NEONATAL JAUNDICE:
Bilirubin and Phototherapy

MOSES CAMILLERI

1st Year (Final) Medical Student

Bilirubin is a product of hemoglobin breakdown. Catabolism of the heme group of haemoglobin involves the opening of the porphyrin ring, usually at the *a* position, and loss of the *a* carbon atom to yield bilirubin IX-*a*. In normal adults the latter is excreted in the bile primarily as a conjugate with glucuronic acid. In adults a red blood cell has an average life span of 120 days; in babies, the value is only 70 days. The rate of bilirubin production in the newborn is, therefore, several times that of the adult on body weight basis. This rapid turnover implies that metabolism and eventual excretion of bilirubin is more critical in babies if accumulation and subsequent damage due to its toxicity are to be avoided. A potential consequence of hyperbilirubinemia in the newborn is irreparable damage to the central nervous system due to precipitation of this substance in certain areas of the brain (kernicterus). *This follows damage to the blood-brain barrier precipitated by situations such as asphyxia in an ill premature baby.* Lesser, but still hazardous complications include mild types of encephalopathy and damage to the auditory nerve.

A high percentage of newborn babies develop hyperbilirubinemia which is clinically known as physiological jaundice. Levels of bilirubin around 1.5 mg/dl (normal adult level 0.2-1.2 mg/dl) are almost universal during the first week of life, because the liver is still not developed enough to conjugate bilirubin to glucuronic acid. Unconjugated bilirubin accumulates in the plasma and extracellular fluid until the liver is in a position to tackle the workload expected of it. This condition, which appears at birth, usually disappears within a few days.

Some babies, however, have more elevated plasma bilirubin levels or levels which keep rising after the first weeks of life. In these individuals there is usually a higher rate of bilirubin production due to increased red blood cell breakdown. The commonest cause is the condition known as erythroblastosis fetalis or hemolytic disease, which arises due to Rh-incompatibility between the mother and the baby and which results in massive breakdown of the latter's red blood cells.

Bilirubin levels of 18 to 20 mg/dl are considered by some to be the point at which an exchange transfusion is indicated, but plasma bilirubin levels as low as 10 mg/dl may be dangerous.

Bilirubin has two carboxyl groups and can exist either as an unionized acid or as a bivalent anion (Figure 1a). In a polar solvent such as water, the un-ionized acid, as a result of the formation of intramolecular hydrogen bonds, assumes a conformation known as a knot structure (Figure 1b). This form is insoluble in aqueous solutions since its NH and CO groups are no longr available to hydrogen bond with the water molecules of the solvent. It binds tightly to the phospholipids in biological membranes, and is the toxic species. The bilirubin anion binds to serum albumin to form a soluble complex which is non-toxic since it will not diffuse through the plasma membrane. The distribution of bilirubin in the body may be represented as follows:

bilirubin-albumin complex		bilirubin anion	+2h+ —2H+ bilirubin acid

tissue bilirubin

It is its poor water solubility that makes bilirubin difficult to excrete. The adult solves this problem by conjugating bilirubin to glucuronic acid, thus rendering it more soluble and more readily excreted, but the newborn's liver, due to its immature enzyme systems, does not conjugate bilirubin effectively and this toxic waste product accumulates. Thanks to albumin's buffering capacity and the body's functional reserve, a certain amount of bilirubin can be accumulated without harmful consequences. The albumin sink is not infinite, however, and when a certain threshold is exceeded, the buffering capacity breakds down. The amount of bilirubin which may be safely accumulated is generally less for a premature or critically ill infant than for a normal newborn baby.

Several factors are known to influence the distribution of bilirubin in the body:

1. Some drugs bind to albumin and reduce its affinity for bilirubin. The effect of these drugs will be to shift the equilibrium in favour of transfer of bilirubin from the plasma to tissues. Care should be taken when prescribing drugs to neonates and also in pregnant and lactating women. Examples of drugs having this effect are sulfisoxazole (Gantrisin), injectable preparations of diazepam (Valium), frusemide (Lasix) and gentamicin (Genticin).
2. The bilirubin-binding capacity is known to be reduced to premature babies and in ill newborn infants.
3. Changes in pH will influence the transfer of bilirubin from albumin to tissue and vice-versa. Since the bilirubin molecule takes up two hydrogen ions in its movement from the plasma to the tissues, acidosis could be an important factor in precipitating hyperbilirubinemia with its possible consequences.
4. Another factor which can cause the accumulation of bilirubin in tissues is hypoxia. Mitochondria in normal brain cells and other tissues of the body, are equipped with an enzyme system which oxidizes bilirubin to yield products which can be excreted. This enzyme system is com-

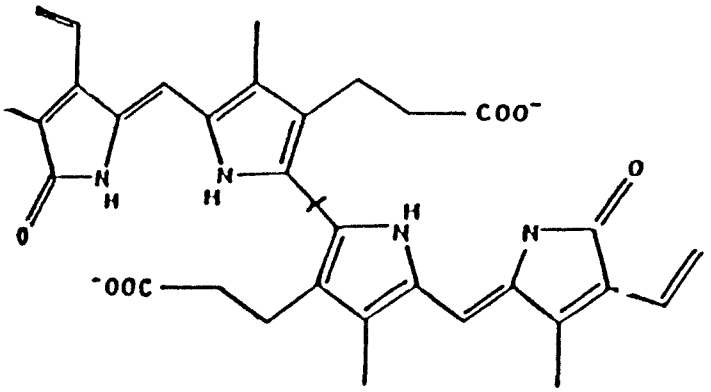

Figure 1a: Structure of Bilirubin Anion.

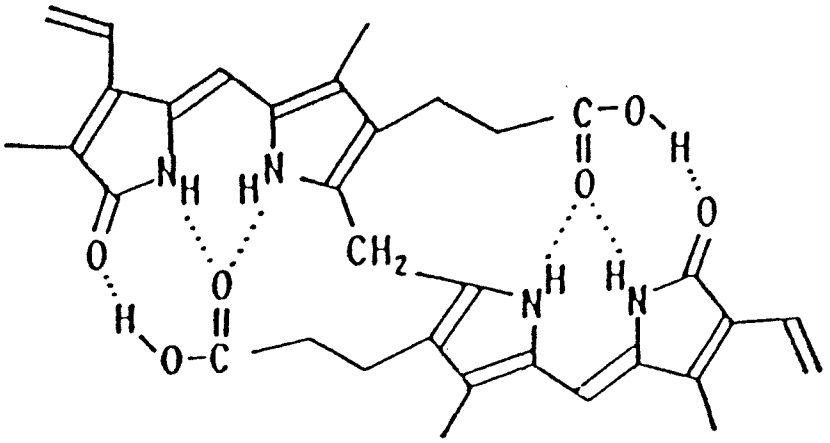

Figure 1b: Structure of Bilirubin Acid known also as the Knot Structure; this is the Toxic Species.

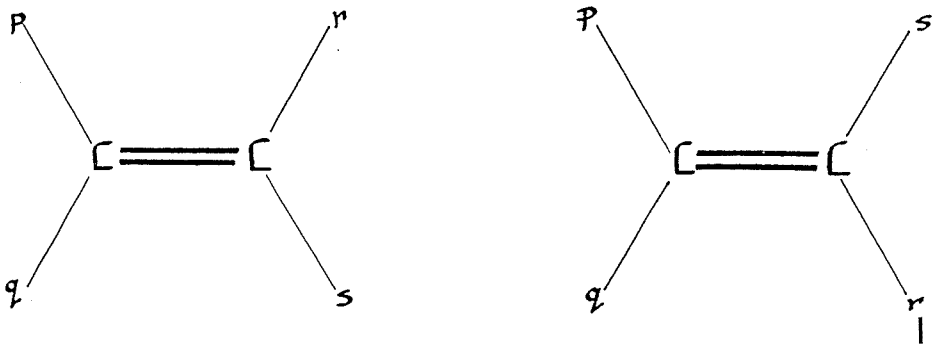

Figure 2: Configurational Isomers.

promised in a hypoxic neonate.

For many years, the only practical solution for the jaundiced neonate was an exchange transfusion. Today, phototherapy can also be employed. This achieves what a liver capable of glucuronidation does, that is, it increases the solubility of bilirubin in water to an extent that it may be excreted. Originally, phototherapy was believed to degrade bilirubin via photo-oxidation; recent evidence suggests that it works via photoisomerization.

At this point, a review of some general chemistry may be helpful. If two carbon atoms are joined by a single bond, there is free rotation about the C—C axis. If they are joined by a double bond, however, two structures are possible since the double bond prevents free rotation (Figure 2). The two structures shown in Figure 2 are configurational isomers, and though they have the same composition, they have a different arrangement of atoms in space and different chemical and physical properties. By convention, one isomer is denoted by the postscript Z, the other by the postscript E. When such a configurational isomer absorbs a photon of light, it assumes an excited state in which the double bond behaves transiently as a single bond, thus making possible rotation around the bond and conversion to the related isomer. This is the basis for phototherapy.

The bilirubin molecule has two carbon-carbon double bonds outside the four pyrrole rings (at C4 and C15). Thus, isomerization is possible at these two double bonds which connect the outer rings to the methin groups (Figure 3). Bilirubin produced naturally in the body has the most stable configuration possible and is denoted as the 4Z, 15Z isomer.

When bilirubin is irradiated, there is a change in configuration as one or both of the terminal porphyrin rings undergoes a 180-degree rotation about the double bond connecting the ring to the methin group (Figure 4). Blue light of wavelengths between 400 and 500 nm has been found to be most efficient at bringing about this transition.

There are four possible configurational isomers of bilirubin IX-*a*, isomerization can occur at the C4 double bond resulting in the 4E, 15Z isomer: at C15 giving the 4Z, 15E isomer; or at both C4 and C15 producing the 4E, 15E isomer.

In the newborn undergoing phototherapy, it has been established using

high-performance liquid chromatography, that isomerization produces predominantly the 4Z, 15E isomer from the natural 4Z, 15Z isomer (Table I). There is as yet no explanation for this.

Table I

Configurational isomers of bilirubin IX-a after 12 hours of phototherapy

Site of isomerization	Notation	Relative amount present
None	4Z, 15Z	80%
C15	4Z, 15E	20%
C4	4E, 15Z	Negligible
C4 and C15	4E, 15E	Negligible

The importance of photoisomerization of bilirubin is that the 4Z, 15E-bilirubin IX-*a* isomer (photobilirubin) is much more soluble than the naturally produced isomer. This increased solubility has two very important consequences:

1. The soluble isomer is much less toxic:
2. Excretion of bilirubin without conjugation is now possible.

The effect on solubility brought about by photoisomerization can be explained on the basis of the intramolecular H-bonding in the acid-form of the bilirubin molecule. The 180-degree rotation disrupts this intramolecular H-binding and in the process exposes the NH and 0 groups of the terminal porphyrin ring to the solvent, thus rendering the molcule polar and permitting formation of H-bonds between water molecules and photobiolirubin (Figure 5; compae with 2b). The result is an increase in solubility.

Photoisomerization probably occurs in the extravascular tissue below the newborn's skin. The 4Z, 15E-bilirubin (photobilirubin) moves across the plasma membrane to be taken up by the blood and is replaced by the natural isomer, since the two are in dynamic equilibrium. In the blood bilirubin is bound to a carrier protein, usually albumin. This is very important since photobilirubin is less stable than the naturally produced bilirubin and will revert to the natural state with time. Binding to photobilirubin retards this process. Thus, albumin acts as a stabilizing protein.

On reachng the liver, the photobilirubin is sequested from the general circulation and secreted into the bile canaliculi. In the bile, the photo-

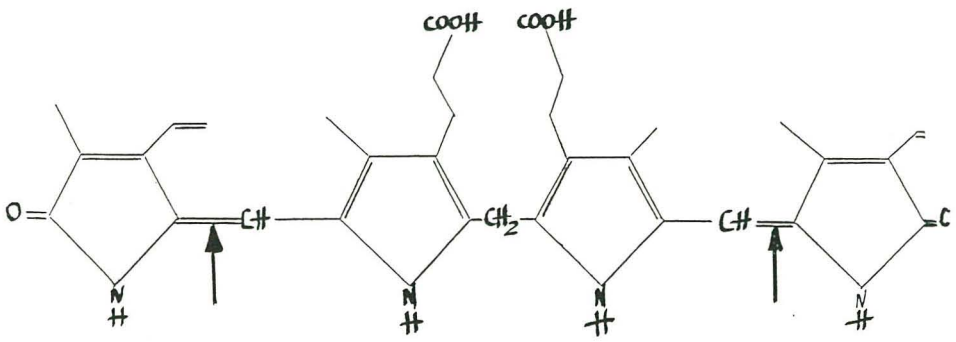

Figure 3: Bilirubin can Undergo Photoisomerization at two sites — C4 and C15 — which are indicated by Arrows.

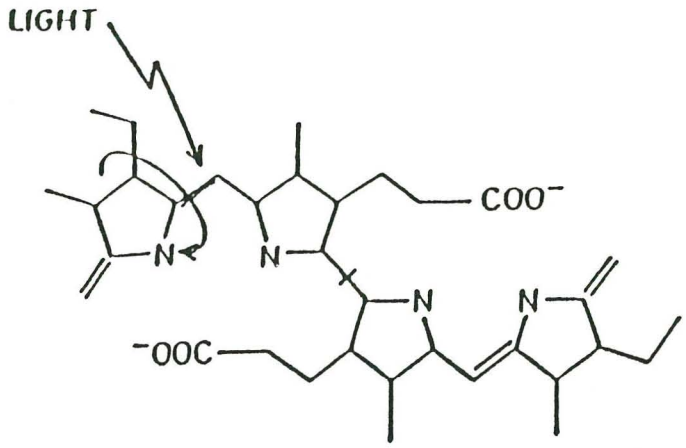

Figure 4: Photoisomerization at C4 by turning one of the Outer Pyrrol Rings at the Double Bond.

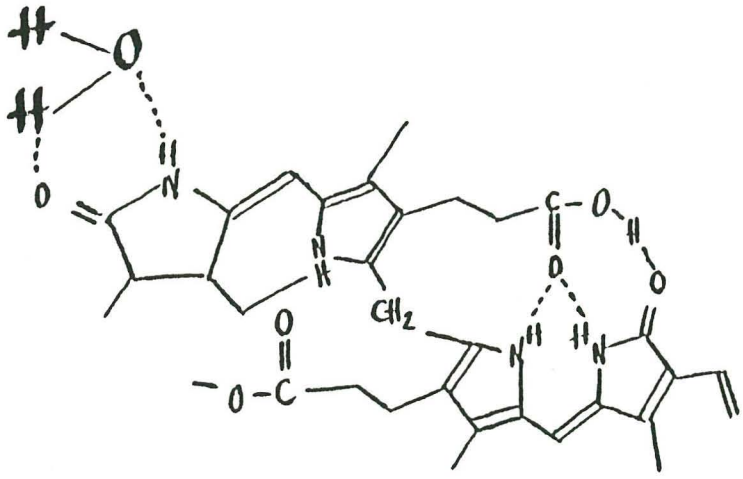

Figure 5: Photobilirubin which is able to form bonds with Water.

bilirubin probably reverts to the natural isomer. From the gall bladder it enters the intestines and is either lost with indigestible food, or is reabsorbed. In the latter case, the overall effect of phototherapy will be reduced.

Because of the path followed by photobilirubin, the effect of phototherapy will be reduced if the newborn is suffering from liver dysfunction, biliary atrasia or cholestasis. It has been suggested that bilirubin reaching the intesines inhibits lactase, thus causing diarrhoea. This may, in fact, be beneficial since it causes a more rapid emptying of the intestinal contents and thus decreases the amount of bilirubin that can be reabsorbed.

Phototherapy has been considered by many to be useful only in mild cases of hyperbilirubinemia where no real danger for the neonate exists. Acute cases have been treated using exchange transfusion. There is an increasing awareness of the benefits of phototherapy especially since phototherapy has not been shown to have adverse side effects provided that the following rules are observed:

(a) To enhance effectiveness of therapy, use "intensive phototherapy" with light placed above and below mattress.
(b) Shield baby's eyes to prevent any damage to retina.
(c) During visiting hours allow visual contact between parent and baby.
(d) During therapy, use a yellow trasnparent acetate sheet in order to filter most of the blue radiation thus avoiding distressing visual effects, headache and nausea experienced by the staff.

Its use is now more common especially in view of the risks associated with exchange transfusion. These are:

1. Acid-base and electrolyte disturbances.
   eg. hypokalaemia
2. Metabolic disturbances.
   eg. hypothermia
3. Mechanical damage.
   eg. umbilical vein perforation
4. Infections.
   eg. hepatitis
5. Thromboembolic episodes.
6. Cardiac disturbances.
   eg. volume overload
7. Hematologic disturbances.
   eg. thrombocytopenia
8. Others.
   eg. hydrothorax

Summary

Phototherapy which is being increasingly used to treat neonatal jaundice, brings about the photoisomerization of bilirubin. The photobilirubin thus formed is less toxic and more soluble than the natural isomer of bilirubin and can be excreted without prior conjugation to glucuronic acid.

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THE DEAFENING SMELL OF POLLUTION

We've all by now grown used to the idea that excessive noise can cause deafness. Avove a certain threshold it is very nearly a product of amplitude and duration. But of all the factors likely to induce hearing loss, airborne pollution hasn't up till now been a front runner.

Disturbing experiments conducted at Johns Hopkins University in the USA and at two Japanese institutions show that, in rats, noise and atmospheric carbon monoxide levels have an additive effect. Laurence Fechter, Associate Professor of Environmental Health Sciences, has produced data to show that the danger threshold level for noise is considerably lowered in the presence of carbon monoxide. Moreover, noise levels and carbon monoxide concentrations (500ppm), harmless on their own, can cause permanent ear damage when combined.

Although there's no direct means of proving that the same is true for humans, Fechter is convince that the risk is a very real one. This is especially so in view of the tendency of high ambient noise levels to occur in the same situations as high carbon monoxide levels. Examples that come to mind include smokers in discos, furnace operators and transport workers. Smokers have carbon monoxide levels in their lungs of around 350ppm — not much below the level that causes serious problems for rats.

Providing conclusive proof of a risk to human health will not of course be easy. But if it does emerge, it will be immensely complicating for occupational health legislators. No longer will it be reasonable, for example, to specify blanket intensity/time limits on noise in all environments. And of course the famous orange warning light, bane of concert promoters, could well in future need to have a second input ... from a gas detector.

ZAPPED BY MICROWAVES

A little knowledge of RF can be a dangerous thing, at least to judge from a nationwide survey conducted by Cambridge University safety adviser, John Williams. Following informed rumours about accidents involving microwave ovens in laboratories, Williams decided to find out for himself. A questionnaire sent to members of the University Safety Association has now been analysed and the results published in *Laboratory News*, 31.10.88.

The microwave oven, it appears, is now being used indiscriminately as a sort of up-market bunsen burner. But no-one is reading the instructions — at least not many. 14% of those returning questionnaires said that they could recall a damaging incident of some kind. Worse still, thirteen universities had experienced explosions or accidents that were actual or potential causes of serious injury. In two such incidents microwave ovens had their doors blow clean off.

Of some wry satisfaction to well-educated engineers is the fact that all thirteen of the serious accidents took place in biology (sorry, life sciences) laboratories. They involved heating closed containers of liquids, leaving metal clips on glassware and failing to allow time for superheated liquids to cool down.

I can only surmise that either they've changed the 'O' level physics syllabus since my day or else that they've resurrected the old lady who warmed up a cold bath with the aid of a suitably immersed one-bar electric fire.