Virtual Free Radical School

Bilirubin: Friend or Foe?

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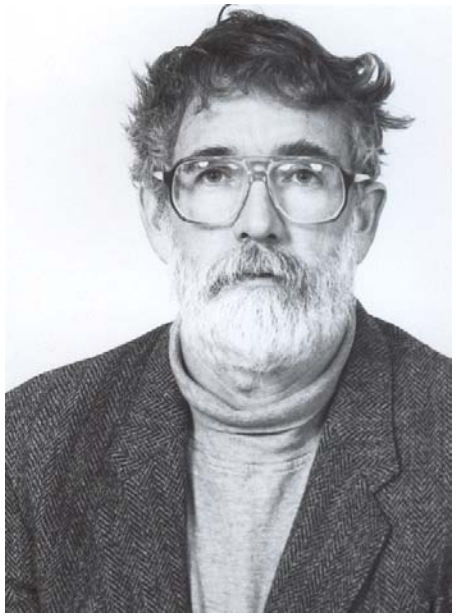

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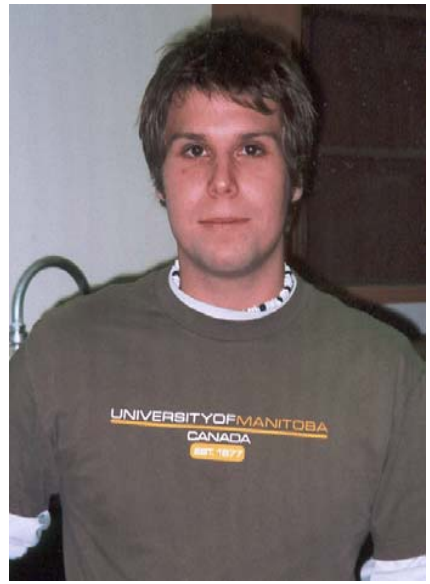
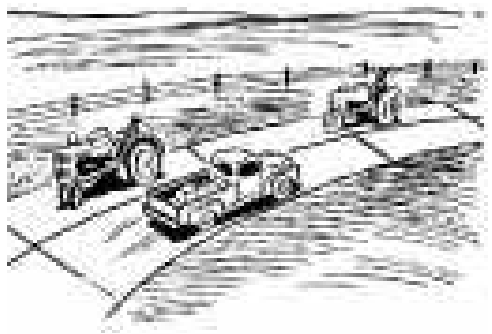


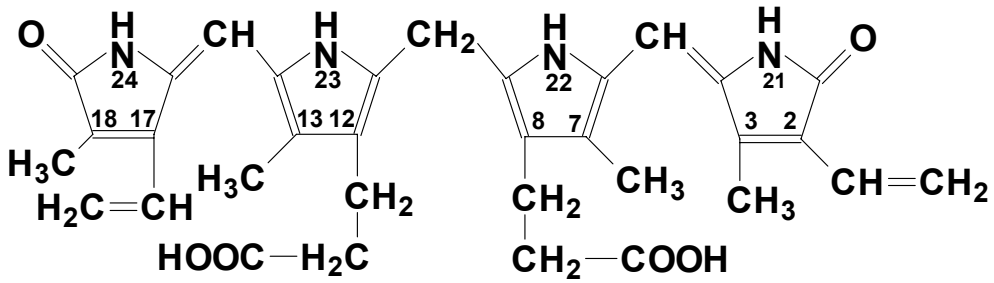
"Learning is my home."

The Road Ahead

- Function of Bilirubin
- Bilirubin as Antioxidant
- Bilirubin as Toxin
- Jaundice
- Hyperbilirubinemia
- The Premature Infant
- Now what?



Bilirubin

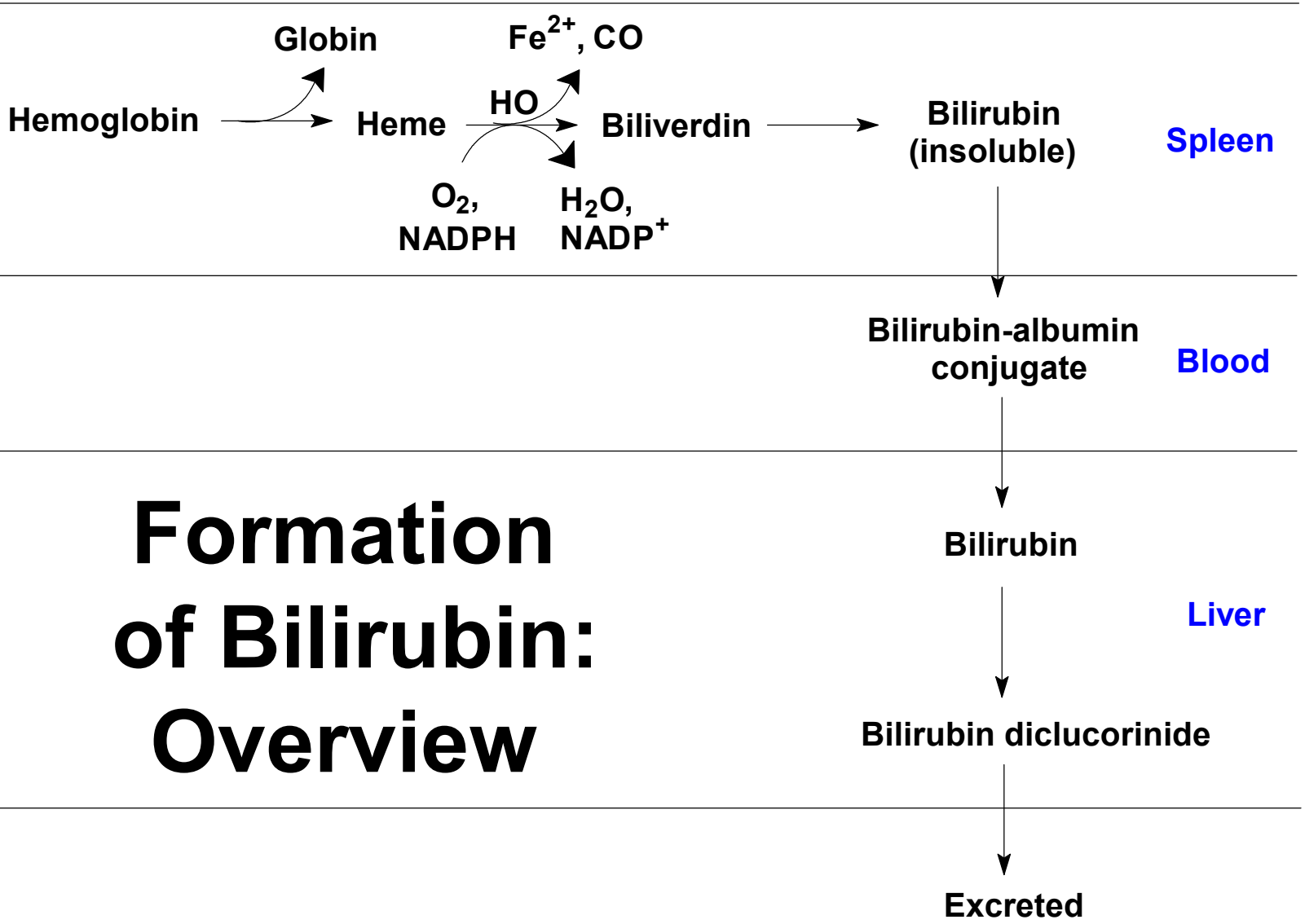


Bilirubin

- Is a bile pigment.
- Results from the degradation of heme, one of the breakdown products of red blood cells.
- It is thought to be a toxin because it is associated with neonatal jaundice, possibly leading to irreversible brain damage.

Tomaro ML, Batlle AM del C. (2002) Bilirubin: its role in cytoprotection against oxidative stress. *Int J Biol Cell Biol.* **34:**216-220

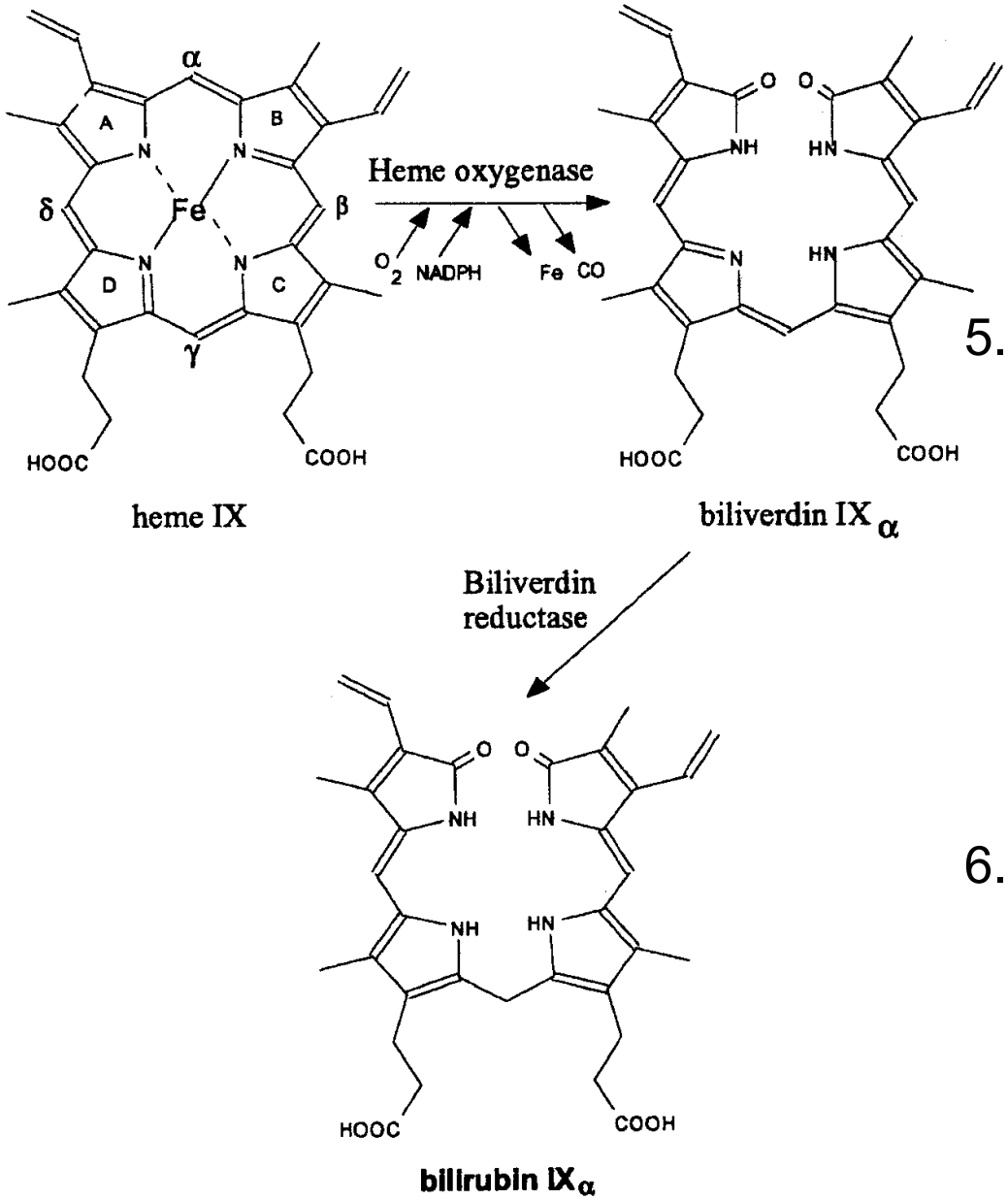
Formation of Bilirubin: Overview



Formation of Bilirubin

1. Hemoglobin from senescent or hemolyzed red cells is broken down, releasing heme.
2. Heme is then degraded in humans by the enzyme heme oxygenase (HO), which is the rate-limiting step in the formation of bilirubin.
3. HO converts heme to biliverdin IX.
4. Biliverdin is a hydrophilic compound that is reduced by biliverdin reductase into the hydrophobic compound bilirubin.

Formation of Bilirubin



5. HO catalyses an oxidase reaction opening the heme ring to convert one of the bridge carbons to carbon monoxide. This step releases iron from the now linear tetrapyrrole yielding biliverdin.

6. Biliverdin reductase reduces the double bond on nitrogen inside one of four of the pyrrole rings leading to the formation of bilirubin.

Bilirubin as an antioxidant:

As early as 1959, it was suggested that bilirubin might be a an antioxidant.

Bilirubin can suppress oxidation of lysosomes at oxygen concentrations that are physiologically relevant.

Bilirubin can act as an important cytroprotector of tissues that are poorly equipped with antioxidant defense systems, including myocardium and nervous tissue.

Temme EHM, Zhang J, Schouten EG, & Kesteloot H. (2001). Serum bilirubin and 10-year mortality risk in a Belgian population. *Cancer Causes and Control.* **12**: 887-894.

Bilirubin as an Antioxidant

The proposed mechanism is:

- Bilirubin can **scavenge the chain-carrying peroxyl radical** by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule to form a carbon-centered radical Bil•

$$LOO^{\bullet} + Bil \rightarrow LOOH + Bil^{\bullet}$$

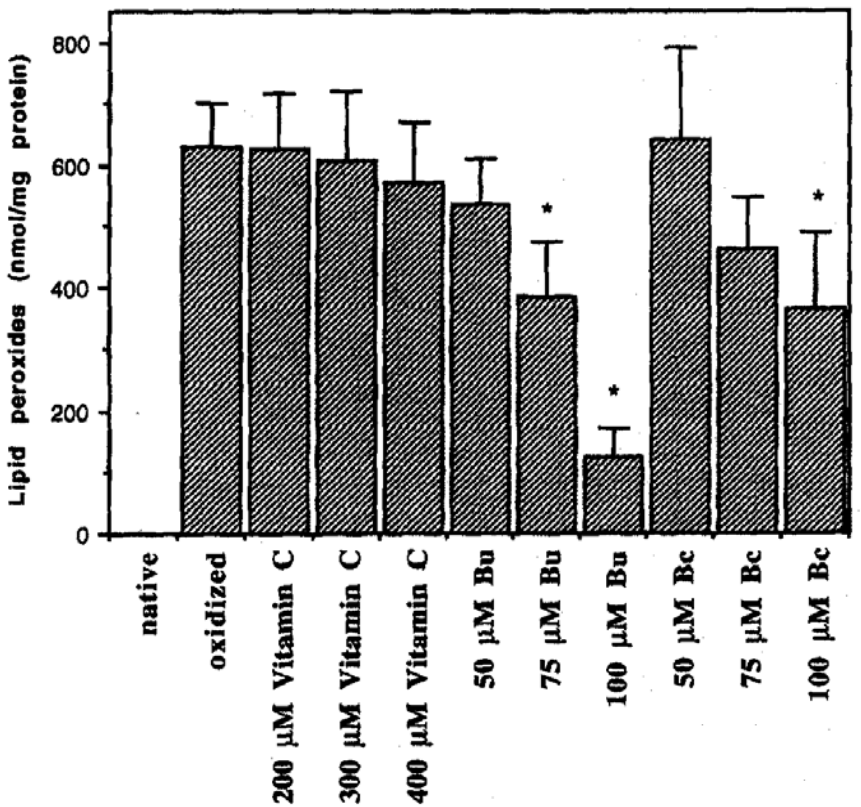
$$Bil^{\bullet} + LOO^{\bullet} \rightarrow Bil\text{-}OOL$$

$$Bil^{\bullet} + O_2 \leftrightarrow Bil\text{-}OO^{\bullet}$$

$$LOO^{\bullet} + BV \rightarrow LOO\text{-}BV^{\bullet}$$

Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, & Ames BN. (1987). Bilirubin is an antioxidant of possible physiological importance. *Science.* **235:** 1043-1046.

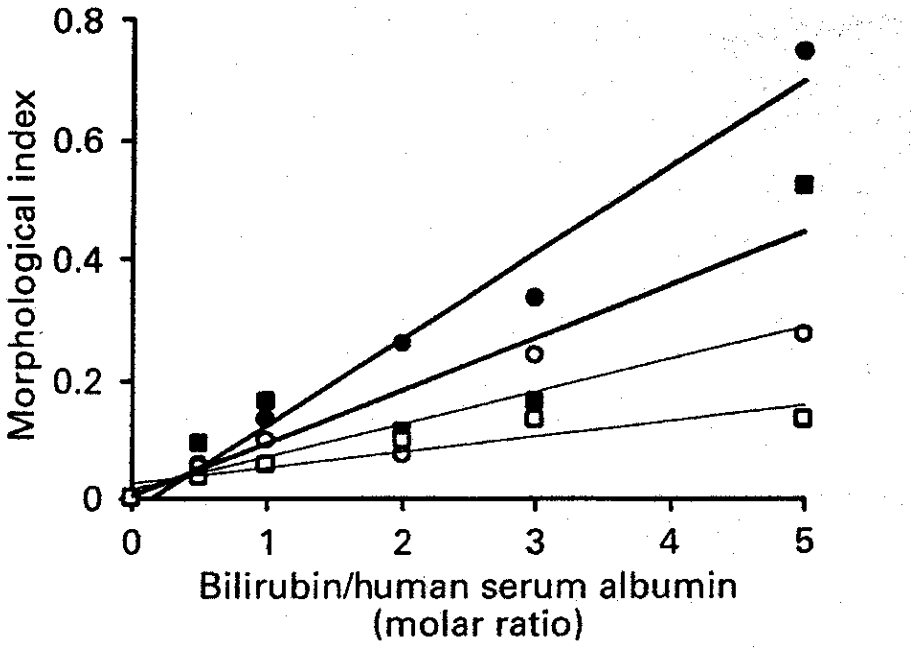
Unconjugated & Conjugated Bilirubin Serve as Antioxidants in Lipid peroxidation



Both unconjugated bilirubin (Bu) and conjugated bilirubin (Bc) can serve as antioxidants, protecting human LDL from lipid peroxidation *in vitro* against peroxyl radicals (generated by 2,2'-*azobis* (2-amidinopropane) dihydrochloride.

Wu T-W, Fung KP, Wu J, Yang C-C & Weisel RD (1996). Antioxidation of human low-density lipoprotein by unconjugated and conjugated bilirubins. *Biochem Pharmacol* **51**: 859-862.

The Toxic Side of Bilirubin



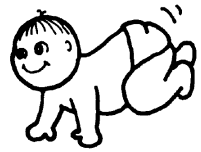
Brito MA, Silva R, Tiribelli C & Brites D. (2000). Assessment of bilirubin toxicity to erythrocytes. Implication in neonatal jaundice management. *European J Clinical Invest* **30:** 239-247.

Erythrocyte morphological changes have been seen with incubation of cells with different molar ratios of unconjugated bilirubin.

These changes occur as the bilirubin/human serum albumin molar ratio increases.

This indicates that bilirubin can illicit toxicity in the erythrocyte membrane in a concentration and temperature-dependent manner.

Bilirubin and Jaundice



- Neonatal jaundice is a yellowing of the skin and eyeballs and may lead to deposition of bilirubin in brain cells.
- Normally bilirubin is bound (conjugated) by a transport molecule and excreted.
- However “unconjugated” bilirubin can induce a loss of neurons and atrophy of involved fiber systems (called Kernicterus).
- Jaundice has become one of the most common problems in the neonatal period, which is not restricted to premature infants (< 37 weeks gestation).

Gurses D, Kilic I, Sahiner T. (2002) Effects of hyperbilirubinemia on cerebrocortical electrical activity in newborns. Pediatr Res 52:125-130.

Hyperbilirubinemia:
Elevated Bilirubin in the Blood

Neonatal hyperbilirubinemia results from a predisposition to the production of unconjugated bilirubin in newborn infants and their limited ability to (conjugate it or) excrete it.

These limitations lead to physiologic jaundice, where high serum bilirubin concentrations in the first days of life will color the skin yellow.

Hyperbilirubinemia (cont.)

- Hyperbilirubinemia has the potential for neurotoxic effects.
- Bilirubin can enter the brain if it is not bound to albumin or is unconjugated or if there has been damage to the blood brain barrier.
- Once inside the brain, precipitation of bilirubin at low pH may have toxic effects. Neurons undergoing differentiation are particularly susceptible to injury from bilirubin, suggesting that prematurity predisposes infants to bilirubin encaphalopathy.

Dennery PA, Seidman DS, & Stevenson DK. (2001). Neonatal Hyperbilirubinemia. *New England Journal of Medicine* **344:** pp 581-590.

Treatment of Hyperbilirubinemia

- Phototherapy with fluorescent white light to reduce serum bilirubin.
- Exchange blood transfusions to eliminate bilirubin from the circulation.
- Phenobarbital: given to mothers during the last week of pregnancy to increase conjugation and excretion in high-risk newborns (with some success).
- Disadvantages: known risks of blood transfusion; damage to eyes by UV light.

Bilirubin and the Premature Infant

- Premature infants have **higher rates of bilirubin production** than do full term infants or adults because their red blood cells have a higher turnover rate and shorter life span.
- Premature infants are also at **increased risk of oxidative stress** from hypoxia due to the immaturity of the lungs, followed by risk of hyperoxia once mechanical ventilation proceeds. Infants are often exposed to oxygen concentrations as high as 95%.

Friel JK, Martin SM, Langdon M, Herzberg G & Buettner GR. (2002) Human milk provides better antioxidant protection than does infant formula. *Pediatr Res* **51**;612-618.

Oxidative Stress and Prematurity

- Neonates have **impaired antioxidant defenses** and are susceptible to the development of oxygen free radical mediated diseases.
- Neonatal blood has low content of glutathione peroxidase, superoxide dismutase, β-carotene, riboflavin, α-proteinase, vitamin E, selenium, copper, zinc, ceruloplasmin and other plasma factors.
- The premature **brain is rich in polyunsaturated fatty acids** that are easily oxidized compared to monounsaturated fatty acids.

Hammerman C. Goldstein R, Kaplan M, Eran M, Goldschmidt D, Eidelman AI, & Gartmer LM. (1998). Bilirubin in the premature: Toxic waste or natural defense? *Clinic Chem* **44**: 2551-2553.

Gitto E, Reiter RJ, Karbownik M, Tan D, Gitto P, Barberi S & Barberi I. (2002). Causes of oxidative stress in the pre-and perinatal period. *Biol Neonate* **81**:146-157.

Oxidative Stress and Prematurity (continued)

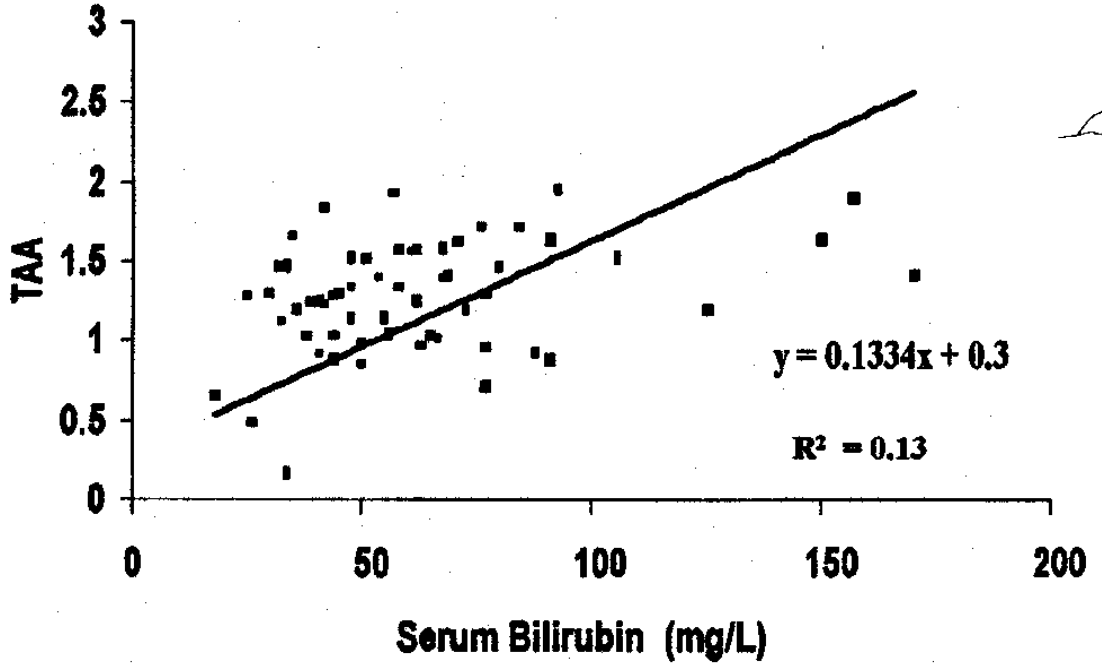
For the premature infant, bilirubin has always been considered a toxin. More recently bilirubin's antioxidant properties have been characterized. It is possible therefore, that **elevated bilirubin is an attempt by an immature fetus to cope with increased exposure to ROS.**

Ironically in an attempt to rid the premature of bilirubin, we may be eliminating a powerful antioxidant that could assist the immature defense system under attack.

Should we "breathe a sigh of relief that bilirubin is probably good for the little one?"

Hansen TWR. (2001) Bilirubin production, breast-feeding and neonatal jaundice. *Acta Paediatrica* **90**:716-723.

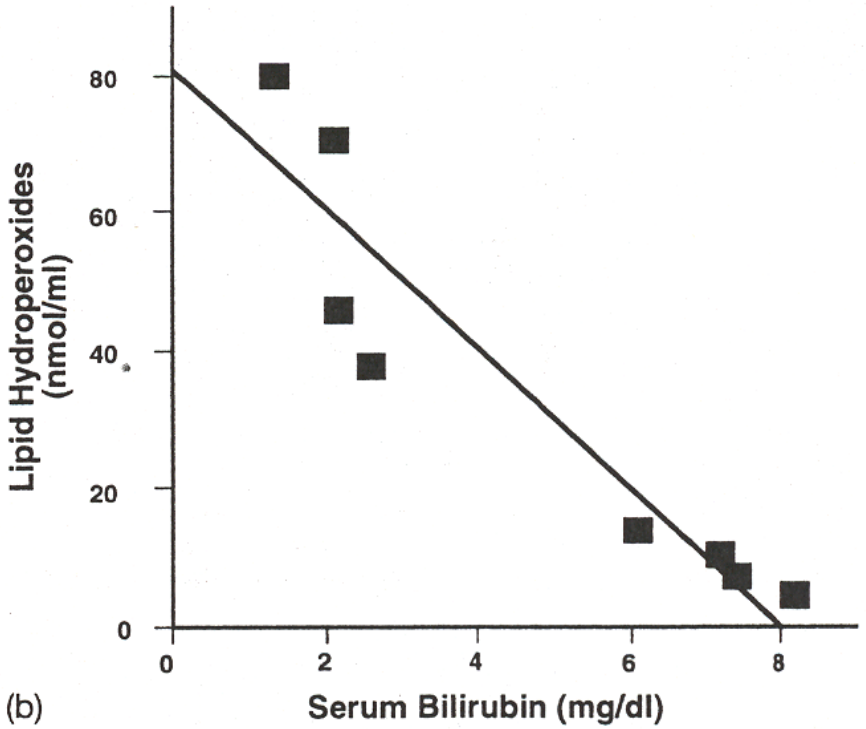
Serum Bilirubin in Neonates correlated with Total Antioxidant Activity



(Hammerman *et al.,* 1998) In contrast we did not find a relation between bilirubin and tissue damage or antioxidant status in small premature infants in the first month of life.

Friel J,Widness J, Jiang T, Belkhode SL, Rebouche CJ, Ziegler EE. (2002) Antioxidant status and oxidant stress are associated with vitamin E intakes in VLBW infants in early life. *Nutr Res* **22**:55-64.

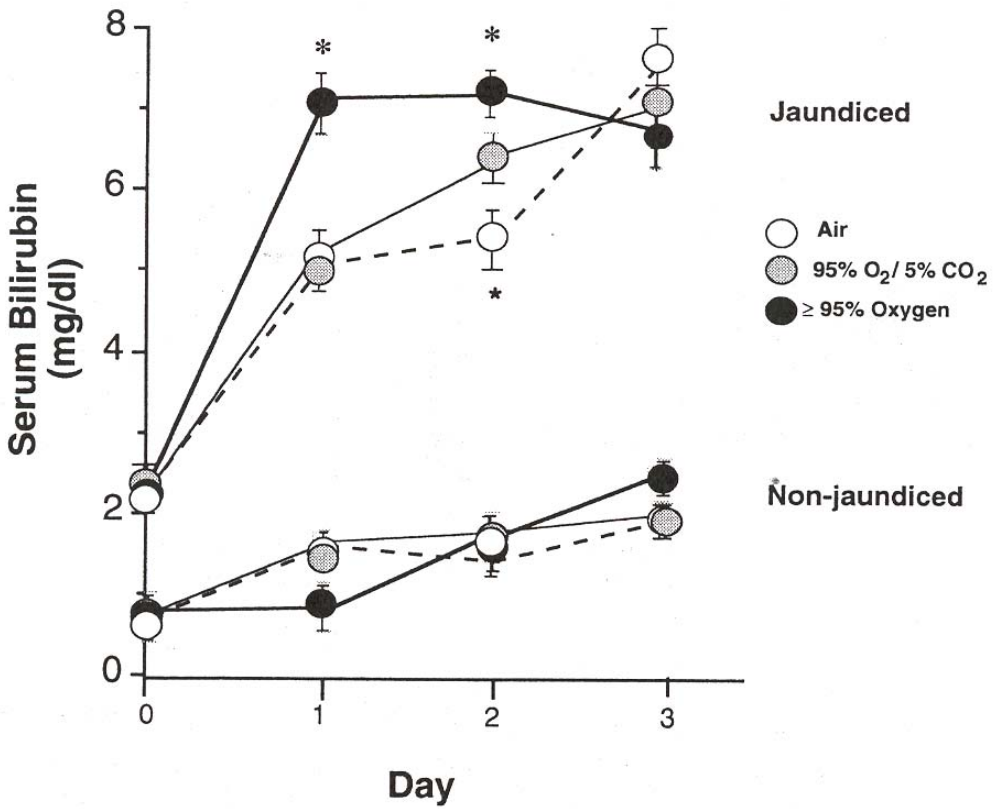
Hyperbilirubinemia protects against lipid peroxidation in Neonatal Gunn Rats Exposed to Hyperoxia



Serum bilirubin in jaundiced and non-jaundiced pups exposed to 95% O_2 shows a negative correlation with lipid hydroperoxides at 3 days of exposure. Higher serum bilirubin concentrations resulted in lower lipid hydroperoxide levels. This is good!!!!

Dennery PA, McDonagh AF, Spitz DR, Rodgers PA, & Stevenson DK. (1995). Hyperbilirubinemia results in reduced oxidative injury in neonatal Gunn rats exposed to hyperoxia. *Free Radic Biol Med.* **19**: 395-404.

The Effect of Hyperbilirubinemia in Neonatal Gunn Rats Exposed to Hyperoxia



Dennery also showed that jaundiced rats exposed to >95% O_2 showed higher mean serum bilirubin levels than jaundiced rats exposed to 95% O_2 and 5% CO_2 or room air.

It is not yet clear how knowledge about Bilirubin as an antioxidant can be used to assist in defense against oxidative stress

Do we allow elevated levels in the neonate to persist for an unknown time to protect the infant?

Where is the crossover to irreparable harm?

Should we market bilirubin as an antioxidant?

Are there others besides infants for whom this molecule may be important?