The Physiological Role of Bilirubin in Neonatal Free Radical Generating Diseases

Thesis
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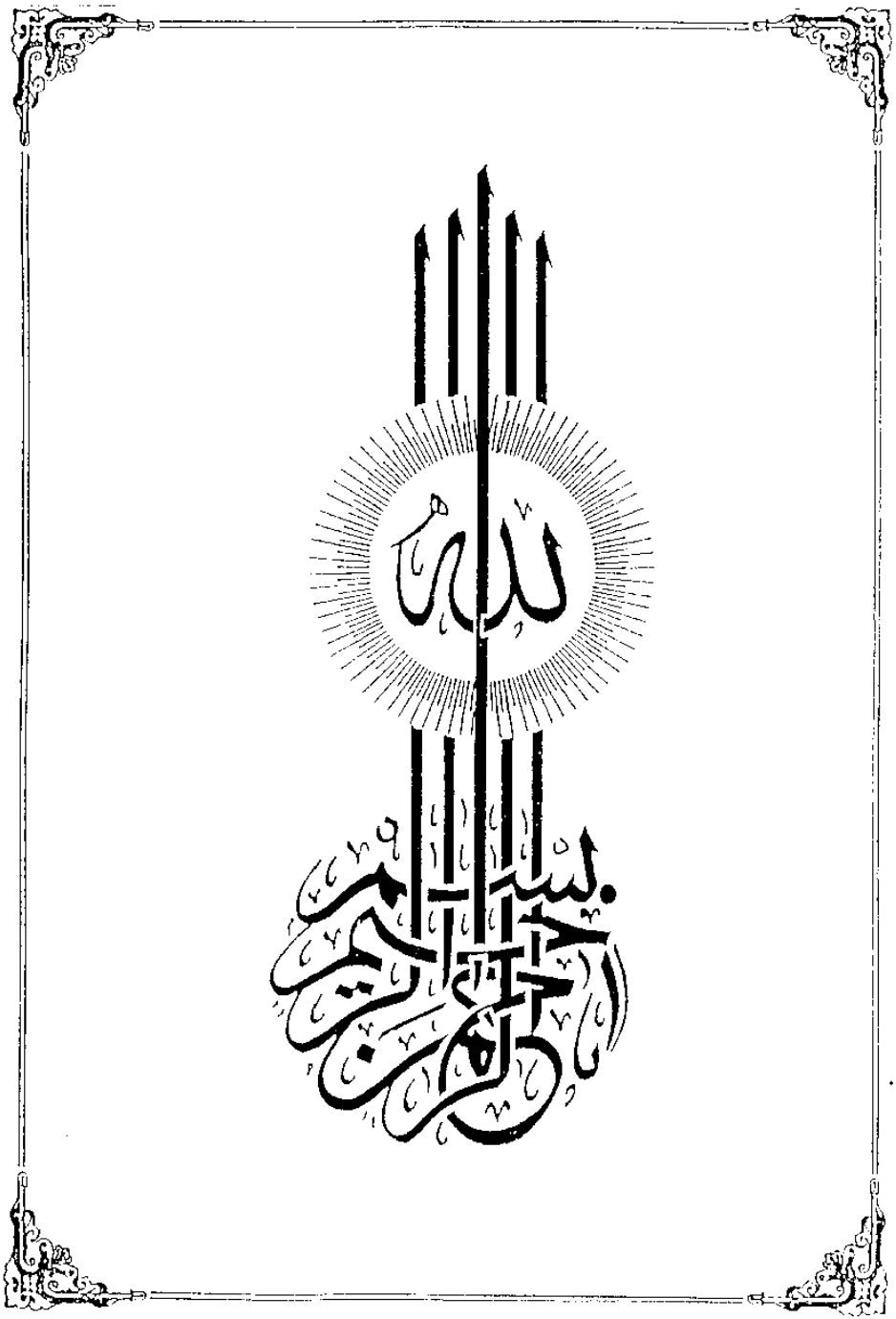

List of Abbreviations

	Abbreviation	Full name
1.	**Alb-BR**	Albumin-bound bilirubin
2.	**α-1-PI**	Alpha-1-proteinase inhibitor
3.	**BPD**	Bronchopulmonary dysplasia
4.	**BR**	Bilirubin
5.	**BR-DT**	Bilirubin ditaurine
6.	**BV**	Biliverdin
7.	**H_2O_2**	Hydrogen peroxide
8.	**LH**	Linoleic acid
9.	**LOO**	Linoleic acid peroxyl radical
10.	**LOOH**	Linoleic acid hydroperoxide
11.	**MAS**	Meconium aspiration syndrome
12.	**MASF**	Meconium-stained amniotic fluid
13.	**NICU**	Neonatal intensive care unit
14.	**O_2**	Superoxide
15.	**OH**	Hydroxyl radical
16.	**PUFA**	Polyunsaturated fatty acid
17.	**RDS**	Respiratory distress syndrome
18.	**SOD**	Superoxide dismutase

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INTRODUCTION AND AIM OF WORK

The Physiological Role of Bilirubin in Neonatal Free Radical Generating Diseases

Introduction :-

Jaundice is common in the new born nursery *(Maisels et al., 1988)*. Over 6% of healthy, full term, white new born infants will have peak serum bilirubin levels of 220 µmol/L or higher *(Hardy et al., 1979)*. No beneficial role for this excess of neonatal bilirubin has been shown. Bilirubin, in fact, is viewed as toxic metabolic waste, devoid of benefit *(Maisels, 1987)*.

Hyperbilirubinemia in newborn infants is generally regarded as a problem, but the high frequency in newborn infants suggest that the excess of neonatal bilirubin may have a positive function. There may be a physiologic bilirubin-consuming process in infants with illnesses that enhance free radical formation *(Benaron and Bowen, 1991)*.

It was postulated that bilirubin is consumed in vivo as an antioxidant *(Benaron and Bowen, 1991)*. One mol of albumin-bound bilirubin can scavenge 2 mol of peroxyl radicals and that small amounts of plasma bilirubin are sufficient to prevent oxidation of albumin-bound fatty acids as well as of the protein itself. The data indicates a role for albumin-bound bilirubin as a physiologic antioxidant in plasma and the extravascular

space *(Stocker et al., 1987a)*. Conjugated bilirubin may be an important chain-breaking antioxidant preventing lipid peroxidation *(Stocker and Ames, 1987)*. Furthermore, under 2% oxygen, in liposomes, bilirubin suppresses the oxidation more than α-tocopherol, which is regarded as the best antioxidant of lipid peroxidation *(Stocker et al., 1987b)*.

Aim of the work :-

Aim of the present study is to determine the role of bilirubin as a free-radical scavenger in illnesses believed to enhance free radical production.

REVIEW OF LITERATURE

Bilirubin Metabolism

Bilirubin metabolism

Bilirubin formation and excretion :-

Bilirubin is derived from the catabolism of heme proteins. Heme-containing proteins include hemoglobin, myoglobin, and heme-containing enzymes such as the cytochromes, catalase, and tryptophan pyrrolase *(Oski, 1991).*

Approximately 35 gm hemoglobin are broken down daily and 300 mg bilirubin are formed and production takes place in reticulo-endothelial cells *(Sherlock and Dooley, 1993).*

The catabolism of 1 gm of hemoglobin results in the production of 34 mg of bilirubin *(Oski, 1991).*

About 75% of bilirubin is derived from red cell hemoglobin, while the remaining 25% is called early-labeled hemoglobin *(Cloherty, 1991).*

Early-labeled hemoglobin comes from;

- a) The breakdown of free heme, heme proteins, myoglobin, and heme-containing enzymes in the liver, and
- b) ineffective erythropoiesis with destruction of non-senescent red cell precursors in the bone marrow *(Stevenson, 1983).*

Bilirubin, is ultimately derived from enzymatic opening of the protoporphyrin ring of heme at α carbon bridge with the initial formation of carbon monoxide and biliverdin *(Berlin and Berk, 1981).*

This oxidation is catalyzed by the enzyme microsomal heme-oxygenase. Biliverdin, the initial tetrapyrrolic product of the ring-opening reaction, is then rapidly reduced to bilirubin by the enzyme biliverdin reductase. This pathway represents the only known source of bilirubin in humans *(Stevenson, 1983).*

Unconjugated bilirubin is transported in the plasma tightly bound to albumin and a very small amount is dialysable. The dialysable amount can be increased by substances such as fatty acids and organic anions which compete with bilirubin for albumin binding. This is important in neonate where such drugs as sulfonamides and salicylates facilitate diffusion of bilirubin into the brain and so increase the risk of kernicterus *(Sherlock and Dooley, 1993).*

The albumin binding capacity is 0.5-1.0 mmol bilirubin per mol albumin, at bilirubin concentration of 340 µmol / l, the molar ratio exceeds 1:1 and dissociation of bilirubin occurs readily *(Fenton, 1992).*

Binding to albumin is essential for transport, because the solubility of unbound bilirubin at pH 7.4 is extremely low, averaging 0.4 µg / dl *(Oski, 1991).*

The liver extracts such organic anions as fatty acids, bile and non-bile acid cholephils, such as bilirubin, despite tight albumin binding. This results from interaction with the liver cell plasma membrane, perhaps involving a specific albumin receptor. Binding protein, ligandin may be concerned with the transport of bilirubin from the plasma membrane to the endoplasmic reticulum *(Stremmel et al., 1983).*

Bilirubin within the hepatocyte is bound primarily not only to ligandin (Y protein, glutathione S-transferase B), but also to other glutathione S-transferase and to Z-protein. This binding within the cell prevents backflow of bilirubin into the circulation *(Wolkoff et al., 1979).*

Ligandin levels are low at birth, maturing over the first 5-10 days and are inducible by phenobarbitone *(Tanner, 1989).*

Unconjugated bilirubin is non-polar (lipid-soluble). It is converted to a polar (lipid-soluble) compound by conjugation. This allows its excretion into the bile *(Sherlock and Dooley, 1993).*

In adults, the major product of conjugation is bilirubin diglucuronide while in newborns during the first 48 hours of life, only monoglucuronides are formed. After 48 hours of life, bilirubin diglucuronide is the major excretory product *(Maisels, 1982)*.

It appears that two separate enzymes participate in the conjugation process, the first is bilirubin uridine diphosphate glucuronyl transferase (UDPG-T), an enzyme associated with the smooth endoplasmic reticulum and inducible by Phenobarbital. UDPG-T catalyzes the formation of bilirubin monoglucuronide which may be excreted, stored or converted to the diglucuronide. The formation of the bilirubin diglucuronide appears to be catalyzed by a transferase enzyme located in the plasma membrane of the hepatocyte, this is the second enzyme of conjugation *(Schmid, 1978)*.

With a high bilirubin load, as in hemolysis, monoglucuronide formation is favored whereas if bilirubin level is low or following enzyme induction the diglucuronide increases *(Chowdhury and Chowdhury, 1983)*.

After conjugation, bilirubin is excreted into the bile. This excretion is an active, energy-dependent process because it occurs against a large concentration gradient. The conjugated bilirubin is not reabsorbed once it enters the intestinal tract *(Oski, 1991)*.

In the normal adult, most of the conjugated bilirubin is reduced to stercobilin by bacteria and only a very small fraction is hydrolyzed to unconjugated bilirubin and reabsorbed via the enterohepatic circulation. On the other hand, in the sterile intestine of the new born, the reduction of bilirubin to stercobilin does not occur. In addition, the newborn gut is rich in β-glucuronidase. This enzyme hydrolyzes the ester linkage of bilirubin glucuronide yielding unconjugated bilirubin *(Maisels, 1982).*

Unconjugated bilirubin will be reabsorbed and returned to circulation, where it must again be transported to the liver for conjugation and excretion *(Oski, 1991).*