Understanding Neonatal Jaundice: A Perspective on Causation

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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
series of oxidations and reductions, ultimately leading to the breakage of the α-methene bridge of the heme ring, releasing carbon monoxide (CO), iron (Fe⁹⁺) and biliverdin. Biliverdin is subsequently reduced to form bilirubin. Modified and reproduced with permission from Reference 4.

Neonatal jaundice can be understood by analogy with a sink (Figure 2). 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. 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; Fe⁹⁺ can cause mitochondrial dysfunction and death; CO can cause 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.\textsuperscript{11} CO has a myriad of effects, including vessel relaxation through several possible mechanisms,\textsuperscript{12-14} inhibition of platelet aggregation,\textsuperscript{15} and anti-apoptotic and anti-proliferative effects.\textsuperscript{16} It may also inhibit pro-inflammatory cytokines,\textsuperscript{17,18} and play a role in neurotransmission.\textsuperscript{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.\textsuperscript{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\textsuperscript{21,22} and lipid peroxidation.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{6,25-27} The distribution of corrected ETCO values has been described previously, with easy identification of babies outside the normal distribution.\textsuperscript{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.\textsuperscript{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).\textsuperscript{9}

### Table 1

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<th>Jaundice risk profiles based on an infant's rates of bilirubin production and elimination</th>
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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.\textsuperscript{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. 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). 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. The most well studied compounds include tin protoporphyrin, tin mesoporphyrin, zinc protoporphyrin, zinc deuteroporphyrin bis glycol, 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 anti-hyperbilirubinemia drugs. 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), negligible photoreactivity, 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. The HO-1-luc transgenic mouse model has been used for this purpose (Figure 3). Such studies have revealed that metalloporphyrins differ in their abilities to inhibit HO and upregulate HMOX-1. For example, tin mesoporphyrin is a potent inducer of HMOX-1, despite its inhibitory potency. Zinc deuteroporphyrin bis glycol and chromium mesoporphyrin are nearly as potent, but cause minimal upregulation of the HMOX-1 system at effective inhibitory doses. They have other attractive properties as well, such as oral absorption, which make them possible alternatives for clinical use.

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 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. 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. 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. 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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References


