Molecular Basis of Hyperbilirubinemia and Phototherapy

THOMAS R.C. Sisson, M.D.
Department of Pediatrics, Perth Amboy General Hospital, Perth Amboy, New Jersey, U.S.A.
and Rutgers Medical School

Hyperbilirubinemia in the newborn results not only in visible yellow discoloration of the skin but, in high concentration, may cause bilirubin encephalopathy. Such damage to the central nervous system may be subtle and not apparent for several years, as with visual-motor perceptive defects; or it may cause severe neurologic damage (Kernicterus)—even death. Sick and immature infants are the most vulnerable to bilirubin toxicity. Although this condition affects nearly half of all newborns to some degree, only about 10% require treatment.

Two methods of treatment are really effective in correcting hyperbilirubinemia, exchange blood transfusions, and/or phototherapy with light radiation in the blue part of the visible spectrum.

If the rate of production of bilirubin is excessive or an infant’s capacity to conjugate and excrete the pigment is deficient, bilirubin will accumulate in plasma, and will be taken up by other lipid-containing tissues and collagen. Bilirubin is normally bound to albumin at 2 sites, a primary site with a binding constant of $3 \times 10^9$ (80% or more bound here), and a secondary site with binding constant of $10^9$ [3]. This secondary site of weaker binding is occupied if the albumin: bilirubin ratio exceeds 1, or if other compounds compete for the stronger primary site. If bilirubin is bound to albumin, whether conjugated or not, it will not cross the blood brain barrier and thus cannot damage cells of the CNS. However, if unbound, the entry of divalent bilirubin (acid) into neuronal membranes by binding to phospholipids in these structures will cause distortion of the cell, and by aggregating cause its eventual destruction [4].

Many factors combine to raise plasma levels of bilirubin to toxic levels; for example, acidosis, sepsis, hypoxia, hemolysis, hypoalbuminemia, and certain competitive albumin binders.

Bilirubin is photolabile in vivo, and if the whole body is irradiated with visible light in the absorption band (450-490 nm) of bilirubin, the pigment will undergo photocatabolism. Under phototherapy bilirubin undergoes photoisomerization at the meso double-bond to conformations less lipophilic. It is now known that the major photo products of bilirubin IX-α are an unresolved mixture of its E, Z and E, Z isomers, easily excreted by the liver. Thus, phototherapy will reduce the accumulation of bilirubin in skin and other tissues and in circulating plasma.

Visible jaundice in the skin and sclerae of newborn infants is the result of hyperbilirubinemia, that is to say, concentrations of bilirubin in plasma that exceed 5-6 mg/dl. This condition affects roughly half of all infants born, to a greater or lesser degree, and is one of the very few symptoms in the neonate which itself may cause damage unrelated to the underlying illness.

Bilirubin is a straight chain tetrapyrrole derived principally from the breakdown of heme. This occurs when heme is catabolized, by oxidation of its alpha-methene bond, in the reticuloendothelial cells of the liver and spleen and bone marrow, as red blood cells age, are incompletely formed, or are for other reasons destroyed [1].

Bilirubin is a lipid-soluble, nonpolar pigment which enters the circulation in an unconjugated form and is bound largely to albumin, and to a lesser extent to red cell membranes, during its transport. In the liver cell it is normally taken up by protein Y, called ligandin [2], which binds organic ions, among its functions, and which holds the bilirubin molecule within the cytoplasm. In this location, bilirubin is normally conjugated with glucuronic acid by UDP-glucuronyl transferase, and thence excreted in the bile.

If the rate of production of bilirubin is excessive, or the capacity to conjugate and excrete the pigment is deficient, unconjugated bilirubin accumulates in the plasma and will be taken up by other lipid-containing tissues and collagen. Bilirubin is normally bound to albumin at 2 sites, a primary site with a binding constant of $3 \times 10^9$ (80% or more bound here), and a secondary site with binding constant of $10^9$ [3]. This secondary site of weaker binding is occupied if the albumin: bilirubin ratio exceeds 1, or if other compounds compete for the stronger primary site. If bilirubin is bound to albumin, whether conjugated or not, it will not cross the blood brain barrier and thus cannot damage cells of the CNS. However, if unbound, the entry of divalent bilirubin (acid) into neuronal membranes by binding to phospholipids in these structures will cause distortion of the cell, and by aggregating cause its eventual destruction [4].

Many factors combine to raise plasma levels of bilirubin in the newborn, especially in immature infants and those small for their gestational age [5]: acidosis and hypoalbuminemia (common in the premature), hypoxia, hemolytic disease of the newborn, dehydration, sepsis, certain drugs and antibiotics, steroids and other compounds which compete for binding sites on albumin, red cell enzyme deficiencies, etc.

Whatever factors are at play to produce hyperbilirubinemia, the effect of levels above 9 mg/dl in the immature infant and above 15 mg/dl in the full-term infant are potentially if not actually damaging to the central nervous system [6-11].

This damage has been termed bilirubin encephalopathy, and may be expressed as visual-motor perceptive defects, all the way to deafness, cerebral palsy and other massive neurologic defects called “kernicterus,” to actual death. It is these irreversible injuries to the brain that result from uncontrolled and elevated levels of bilirubin in the plasma that must be treated.

Previous to the studies of Cremers, Perryman, and Richards [12], which inaugurated the technique of phototherapy, the only effective treatment of neonatal hyperbilirubinemia was exchange transfusion [13]. Now, however, it is possible to reduce the plasma concentration of bilirubin by the whole body irradiation of the jaundiced infant with visible light. However, its spectral output must contain light in the range of 440-490 nm, in order to assure its absorption by bilirubin attached to albumin, lipids and collagen, at a variety of plasma pH—generally absorption is 450-460 nm. Phototherapy units, banks of 8 to 10 fluorescent lamps placed about 2 ft above the skin surface of infants, are effective light sources. It is now known that there is a dose: response relationship of light and bilirubin photodecomposition [14-16], and that fluorescent light sources containing 420-470 nm wavelengths are more efficient than broad-spectrum fluorescent lamps having an output of energy between 400 and 700 nm. with but one-quarter the energy in the range of absorption of bilirubin.

Since the discovery of phototherapy, much effort has gone into the determination of the actual photocatabolism of bilirubin. It is clear today that, both in vitro and in vivo, bilirubin

Reprint requests to: Thomas R. C. Sisson, M.D., Dept. of Pediatrics, Perth Amboy Gen'l Hospital, Perth Amboy, NJ 08861.

Abbreviations:

G6PD: glucose-6-phosphate dehydrogenase
GSH: glutathione reductase
HGH: human growth hormone
REM: rapid eye movements
undergoes a number of photochemical reactions that depend on the chemical environment [17]. The lability of bilirubin and the intermediate compounds of its photobreakdown complicates the determination of the pathway end-products. It is enough to say, however, that following the work of Ostrow et al [17-20] and Lightner, McDonagh et al [21-24] we have conclusive evidence that under phototherapy, bilirubin undergoes photodimerization at the meso double-bond to a conformation less lipophilic, a less internally hydrogen bonded form. Thus, it is now known that the main photoproduct of IXα-bilirubin are an unresolved mixture of its E, Z and Z, E isomers (Fig 1).

The liver is able to excrete such isomers, and so the marked increase in the biliary excretion of unconjugated, as well as normally conjugated, forms of bilirubin and related bile pigments when both jaundiced rats [20, 25] and jaundiced babies [26] are irradiated with visible light of appropriate wavelength is now explained.

Phototherapy has effects upon organ systems and profound influence on intrinsic biologic behavior as well as upon cellular and molecular events. It has been shown, for instance, that during phototherapy the sleep states of newborns is altered; that rapid eye movement (REM) sleep increases not only in frequency but duration, compared with infants in a normal nursery environment [27]. It is believed that protein synthesis in neurones occurs mainly during periods of REM sleep, and one can hope that promotion of this state in the developing brain of the newborn is an advantage.

Still another phenomenon of significance that is influenced by exposure of infants to visible light is biologic rhythm. Plasma somatotropin (human growth hormone or HGH) is a representative marker for biorhythms in the neonate. A series of studies [28-30] showed the pattern of rise and fall of plasma HGH under conditions of cycled light: dark, constant nursery light, and constant phototherapy with eyes covered by light-opaque shields.

It was found that under cycled light with intervals of darkness or very dim illumination, the newborn has an ultradian rhythm of 4-6 hr interval and with a wide circadian rhythm peaking at midnight to 2:00 AM, as in the adult. Under constant nursery light, however, the major circadian rhythm disappears and only the 4-6 hr ultradian rhythms persist. Both circadian and ultradian rhythms of HGH, on the other hand, are obliterated in infants under phototherapy (Fig 2).

These findings indicate that the common practice of keeping nurseries constantly illuminated can eliminate normal rhythms of growth hormone observed under cycled lighting. The erasure of all biorhythm of this important developmental hormone during phototherapy is not physiologic; although its temporary nature may not produce a long-term effect, it does delay the establishment of a biorhythm normally present in the first day of life, and the consequence of this is uncertain.

Certain short-term effects of phototherapy indicate that fundamental metabolic processes are vulnerable to visible light irradiation. Rubaitelli, et al [31] have shown that the tryptophane-kynurenine pathway is disturbed because of the photodestruction of the metabolites of tryptophane.

The normal concentration of riboflavin, vitamin B2, in whole blood is rapidly reduced by phototherapy of the newborn (Fig 3) [32]. The in vitro and in vivo reduction of riboflavin has been associated with reduction of erythrocyte glucose-6-phosphate dehydrogenase(G6PD) and glutathione reductase (GSH) [32-36]. When such enzyme deficiencies are essentially light-induced they lead to increased red cell breakdown and consequent increase of plasma bilirubin rather than an expected phototherapeutic decrease. As riboflavin is an essential co-

---

**Fig 1.** Light converts bilirubin IX-α (Z, Z) to photobilirubin IX-α (E, Z) by turning one of the outer pyrrol rings at the double-bond. Photobilirubin does not form an insoluble acid, is nontoxic, and is excreted rapidly in the bile without conjugation.

**Fig 2.** The effect of cycled light/dark environments and constant phototherapy radiation upon plasma Human Growth Hormone in vivo.

**Fig 3.** Effect of phototherapy upon whole blood riboflavin levels in newborn infants.
Table 1. Enzyme activity of perfused Gunn rat livers—\( \mu \text{mol/gm} \)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dark</th>
<th>Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( i )</td>
<td>( j )</td>
</tr>
<tr>
<td>Aminopyrene demethylation</td>
<td>3.028</td>
<td>3.77</td>
</tr>
<tr>
<td>Aniline dehydroxylation</td>
<td>0.151</td>
<td>0.187</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>0.435</td>
<td>0.442</td>
</tr>
<tr>
<td>Cytochrome b5</td>
<td>0.792</td>
<td>0.551</td>
</tr>
<tr>
<td>Benzo (a) pyrene hydroxylation</td>
<td>0.569</td>
<td>0.772</td>
</tr>
</tbody>
</table>

22. McDonagh AF: Phototherapy and hyperbilirubinemia. Lancet 1: 389 (February 2) 1975
33. Sisson TRC, Fiorentino T: unpublished data
36. Johnson L: personal communication
37. Hola-Pleszczynski M, Hensen SA, Vincent MM, Bellanti J: Inhibi-
Nancy J.1981 MOLECULAR BASIS OF HYPERBILIRUBINEMIA AND PHOTOTHERAPY 161


ACKNOWLEDGEMENT

In order for The Society for Investigative Dermatology to generate additional funds and further expand its activities in the field of Dermatology, a new class of membership, Corporate Sustaining Membership, has been established. The Society wishes to acknowledge the support of the following companies, who are Corporate Sustaining Members:

BURROUGHS WELLCOME COMPANY
DERMIX LABORATORIES, INC.
HOFFMAN-LaRoche, INC.
ELI LILLY AND COMPANY
HOECHST-ROUSSEL PHARMACEUTICALS, INC.
NEUTROGENA CORPORATION
OWEN LABORATORIES
ORTHO PHARMACEUTICALS, INC. (DERMATOLOGICS DIVISION)
PROCTER AND GAMBLE COMPANY
REED AND CARNICK PHARMACEUTICALS
RICHARDSON MERRELL, INC. (VICK TOILETRIES DIVISION)
SCHERING LABORATORIES
STIEFEL LABORATORIES, INC.
SQUIBB INSTITUTE FOR MEDICAL RESEARCH
SYNTEx LABORATORIES
UPJOHN COMPANY
This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.