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REVIEW ARTICLE

# Understanding Neonatal Jaundice: A Perspective on Causation

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Neonatal jaundice can be best understood as a balance between the production and elimination of bilirubin, with a multitude of factors and conditions affecting each of these processes. When an imbalance results because of an increase in circulating bilirubin (or the bilirubin load) to significantly high levels (severe hyperbilirubinemia), it may cause permanent neurologic sequelae (kernicterus). In most infants, an increase in bilirubin production (e.g., due to hemolysis) is the primary cause of severe hyperbilirubinemia, and thus reducing bilirubin production is a rational approach for its management. The situation can become critical in infants with an associated impaired bilirubin elimination mechanism as a result of a genetic deficiency and/or polymorphism. Combining information about bilirubin production and genetic information about bilirubin elimination with the tracking of bilirubin levels means that a relative assessment of jaundice risk might be feasible. Information on the level of bilirubin production and its rate of elimination may help to guide the clinical management of neonatal jaundice.

## 1. Etiology of Neonatal Jaundice

Neonatal jaundice is usually a normal physiologic condition occurring during the transitional period after birth. It is not a singular disease in itself, but a physical finding associated with multiple possible etiologies. Severe neonatal jaundice is considered to be pathophysiologic. Jaundice reflects the accumulation of the yellow-orange pigment bilirubin in the skin, sclerae, and other tissues; it does not imply any particular causation. Thus, preventive and therapeutic approaches to pathophysiologic neonatal jaundice or hyperbilirubinemia have typically been nonspecific. They include phototherapy and blood exchange transfusions, both of which facilitate the removal of bilirubin after it has been produced in the body. More specific or targeted approaches require a better understanding of the pathophysiology involved in each individual case.

The production of bilirubin as a result of the degradation of heme arising from normal red blood cell (RBC) turnover is a normal part of our physiology. The first step in this two-step process is an ancient one in the biology of this planet; it occurs in both plants and animals. In the first step, heme is catalyzed by the membrane-associated enzyme, heme oxygenase (HO).[1] There are at least two forms of this enzyme: HO-1, the inducible form, and HO-2, the constitutive form.[2] The ratio between the two forms varies in different tissues. It is possible that a third form also exists, but much less is known about this protein.[3] The first enzymatic step requires molecular oxygen ($O_2$) and NADPH donated from the cytochrome P450 system (Figure 1[4]). It involves a

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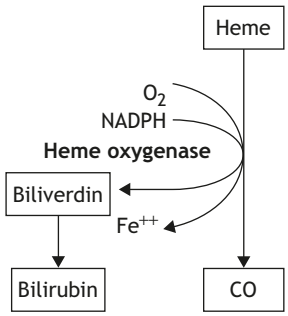


**Figure 1** Heme degradation pathway. The turnover of hemoglobin and other hemoproteins yields heme, which is metabolized to equimolar quantities of carbon monoxide (CO), iron ($Fe^{++}$) and biliverdin. Biliverdin is subsequently reduced to form bilirubin. Modified and reproduced with permission from Reference 4.

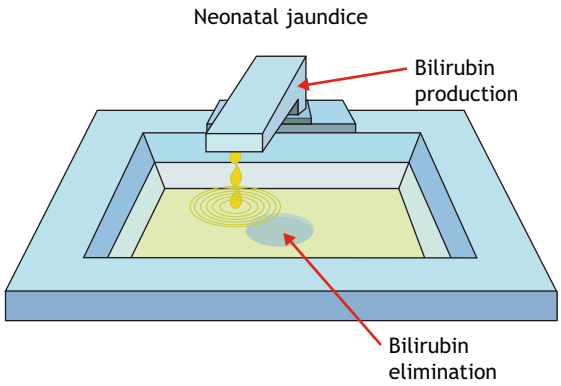


**Figure 2** Diagram of bilirubin production and elimination. Reproduced with permission from Reference 8.

series of oxidations and reductions, ultimately leading to the breakage of the α-methene bridge of the heme ring, releasing carbon monoxide (CO) and ferrous iron ($Fe^{++}$), giving rise to the green pigment, biliverdin. In most mammals, biliverdin is rapidly reduced in the cytosol by biliverdin reductase in the presence of NADPH to produce the yellow pigment, bilirubin. CO, $Fe^{++}$ and bilirubin are produced in equimolar amounts. Therefore, based on this stoichiometry and in the absence of any other source of bilirubin, CO production can serve as an index of bilirubin production.[5] CO diffuses from the cell, binds to hemoglobin (Hb), thereby forming carboxyhemoglobin. This complex then circulates to the lungs, where the CO is exchanged for oxygen and then excreted in breath. End-tidal CO (ETCO)[6] or the excretion rate of CO (VeCO)[5,7] can therefore be used to estimate total bilirubin production in neonates.

Once produced, bilirubin is normally conjugated and then excreted via the liver. It is transported in the bloodstream bound to albumin, and is actively transported across the sinusoidal cell membrane of hepatocytes facing the blood stream (space of Disse). Once inside hepatocytes, it is conjugated with glucuronic acid to form bilirubin glucuronide, also known as either "conjugated" or "direct" bilirubin. The enzyme bilirubin uridine diphosphate glucuronosyltransferase (bilirubin UGT; UGT1A1) does this by a two-step process, producing bilirubin monoglucuronide first, and following a second glucuronidation, producing bilirubin diglucuronide. The conjugated bilirubin is then transported across the canalicular membrane into the biliary tree by specific proteins, most notably multidrug resistance-associated protein 2. Conjugated bilirubin proceeds down the biliary tree to the lumen of the gut for excretion. Newborn infants, particularly premature ones, have an immature bilirubin conjugation and excretion system.

Neonatal jaundice can be understood by analogy with a sink (Figure 2[8]). The turned-on spigot represents the process of bilirubin production, and the drain represents the process of bilirubin elimination. If the rate at which bilirubin is produced exceeds the rate at which it is eliminated, then the level in the sink begins to rise. In this analogy, the sink represents the circulation and the capacity of the sink is determined largely by how much bilirubin can be bound to albumin. However, bilirubin is always moving from the circulation into the tissues, depending on the binding conditions. Therefore, the amount of albumin in the circulation is relevant, but the affinity of albumin for bilirubin is also a factor, with lower affinity reducing the amount of bilirubin retained in circulation.

The bilirubin load in the body is thus the result of the imbalance between bilirubin production and elimination.[9] Neonatal jaundice reflects an increase in total body bilirubin load after birth, but the apparent or "visible" jaundice is not a good predictor of the level of bilirubin in circulation or the amount of bilirubin in the various tissues. Because bilirubin can have toxic effects under some conditions, its levels need to be closely monitored in the first weeks after birth.

## 2. Role of Carbon Monoxide

Nature has two dispositions, one that is toxic and one that is beneficial, and under physiologic conditions, are usually balanced. For example, in the case of heme degradation, its products can be characterized as toxic: bilirubin can cause neurologic disturbances, such as acute bilirubin encephalopathy that can result in permanent damage or kernicterus and occasionally death;[10] CO can cause mitochondrial dysfunction and death; $Fe^{++}$ can generate reactive oxygen species and contribute to oxidative injury. However, these same products can

be characterized differently as having a variety of important roles in biology. The biliverdin/bilirubin oxidation-reduction shuttle plays an important role in antioxidant defense.[11] CO has a myriad of effects, including vessel relaxation through several possible mechanisms,[12–14] inhibition of platelet aggregation,[15] and anti-apoptotic and anti-proliferative effects.[16] It may also inhibit pro-inflammatory cytokines,[17,18] and play a role in neurotransmission.[19,20] Moreover, the interactions between the HO/CO and nitric oxide synthase/nitric oxide systems are especially complex, and may involve both positive and negative effects.[16] Finally, iron is an essential element for much of life on the planet.

Under most of the conditions encountered in the newborn, the predominant endogenous source of CO (about 80%) comes from the degradation of heme, with 70% arising from RBC senescence, approximately 10% from ineffective erythropoiesis and 20% from the degradation of other hemoproteins. Nonetheless, it is important to recognize other potentially important sources of this gas, including photo-oxidation[21,22] and lipid peroxidation.[23] Only 14% of CO or less typically comes from lipid peroxidation and photo-oxidation, although these sources become more significant under certain conditions, such as oxidation or light exposure.[21,22]

Bilirubin production has been estimated in a variety of species by measuring CO production. Various methods have been used for this purpose, but gas chromatography using a reduction gas detector is one of the most sensitive methods.[24] Heme administered as damaged RBCs can be recovered completely as CO over a timeframe, reflecting splenic sequestration, RBC breakdown and heme catabolism. The same technology has been used in human studies; however, because the other sources of CO cannot be known precisely, VeCO or ETCO measurements should only be considered as an index of total bilirubin production. Despite this limitation, such measurements can be useful for identifying differences in bilirubin production between infants and adults, and in infants with conditions such as hematoma, polycythemia, hemolysis and having a diabetic mother. VeCO measurements are not easily performed in the clinical setting. However, measurements of ETCO corrected for ambient CO are a good index of the CO production rate and correlate well with the carboxyhemoglobin corrected for ambient CO as well.[6,25–27] The distribution of corrected ETCO values has been described previously, with easy identification of babies outside the normal distribution.[6,28] It is technically possible to produce a handheld device for commercial use that would provide accurate, reliable as well as rapid and easy bedside end-tidal breath sampling for estimating CO production as an index of bilirubin production. An infant could then be classified as "high" or "low" jaundice risk on the basis of an increased bilirubin load. Finally, the hour-specific total serum bilirubin nomogram reflects increased production of bilirubin in the context of impaired elimination, with the highest risk zones reflecting increased bilirubin loads often observed in infants with increased bilirubin production rates.[29] Infants with hemolysis or other causes of increased bilirubin production are those most likely to demonstrate a trajectory that enters the risk zone early. Other infants appear to enter the risk zone over a longer period of time with low or normal bilirubin production, suggesting impaired conjugation. The worst-case scenario is of an infant with the combination of increased production and impaired elimination, with an unpredictable trajectory depending upon the degree of imbalance (Table 1).[9]

**Table 1** Jaundice risk profiles based on an infant's rates of bilirubin production and elimination

| | Fast eliminator | Slow eliminator |
|---|---|---|
| Low producer | Low risk | Medium risk |
| High producer | Medium risk | High risk |

## 3. Diagnosis and Management of Neonatal Jaundice

Clinically, the differential diagnosis of neonatal jaundice varies during the first weeks of life. For instance, because virtually all neonates have decreased conjugation and excretion capabilities at birth, hyperbilirubinemia in the first 1–3 days of life almost always reflects an increase in bilirubin production.[30,31] Unconjugated hyperbilirubinemia on day 1 generally reflects hemolysis, usually due to minor blood-type incompatibilities such as rH or Kell, or intrauterine infection. Less commonly, the increase in bilirubin production may be due to a large hematoma (e.g., subcapsular hepatic or subgaleal). Jaundice presenting from days 3–7 more often reflects more modest increases in production, such as those associated with ABO incompatibilities or maternal diabetes. This is also the time when problems with an impaired ability to upregulate conjugation start to appear; the classic form of this is Gilbert's disease, where there is impaired UGT1A1 activity. When combined with even modest increases in production such as those seen with glucose-6-phosphate dehydrogenase (G6PD)-deficiency, the consequences can be devastating, with a dramatic and rapid rise in bilirubin levels. Infants with decreased food intake, and thus decreased stooling,

may also present with increased jaundice at this time, because of the increased re-uptake of excreted bilirubin by the intestines (enterohepatic circulation of bilirubin). This is seen with so-called "breast-feeding failure jaundice", when an infant's milk intake is too low. Jaundice presenting after the first week of life rarely represents increased bilirubin production (more flow at the spigot), but rather implies decreased excretory ability (prolonged inability to open the drain). This is when problems with liver disease and biliary obstructions tend to present. Failure to recognize this transition represents a serious clinical error: once jaundice persists beyond 7–10 days, and certainly beyond 14 days, conjugated hyperbilirubinemia must be considered and ruled-out to avoid delaying the diagnosis of biliary atresia.

Because increased bilirubin production is a contributory factor in all kinds of neonatal hyperbilirubinemia, modulation of bilirubin production represents a rational approach to managing the condition.[32,33] The target is HO, although it is important to understand that this target is not singular but part of a biochemical system with connections to other important biological processes through the products of the heme catabolic pathway (Figure 1).[4] Thus, inhibition of HO as a means of controlling bilirubin production must be considered in the context of its other potential ramifications. A variety of metalloporphyrins (heme analogs) have been investigated as competitive inhibitors of the HO reaction.[34,35] The most well studied compounds include tin protoporphyrin, tin mesoporphyrin,[35] zinc protoporphyrin, zinc deuteroporphyrin bis glycol,[36] and chromium mesoporphyrin. Even the naturally occurring zinc protoporphyrin, which has a physiologic role, can be used as a pharmacologic inhibitor of HO. In Rhesus monkeys with neonatal jaundice exacerbated by hemolysis, increased bilirubin production can be lowered to native production rates and hyperbilirubinemia ameliorated following metalloporphyrin administration. Criteria have been developed for potential antihyperbilirubinemia drugs.[34,35] These criteria include the following: biocompatible central metal, potent HO inhibition, negligible degradation, negligible inhibition of other enzymes (such as soluble guanylyl cyclase and nitric oxide synthase),[37] negligible photoreactivity,[21,34] optimal duration of action (several days), and negligible HMOX-1 (HO-1 gene) upregulation. *In vivo* bioluminescence imaging has been used to investigate the upregulation of HMOX-1 in response to competitive inhibition of the protein.[38–42] The HO-1-*luc* transgenic mouse model has been used for this purpose (Figure 3).[43] Such studies have revealed that metalloporphyrins differ in their abilities to inhibit HO and upregulate HMOX-1.[39,40,42] For example, tin mesoporphyrin is a potent inducer of HMOX-1, despite its inhibitory potency.[35] Zinc deuteroporphyrin bis glycol and chromium mesoporphyrin are nearly as potent, but cause minimal upregulation of the HMOX-1 system at effective inhibitory doses.[40,44] They have other attractive properties as well, such oral absorption, which make them possible alternative drugs for clinical use.

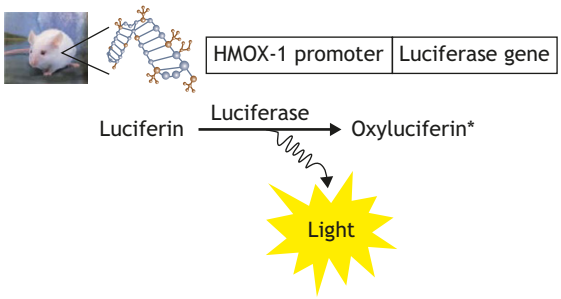


**Figure 3** HO-1-*luc* mouse. The reporter mouse contains a transgene consisting of the full-length (15 kb) HMOX-1 promoter driving expression of the reporter gene, luciferase (*luc*). This model allows us to monitor changes in HMOX-1 transcription noninvasively through proportional changes in luciferase activity. When luciferin is administered, it is rapidly converted to oxyluciferin by luciferase, resulting in the production of light. Light emission, or bioluminescence, therefore reflects luciferase transcription and, hence, HMOX-1 transcriptional activity.

Although bilirubin production is a rational target for the management of neonatal hyperbilirubinemia, bilirubin elimination is an equally important factor in the clinical equation. Kaplan et al[45] demonstrated that in G6PD-deficient neonates only the ones with impaired conjugation became hyperbilirubinemic, even in the absence of a crisis. Thus, the intersection of increased production and impaired elimination creates the most dangerous scenario.[46,47] The genetic vulnerability created by G6PD-deficiency and Gilbert's disease, a polymorphism involving TATA repeats in the promoter of the UGT1A1 gene, represents a real danger to the newborn.[48–50] Thus, genetic information on polymorphisms affecting jaundice could be clinically useful, given it was available in a timely manner. For example, information about the TATA repeats in the HMOX-1 promoter might provide information about the propensity for bilirubin production, with larger expansions being associated with lower production of the protein. In addition, polymorphisms involving UGT1A1 or multidrug resistance-associated protein would be important for understanding the likelihood of impaired elimination.[51,52] A relative assessment of jaundice risk might be possible by looking for the intersection between low/high production and fast/slow elimination. It is possible that combining prenatal genetic information with postnatal information on bilirubin production and the tracking of

bilirubin levels could shift the management of neonatal jaundice to a preventive strategy.

In summary, like all of nature, the heme degradation pathway has two potential dispositions: for the clinician, the potentially toxic disposition is that of concern. However, it is important to understand that the pathway is also involved in many other important biological processes. Newborn jaundice needs to be modulated, rather than totally eliminated, and extremes should be avoided in high-risk circumstances. Maintaining an overall balance is the key to good practice, even if one has to target the pathway under certain risky conditions, in order to control excessive bilirubin production.

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