bilirubin is in equilibrium with the extravascular tissues, and entry of unbound bilirubin into the CNS occurs according to the concentration gradient from the vascular space. Putative bilirubin transporters such as the ATP-binding cassette transporter B1 (ABCB1) at the blood–brain barrier and the ATP-binding cassette transporter C1 (ABCC1) at the blood–cerebrospinal fluid barrier may facilitate bilirubin efflux from the CNS and bilirubin clearance from the brain. Unbound bilirubin in the CNS may also be cleared by bilirubin oxidase and cytochrome P-450 isoenzymes or may bind to cell membranes. Tissue-binding capacity varies among infants and is enhanced by acidosis; there is less tissue-binding capacity in preterm neonates than in term neonates.

in an underestimate of the level of unbound circulating bilirubin.[28,29]

Proxies for assessing the level of unbound circulating bilirubin in plasma have been proposed and used to predict bilirubin-induced CNS injury. The ratio of total serum bilirubin (in milligrams per deciliter) to serum albumin (in grams per deciliter) does correlate with measured unbound circulating bilirubin levels in newborns and has been used as an approximate surrogate measure[23]; this approach was endorsed by the American Academy of Pediatrics.[42] However, preliminary evidence from the prospective, randomized, multicenter Bilirubin Albumin Ratio Trial in the Netherlands indicates that the neurodevelopmental outcome for preterm infants treated according to their total serum bilirubin:albumin ratio in conjunction with the total serum bilirubin level was not superior to that for infants treated according to a threshold total serum bilirubin level alone.[43] This finding underscores the importance of improving, standardizing, and validating techniques to measure unbound circulating bilirubin as well as the importance of conducting controlled trials to examine definitions and thresholds for treatment based on levels of unbound circulating bilirubin as compared with levels of total serum bilirubin.

PATHOBIOLOGIC FEATURES OF BILIRUBIN-INDUCED CNS INJURY

Unbound bilirubin induces a variety of cellular and molecular events that result in neurotoxicity.[2,16] Several aspects of these events are detailed below.

REGIONAL AND CELL-SPECIFIC RESPONSES TO BILIRUBIN IN THE CNS

The regional CNS topography and cell-specific nature of bilirubin-induced CNS injury are striking, since it primarily affects only a subgroup of neurons in selected areas of the basal ganglia, brain stem, and cerebellum. This pattern is notably distinct from the neuropathologic features of hypoxic, ischemic, or hyperoxic brain injury in neonates.[3] Studies have shown region-specific and cell-specific responses to hazardous bilirubin elevations, indicating a greater degree of complexity regarding bilirubin-induced neurotoxicity than was previously recognized.[2,16]

The region-specific nature of kernicterus could reflect differences in neurotoxic bilirubin exposure due to differences in bilirubin uptake, tissue binding, and clearance or to differential cell sensitivity to injury.[34] CNS bilirubin uptake is passive and uniform, with lipophilic unconjugated bilirubin readily permeating the tight blood–brain interfaces.[44] Similarly, there is little evidence to suggest regional differences in bilirubin tissue binding in the CNS.[33,45]

Bilirubin appears to be cleared from the CNS by means of transporter-driven efflux at the blood–brain and blood–cerebrospinal fluid barriers, cellular metabolism, or both. Putative bilirubin plasma-membrane CNS efflux pumps include at least two types of transporters: ATP-binding cassette transporter B1 (ABCB1) P-glycoprotein, which is localized to the luminal (blood-side) face of capillary endothelial cells of the blood–brain barrier, and ATP-binding cassette transporter C1 (ABCC1) multidrug resistance–associated protein 1 (MRP1), which is localized to the basolateral face of the choroid plexus epithelium of the blood–cerebrospinal fluid barrier.[46] In both rodents and humans, ABCB1 and ABCC1 are the most abundantly expressed ABC transporters at their respective CNS interfaces in the developing and mature CNS.[46,47] Although the role of these transporters, particularly ABCC1 MRP1, is clear in vitro,[48] there is no evidence that there are region-specific differences in the expression of either ABCB1 or ABCC1. Thus, their overall effect on bilirubin clearance in vivo is undefined.[49]

Bilirubin-metabolizing enzymes in the brain, such as cytochrome P-450 (CYP), may have a role in setting the cerebral cell-specific and region-specific toxicity of bilirubin.[33] Oxidation of unconjugated bilirubin is catalyzed by CYP monooxygenases 1a1,[50,51] 1a2,[50,51] and 2a3.[52] A recent study showed a close inverse relationship between brain bilirubin content and expression of CYP messenger RNA, suggesting that CYP enzymes may have a role in protecting selected brain areas from bilirubin toxicity. Indeed, in studies involving the Gunn rat (a model of kernicterus), the cerebellum and the inferior colliculus, two regions that are classically affected in kernicterus, had delayed induction of CYP enzymes, as compared with induction in the cerebral cortex and superior colliculus, areas that are typically unaffected.[33] The marked difference in unconjugated bilirubin accumulation between the inferior colliculus and the superior colliculus, which are in close proximity, is unlikely to be due to differential blood supply or blood–brain barriers and is probably linked to regional differences in the cellular mechanisms for unconjugated bilirubin removal.[33]

In vitro studies have shown important neuronal and non-neuronal cell-specific responses to unconjugated bilirubin. These findings suggest that there are additional interacting and intricate mechanisms of unconjugated bilirubin toxicity (Fig. 2).

EFFECT OF BILIRUBIN ON NEURONS

Bilirubin binds avidly to cell membranes, especially myelin-rich membranes, making neurons the principal target of bilirubin toxicity. Exposure of neurons to unconjugated bilirubin in vitro is often accompanied by macroscopic changes, including reduced dendritic and axonal arborization, reduced neurite extension and ramification,[56] reduced cell proliferation,[59] and increased death by apoptosis.[60] Bilirubin delays S-phase progression and leads to cell-cycle arrest in SH-SY5Y neuroblastoma cells.[59] This antiproliferative effect suggests that the cerebellar hypoplasia that is characteristic of murine kernicterus models may result from such cell-cycle arrest.[61,62] Altered cell proliferation may also adversely affect cell migration and synapse formation.

Biochemical perturbations induced by bilirubin include protein oxidation, lipid peroxidation, reduced cellular glutathione content,[53] increased

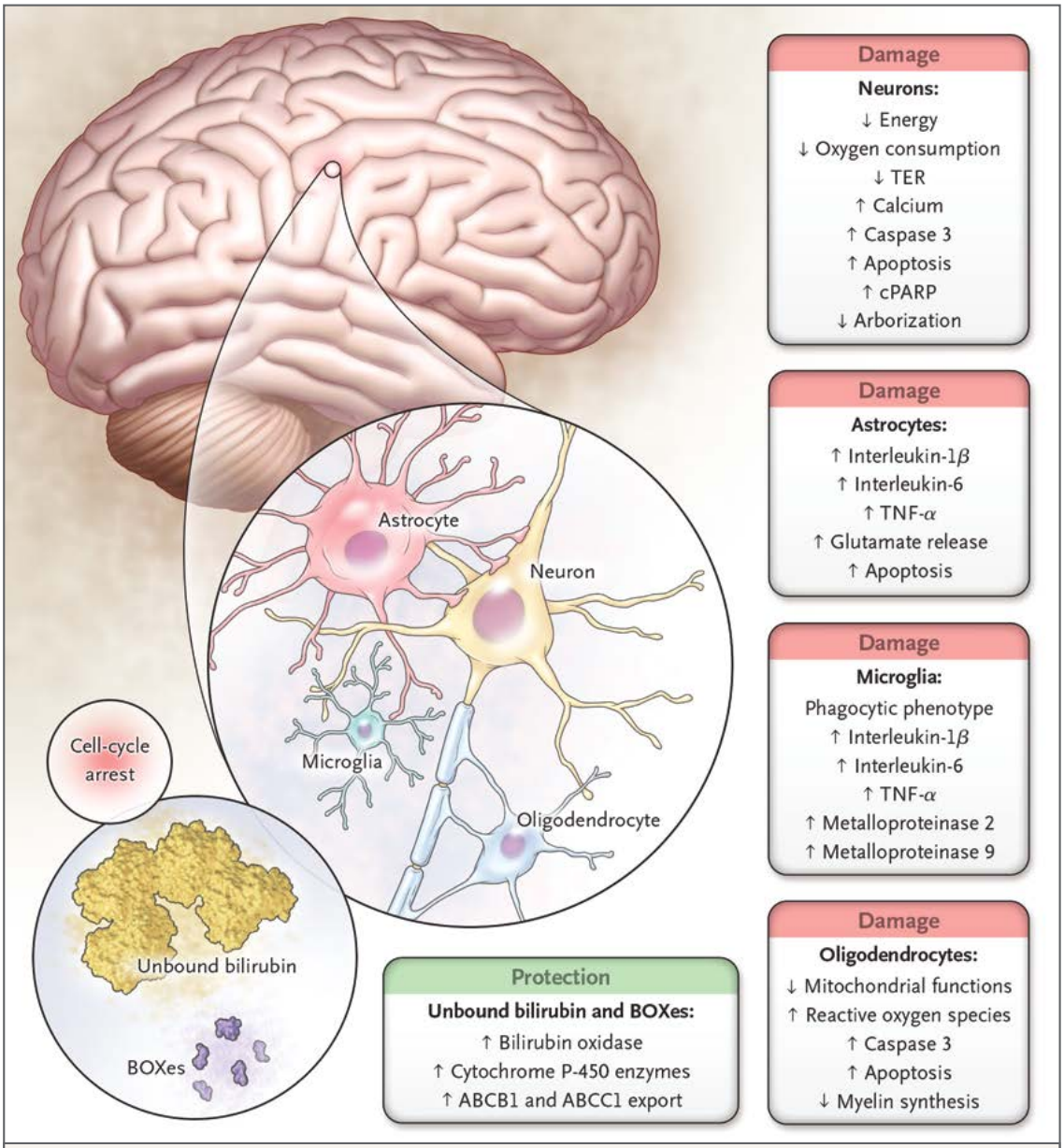

Figure 2. Cell Types and Metabolic Processes Affected by Bilirubin in the CNS.

The main effects of bilirubin on neurons are decreased oxygen consumption and increased release of calcium and caspase 3, resulting in apoptosis.[53-55] There is also decreased dendritic and axonal arborization, suggesting impairment of the intercellular exchange.[56] A similar pattern is observed in oligodendrocytes, with increased apoptosis, impairment of the redox state (oxidative stress), and reduced synthesis of myelin.[57] Microglia react to toxic injury associated with bilirubin by increased release of proinflammatory cytokines and metalloproteinase activity as cells manifest the phagocytic phenotype.[58] A similar proinflammatory pattern is observed in astrocytes, with enhanced release of glutamate and resultant apoptosis.[57] At the same time, cells may reduce the intracellular concentration of bilirubin either by extruding the pigment through the ABC transporters or by increasing the formation of the less toxic bilirubin oxidation products (BOXes) through bilirubin oxidase, cytochrome P-450 enzymes (1a1 and 1a2, in particular), or both.[33,34] These responses are protective, whereas all others result in cell damage; this suggests that once the intracellular concentration of bilirubin exceeds a toxic threshold (still to be defined), the polymorphic metabolic cascade leading to neurotoxicity ensues. The term cPARP denotes cleaved poly(adenosine diphosphate–ribose) polymerase, TNF-α tumor necrosis factor α, and TER transcellular resistance.

lactate dehydrogenase levels, and nitric oxide release (through neuronal nitric oxide synthase activation by engagement of the *N*-methyl-D-aspartate receptors).[63] Thus, bilirubin-induced oxidative stress and mitochondrial changes may be a nexus of neuronal injury. Hazardous unconjugated bilirubin levels are associated in vitro with reduced oxygen consumption, cellular energy failure, reduced inner mitochondrial membrane potential, increased intracellular calcium accumulation, and activation of the mitochondrial apoptotic pathway, with caspase 3 activation and poly(adenosine diphosphate–ribose) polymerase cleavage.[54,55]

Moreover, *N*-acetylcysteine, a glutathione precursor, and glycoursodeoxycholic acid, a bile acid antioxidant, counter adverse alternations in redox status, limit oxidative stress induced by unconjugated bilirubin in vitro, and enhance cell survival.[53] Bilirubin can also induce protective mechanisms, as shown in vitro by the marked up-regulation of expression and activity for the Na^+-independent cystine–glutamate exchanger system Xc(–) (SLC7A11 and SLC3A2) genes resulting in higher cystine uptake and increases in intracellular glutathione content with a consequent protection from an oxidative insult.[64] Whether the effect is protective or toxic depends on the bilirubin concentration; this is also shown in astrocytes, where up-regulation and intracellular reallocation of the ABCC1 MRP1 transporter are effective at a low concentration but fail at higher concentrations of unconjugated bilirubin (>140 nM).[65]

RESPONSES OF NON-NEURONAL CELLS

Non-neuronal cells in the CNS also show sensitivity to unconjugated bilirubin; such cells include astrocytes, microglia, oligodendrocytes, brain microvascular endothelial cells of the blood–brain barrier, and the choroid plexus epithelial cells of the blood–cerebrospinal fluid barrier. The responses of these cells may play a role in modulating bilirubin-induced neurotoxicity.

Primary monotypic astrocyte cultures react to toxic unconjugated bilirubin levels by secreting inflammatory mediators (interleukin-1β, tumor necrosis factor α [TNF-α], interleukin-6 through mitogen-activated protein kinase transduction, and nuclear factor κB), releasing glutamate and ultimately undergoing apoptosis.[66] Notably, astrocytes are less sensitive than neurons to damage from unconjugated bilirubin.

Similarly, microglia are directly activated by unconjugated bilirubin when placed in monotypic primary culture, assuming a phagocytic phenotype, secreting pro-inflammatory cytokines TNF-α and interleukin-1β, and showing increased activity of matrix metalloproteinases 2 and 9.[58] Astrocytes and microglia in culture show evidence of a rapid response. Immunoreactive cytokines detected in culture medium suggest, by extension, that there is probably a strong neuroinflammatory response during bilirubin encephalopathy.

Oligodendrocytes are also susceptible to unconjugated bilirubin toxicity, with reduced mitochondrial function, increased levels of reactive oxygen species, and increased caspase 3–mediated apoptosis in the presence of unconjugated bilirubin in vitro.[57] Studies are needed to determine whether oligodendrocyte damage impairs myelin synthesis and proper axonal function — phenomena observed in brain areas that are generally affected by kernicterus.[67]

In addition to expressing ABCB1, cultured vascular endothelial cells of the blood–brain barrier respond to hazardous levels of unconjugated bilirubin with an early increase of caveolae, caveolin-1, vascular endothelial growth factor (VEGF), and VEGF-receptor expression, followed by a reduction in tight-junction protein expression; the latter suggests an adverse alteration in barrier properties.[68] However, an alteration of the blood–brain barrier has not been observed in vivo during severe spontaneous hyperbilirubinemia. When exposed to high bilirubin concentrations, epithelial cells of the choroid plexus blood–cerebrospinal fluid interface show down-regulation of ABCC1 expression both in vitro and in vivo without an alteration in integrity of the barrier.[49]

COCULTURE STUDIES

Although monotypic cell cultures are valuable in characterizing cell-specific responses to unconjugated bilirubin, they are less informative than coculture studies, which allow exploration of cell–cell interactions that are probably critical for tissue homeostasis and overall CNS function. As noted above, neurons and glial cells respond differently to unconjugated bilirubin toxicity. For example, glial cells may modulate the vulnerability of neurons to injury, as shown in a recent coculture study in which astrocytes limited the

toxic effects of unconjugated bilirubin on neurons by enhancing neuronal viability, preventing apoptosis of neuronal cells, and improving neurite extension and ramification.[69] However, coculture techniques do not fully replicate the complexity of and interplay among the various cell types in the CNS.

ANIMAL MODELS OF BILIRUBIN ENCEPHALOPATHY

Animal models in vivo are necessary to fully capture the breadth of the effects of unconjugated bilirubin. The importance of such confirmatory in vivo studies is illustrated by the recent observation that tauroursodeoxycholic acid, a bile salt shown to be cytoprotective against bilirubin toxicity in vitro, is not neuroprotective in vivo despite its strong antioxidant effects.[70]

Two rodent models of hyperbilirubinemia and kernicterus exist: the Gunn rat and a more recently described mouse (see the Supplementary Appendix, available with the full text of this article at NEJM.org). The Gunn rat emerged spontaneously in late 1936 in a Wistar rat colony. Its genetic basis is now well characterized as a *Ugt1a* single-base deletion frameshift mutation resulting in inactive bilirubin conjugating enzyme and hyperbilirubinemia.[71,72] In contrast, the mouse model is a genetically engineered model created by introducing a premature stop codon in the *Ugt1a1* gene; this results in an inactive enzyme.[73] In spite of the similar gene defect in the two rodent models, they behave very differently, particularly with respect to survival. Severe jaundice develops in homozygous mutant mice soon after birth, and they die within 10 days, whereas Gunn rat pups, despite early hyperbilirubinemia, survive. Both models show significant cerebellar alterations. The striking difference in neurotoxicity is still poorly defined and should be investigated to better define the events leading to CNS damage and death. This investigation may ultimately provide information about how to prevent and treat the toxic effects of unconjugated bilirubin.

PREVENTION AND TREATMENT OF SEVERE HYPERBILIRUBINEMIA

Details on treatment interventions in newborns with hyperbilirubinemia are outlined in Table 1. Phototherapy and exchange transfusion remain the mainstays of therapy. Their effectiveness is based on limiting or reducing unconjugated bilirubin concentrations to nontoxic levels.[74] Improvements in phototherapy have markedly reduced the need for exchange transfusion.[74] Although phototherapy is generally considered a benign intervention and has been in clinical use for decades, studies have raised concerns about the potential toxicity of intensive phototherapy in preterm neonates with extremely low birth weight.[38]

Other interventions are designed to limit bilirubin production, enhance its metabolism and excretion, or both (Table 1). Of these interventions, metalloporphyrin inhibition of heme oxygenase, the rate-limiting step in bilirubin production, is a particularly promising approach to reducing bilirubin levels.[74,76] The use of tin mesoporphyrin has been studied in more than 800 infants and is highly effective in reducing total serum bilirubin levels and the need for phototherapy in both term and preterm neonates. However, an ongoing safety trial must be completed before this treatment is approved by the Food and Drug Administration. Identification and evaluation of other potent yet sufficiently safe metalloporphyrins with a short duration of action and without long-term tissue deposition are awaited.

Pharmacologic agents that provide neuroprotection by directly targeting the adverse effects of unconjugated bilirubin in the CNS are attractive options. Several studies involving the Gunn rat show that minocycline, a second-generation tetracycline with broad neuroprotective properties, prevents bilirubin-induced cerebellar hypoplasia, unconjugated bilirubin–induced abnormalities in brain-stem auditory evoked potentials, and overt signs of neuromotor dysfunction such as ataxia, lethargy, failure of locomotion, and feeding difficulty.[70,77,78] The promise of minocycline in this regard, however, is tempered by the knowledge that this and other tetracyclines are not safe for use in newborns because of their permanent adverse effects on developing bone and dentition. Characterization of the mechanism (or mechanisms) underlying the protective effects of minocycline against bilirubin-induced brain damage may identify new targets for intervention and lead to the development of alternative agents for future clinical investigation.

Table 1. Treatment Interventions to Control Hyperbilirubinemia and Prevent Acute Bilirubin Encephalopathy.

Phototherapy
This intervention, which is used to prevent bilirubin levels from reaching a hazardous range, has greatly reduced the need for exchange transfusion.[74]
Increasing total serum bilirubin levels, despite intensive phototherapy, suggest a hemolytic process underlying the hyperbilirubinemia.[74]
Phototherapy is generally considered to be safe; however, a study suggests potential toxicity of aggressive phototherapy in newborns with extremely low birth weight.[38]
Exchange transfusion
Double-volume exchange transfusion is used to prevent or correct hazardous levels of hyperbilirubinemia and reduce the risk of kernicterus.[74]
The American Academy of Pediatrics recommends immediate exchange when signs of an intermediate-to-advanced stage of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, fever, and high-pitched cry) are present in an infant with jaundice, regardless of the total serum bilirubin level, and even if the total serum bilirubin level is decreasing.[74]
Case series suggest that timely aggressive treatment of infants with intermediate-to-advanced stages of acute bilirubin encephalopathy, including opisthotonos and retrocollis, may avert neurologic damage and adverse neurodevelopmental sequelae in some infants.[75]
In contrast to the findings of earlier studies, opisthotonos and retrocollis are not always markers of permanent injury; further studies are needed to determine how often this may be the case.
Intravenous immune globulin
This pooled blood product has biologic activity against immune-mediated hemolysis and may be useful in direct Coombs-positive hemolytic disease.[74]
The mechanism is unclear, but it may involve Fc receptors.
Carboxyhemoglobin levels are reduced in Coombs-positive hemolytic disease in association with the lowering effect of immune globulin on the total serum bilirubin level.
This agent has a modest but clinically significant overall effect in reducing the need for exchange transfusion.
Pharmacologic therapy
Heme oxygenase inhibitors such as metalloporphyrins reduce bilirubin production.[76]
Phenobarbital increases bilirubin clearance by activating the phenobarbital enhancer module in the promoter sequence of *UGT1A1*, which enhances bilirubin conjugation.
Proof-of-concept studies have shown that pharmacologic agents can directly protect neurons from bilirubin toxicity. For example, minocycline has been shown to have protective effects against bilirubin-induced neuromotor dysfunction, cerebellar hypoplasia, and auditory-pathway abnormalities in Gunn rat pups.[70,77,78]

CONCLUSIONS

Bilirubin-induced brain damage continues to be an important risk among newborns worldwide. Considerable progress has been made in characterizing the molecular, biochemical, and cellular events related to bilirubin neurotoxicity, and the importance of non-neuronal cells and cell–cell interactions is increasingly apparent. The complex multifactorial nature of this injury continues to confound identification of the threshold for neurotoxic bilirubin levels and accurate prediction of the clinical occurrence of bilirubin encephalopathy.

Dr. Watchko reports providing expert testimony in legal cases related to neonatal jaundice. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Silvia Gazzin and Monica Daood for their assistance in preparing an earlier version of the manuscript.

This article is dedicated to the memory of Drs. J. Donald Ostrow and Antony McDonagh.

REFERENCES

1. Shapiro SM. Kernicterus. In: Stevenson DK, Maisels MJ, Watchko JF, eds. Care of the jaundiced neonate. New York: McGraw-Hill, 2012:229-42.
2. Watchko JF. Kernicterus and the molecular mechanisms of bilirubin-induced CNS injury in newborns. Neuromolecular Med 2006;8:513-29.
3. Ahdab-Barmada M. The neuropathology of kernicterus: definition and debate. In: Maisels MJ, Watchko JF, eds. Neonatal jaundice. Newark, NJ: Harwood Academic, 2000:75-88.
4. Bhutani VK, Johnson LH, Jeffrey Maisels M, et al. Kernicterus: epidemiological strategies for its prevention through systems-based approaches. J Perinatol 2004;24:650-62.
5. Johnson L, Bhutani VK, Karp K, Sivieri EM, Shapiro SM. Clinical report from the pilot USA Kernicterus Registry (1992 to 2004). J Perinatol 2009;29:Suppl 1:S25S-S45.
6. Manning D, Todd P, Maxwell M, Platt MJ. Prospective surveillance study of severe hyperbilirubinaemia in the newborn in the UK and Ireland. Arch Dis Child Fetal Neonatal Ed 2007;92:F342-F346.

7. Sgro M, Campbell DM, Kandasamy S, Shah V. Incidence of chronic bilirubin encephalopathy in Canada, 2007-2008. Pediatrics 2012;130(4):e886-e990.
8. Slusher TM, Olusaniya BO. Neonatal jaundice in low-and middle-income countries. In: Stevenson DK, Maisels MJ, Watchko JF, eds. Care of the jaundiced neonate. New York: McGraw-Hill, 2012: 263-73.
9. Maisels MJ, Newman TB. Prevention, screening and postnatal management of neonatal hyperbilirubinemia. In: Stevenson DK, Maisels MJ, Watchko JF, eds. Care of the jaundiced neonate. New York: McGraw-Hill, 2012:175-94.
10. Ogunlesi TA, Dedeke IO, Adekanmbi AF, et al. The incidence and outcome of bilirubin encephalopathy in Nigeria: a bicentre study. Niger J Med 2007;16:354-9.
11. Jamison DT, Breman JC, Measham AR, et al. Disease control priorities in developing countries. 2nd ed. Washington, DC: World Bank and Oxford University Press, 2006.
12. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. Blood Cells Mol Dis 2009;42:267-78.
13. Zipursky A, Paul VK. The global burden of Rh disease. Arch Dis Child Fetal Neonatal Ed 2011;96:F84-F85.
14. Gamaleldin R, Iskander I, Seoud I, et al. Risk factors for neurotoxicity in newborns with severe neonatal hyperbilirubinemia. Pediatrics 2011;128(4):e925-e931.
15. Coda Zabetta CD, Iskander IF, Greco C, et al. Bilistick: a low-cost point-of-care system to measure total plasma bilirubin. Neonatology 2013;103:177-81.
16. Brites D, Brito MA. Bilirubin toxicity. In: Stevenson DK, Maisels MJ, Watchko JF, eds. Care of the jaundiced neonate. New York: McGraw-Hill, 2012:115-43.
17. Ip S, Chung M, Kulig J, et al. An evidence-based review of important issues concerning neonatal hyperbilirubinemia. Pediatrics 2004;114(1):e130-e153.
18. Ahlfors CE, Wennberg RP, Ostrow JD, Tiribelli C. Unbound (free) bilirubin: improving the paradigm for evaluating neonatal jaundice. Clin Chem 2009;55:1288-99.
19. Ahlfors CE. Predicting bilirubin neurotoxicity in jaundiced newborns. Curr Opin Pediatr 2010;22:129-33.
20. Newman TB, Liljestrand P, Jeremy RJ, et al. Outcomes among newborns with total serum bilirubin levels of 25 mg per deciliter or more. N Engl J Med 2006;354: 1889-900.
21. Oh W, Stevenson DK, Tyson JE, et al. Influence of clinical status on the association between plasma total and unbound bilirubin and death or adverse neurodevelopmental outcomes in extremely low birth weight infants. Acta Paediatr 2010; 99:673-8. [Erratum, Acta Paediatr 2013; 102:326.]
22. Ahlfors CE, Parker AE. Bilirubin binding contributes to the increase in total bilirubin concentration in newborns with jaundice. Pediatrics 2010;126(3):e639-e643.
23. Ahlfors CE. Criteria for exchange transfusion in jaundiced newborns. Pediatrics 1994;93:488-94.
24. Cashore WJ, Oh W, Brodersen R. Reserve albumin and bilirubin toxicity index in infant serum. Acta Paediatr Scand 1983; 72:415-9.
25. Wennberg RP, Ahlfors CE, Bhutani VK, Johnson LH, Shapiro SM. Toward understanding kernicterus: a challenge to improve the management of jaundiced newborns. Pediatrics 2006;117:474-85. [Erratum, Pediatrics 2006;117:1467.]
26. Wennberg RP. Measuring free bilirubin: the clinical perspective. Clin Chem 2012;58:811-3.
27. McDonagh AF, Maisels MJ. Bilirubin unbound: déjà vu all over again? Pediatrics 2006;117:523-5.
28. Weisiger RA, Ostrow JD, Koehler RK, et al. Affinity of human serum albumin for bilirubin varies with albumin concentration and buffer composition: results of a novel ultrafiltration method. J Biol Chem 2001;276:29953-60.
29. Roca L, Calligaris S, Wennberg RP, et al. Factors affecting the binding of bilirubin to serum albumins: validation and application of the peroxidase method. Pediatr Res 2006;60:724-8.
30. Lasky RE, Church MW, Orlando MS, et al. The effects of aggressive vs. conservative phototherapy on the brainstem auditory evoked responses of extremely-low-birth-weight infants. Pediatr Res 2012; 71:77-84.
31. Daood MJ, Watchko JF. Calculated in vivo free bilirubin levels in the central nervous system of Gunn rat pups. Pediatr Res 2006;60:44-9.
32. Daood MJ, McDonagh AF, Watchko JF. Calculated free bilirubin levels and neurotoxicity. J Perinatol 2009;29:Suppl 1: S14-S19.
33. Gazzin S, Zelenka J, Zdrahalova L, et al. Bilirubin accumulation and Cyp mRNA expression in selected brain regions of jaundiced Gunn rat pups. Pediatr Res 2012; 71:653-60. [Erratum, Pediatr Res 2012;71: 732.]
34. Gazzin S, Strazielle N, Tiribelli C, Ghersi-Egea JF. Transport and metabolism at blood-brain interfaces and in neural cells: relevance to bilirubin-induced encephalopathy. Front Pharmacol 2012;3: 89.
35. Ghersi-Egea JF, Gazzin S, Strazielle N. Blood-brain interfaces and bilirubin-induced neurological diseases. Curr Pharm Des 2009;15:2893-907.
36. Ostrow JD, Pascolo L, Tiribelli C. Reassessment of the unbound concentrations of unconjugated bilirubin in relation to neurotoxicity in vitro. Pediatr Res 2003; 54:926.
37. Ostrow JD, Pascolo L, Brites D, Tiribelli C. Molecular basis of bilirubin-induced neurotoxicity. Trends Mol Med 2004;10:65-70.
38. Morris BH, Oh W, Tyson JE, et al. Aggressive vs. conservative phototherapy for infants with extremely low birth weight. N Engl J Med 2008;359:1885-96.
39. Bender GJ, Cashore WJ, Oh W. Ontogeny of bilirubin-binding capacity and the effect of clinical status in premature infants born at less than 1300 grams. Pediatrics 2007;120:1067-73.
40. Mreihil K, McDonagh AF, Nakstad B, Hansen TW. Early isomerization of bilirubin in phototherapy of neonatal jaundice. Pediatr Res 2010;67:656-9.
41. McDonagh AF, Vreman HJ, Wong RJ, Stevenson DK. Photoisomers: obfuscating factors in clinical peroxidase measurements of unbound bilirubin? Pediatrics 2009;123:67-76.
42. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316. [Erratum, Pediatrics 2004;114:1138.]
43. Vader-van Imhoff DE. BARTrial: the management of hyperbilirubinemia in preterm infants. Amsterdam: Netherlands Neonatal Research Network (http://www.neonatologiestudies.nl/home/page.asp?page_id=1076).
44. Zucker SD, Goessling W, Hoppin AG. Unconjugated bilirubin exhibits spontaneous diffusion through model lipid bilayers and native hepatocyte membranes. J Biol Chem 1999;274:10852-62.
45. Vítek L, Ostrow JD. Bilirubin chemistry and metabolism; harmful and protective aspects. Curr Pharm Des 2009;15: 2869-83.
46. Gazzin S, Strazielle N, Schmitt C, et al. Differential expression of the multidrug resistance-related proteins ABCb1 and ABCc1 between blood-brain interfaces. J Comp Neurol 2008;510:497-507.
47. Daood M, Tsai C, Ahdab-Barmada M, Watchko JF. ABC transporter (P-gp/ABCB1, MRP1/ABCC1, BCRP/ABCG2) expression in the developing human CNS. Neuropediatrics 2008;39:211-8.
48. Corich L, Aranda A, Carrassa L, Bellarosa C, Ostrow JD, Tiribelli C. The cytotoxic effect of unconjugated bilirubin in human neuroblastoma SH-SY5Y cells is modulated by the expression level of MRP1 but not MDR1. Biochem J 2009;417: 305-12.
49. Gazzin S, Berengeno AL, Strazielle N, et al. Modulation of Mrp1 (ABCc1) and Pgp (ABCb1) by bilirubin at the blood-CSF and blood-brain barriers in the Gunn rat. PLoS One 2011;6(1):e16165.
50. Kapitulnik J, Gonzalez FJ. Marked endogenous activation of the CYP1A1 and CYP1A2 genes in the congenitally jaundiced Gunn rat. Mol Pharmacol 1993;43:722-5.

51. Kapitulnik J, Hardwick JP, Ostrow JD, Webster CC, Park SS, Gelboin HV. Increase in a specific cytochrome P-450 isoenzyme in the liver of congenitally jaundiced Gunn rats. Biochem J 1987;242:297-300.
52. Abu-Bakar A, Moore MR, Lang MA. Evidence for induced microsomal bilirubin degradation by cytochrome P450 2A5. Biochem Pharmacol 2005;70:1527-35.
53. Brito MA, Lima S, Fernandes A, et al. Bilirubin injury to neurons: contribution of oxidative stress and rescue by glycoursodeoxycholic acid. Neurotoxicology 2008; 29:259-69.
54. Vaz AR, Delgado-Esteban M, Brito MA, Bolaños JP, Brites D, Almeida A. Bilirubin selectively inhibits cytochrome *c* oxidase activity and induces apoptosis in immature cortical neurons: assessment of the protective effects of glycoursodeoxycholic acid. J Neurochem 2010;112:56-65.
55. Rodrigues CM, Solá S, Brites D. Bilirubin induces apoptosis via the mitochondrial pathway in developing rat brain neurons. Hepatology 2002;35:1186-95.
56. Fernandes A, Falcão AS, Abranches E, et al. Bilirubin as a determinant for altered neurogenesis, neuritogenesis, and synaptogenesis. Dev Neurobiol 2009;69:568-82.
57. Brites D. Bilirubin injury to neurons and glial cells: new players, novel targets, and newer insights. Semin Perinatol 2011; 35:114-20.
58. Silva SL, Vaz AR, Barateiro A, et al. Features of bilirubin-induced reactive microglia: from phagocytosis to inflammation. Neurobiol Dis 2010;40:663-75.
59. Deganuto M, Cesaratto L, Bellarosa C, et al. A proteomic approach to the bilirubin-induced toxicity in neuronal cells reveals a protective function of DJ-1 protein. Proteomics 2010;10:1645-57.
60. Falcão AS, Silva RF, Pancadas S, Fernandes A, Brito MA, Brites D. Apoptosis and impairment of neurite network by short exposure of immature rat cortical neurons to unconjugated bilirubin increase with cell differentiation and are additionally enhanced by an inflammatory stimulus. J Neurosci Res 2007;85: 1229-39.
61. Ollinger R, Kogler P, Troppmair J, et al. Bilirubin inhibits tumor cell growth via activation of ERK. Cell Cycle 2007;6: 3078-85.
62. Ollinger R, Yamashita K, Bilban M, et al. Bilirubin and biliverdin treatment of atherosclerotic diseases. Cell Cycle 2007; 6:39-43.
63. Brito MA, Vaz AR, Silva SL, et al. N-methyl-aspartate receptor and neuronal nitric oxide synthase activation mediate bilirubin-induced neurotoxicity. Mol Med 2010;16:372-80.
64. Giraudi PJ, Bellarosa C, Coda-Zabetta CD, Peruzzo P, Tiribelli C. Functional induction of the cystine-glutamate exchanger system Xc(-) activity in SH-SY5Y cells by unconjugated bilirubin. PLoS One 2011; 6(12):e29078.
65. Gennuso F, Fernetti C, Tirolo C, et al. Bilirubin protects astrocytes from its own toxicity by inducing up-regulation and translocation of multidrug resistance-associated protein 1 (Mrp1). Proc Natl Acad Sci U S A 2004;101:2470-5.
66. Brites D. The evolving landscape of neurotoxicity by unconjugated bilirubin: role of glial cells and inflammation. Front Pharmacol 2012;3:88.
67. Shapiro SM. Chronic bilirubin encephalopathy: diagnosis and outcome. Semin Fetal Neonatal Med 2010;15:157-63.
68. Palmela I, Sasaki H, Cardoso FL, et al. Time-dependent dual effects of high levels of unconjugated bilirubin on the human blood-brain barrier lining. Front Cell Neurosci 2012;6:22.
69. Falcão AS, Silva RF, Vaz AR, Silva SL, Fernandes A, Brites D. Cross-talk between neurons and astrocytes in response to bilirubin: early beneficial effects. Neurochem Res 2013;38:644-59.
70. Daood MJ, Hoyson M, Watchko JF. Lipid peroxidation is not the primary mechanism of bilirubin-induced neurologic dysfunction in jaundiced Gunn rat pups. Pediatr Res 2012;72:455-9.
71. Chowdhury JR, Kondapalli R, Chowdhury NR. Gunn rat: a model for inherited deficiency of bilirubin glucuronidation. Adv Vet Sci Comp Med 1993;37:149-73.
72. Gunn CH. Hereditary acholuric jaundice in a new mutant strain of rats. J Hered 1938;29:137-9.
73. Bortolussi G, Zentilin L, Baj G, et al. Rescue of bilirubin-induced neonatal lethality in a mouse model of Crigler-Najjar syndrome type I by AAV9-mediated gene transfer. FASEB J 2012;26:1052-63.
74. Maisels MJ, Stevenson D, Watchko JF, McDonagh AF. Phototherapy and other treatments. In: Stevenson DK, Maisels MJ, Watchko JF, eds. Care of the jaundiced neonate. New York: McGraw-Hill, 2012:195-227.
75. Hansen TW, Nietsch L, Norman E, et al. Reversibility of acute intermediate phase bilirubin encephalopathy. Acta Paediatr 2009;98:1689-94.
76. Schulz S, Wong RJ, Vreman HJ, Stevenson DK. Metalloporphyrins — an update. Front Pharmacol 2012;3:68.
77. Lin S, Wei X, Bales KR, et al. Minocycline blocks bilirubin neurotoxicity and prevents hyperbilirubinemia-induced cerebellar hypoplasia in the Gunn rat. Eur J Neurosci 2005;22:21-7.
78. Geiger AS, Rice AC, Shapiro SM. Minocycline blocks acute bilirubin-induced neurological dysfunction in jaundiced Gunn rats. Neonatology 2007;92:219-26.

IMAGES IN CLINICAL MEDICINE

The *Journal* welcomes consideration of new submissions for Images in Clinical Medicine. Instructions for authors and procedures for submissions can be found on the *Journal*'s website at NEJM.org. At the discretion of the editor, images that are accepted for publication may appear in the print version of the *Journal*, the electronic version, or both.