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Kernicterus

Humana Press

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This volume is dedicated to Jeffrey and Steven

Preface

Kernicterus (bilirubin encephalopathy) is a highly interesting example of metabolic encephalopathy. It fills all the characteristics of a metabolic encephalopathy in that it can develop rapidly, produces signature signs and symptoms, and is amenable to successful treatment. In the absence of treatment kernicterus can produce irreversible damage, devastating sequelae, and death.

The present volume will examine the history, biochemistry, and physiology of bilirubin, as well as its hepatic metabolism and renal excretion. Chapters will elaborate on bodily disposition of bilirubin and its neuropathology. Both early treatments and current therapy will be discussed in detail. Phototherapy will be presented, and its efficacy and influence on incidence thoroughly examined. New concepts relating to the way in which bilirubin is toxic, and damage to the acoustic system will be examined in detail. The promising treatment using gene therapy will be discussed. This therapy has been successfully used in Gunn rats.

When red blood cells break down, the resulting components include heme and globin. Bilirubin is contained in the heme moiety. The life span of red blood cells is about 120 days in adults, and about 90 days in the newborn. In utero, this significant amount of bilirubin is transferred across the placenta to be excreted by the maternal liver. At birth, the newborn liver is faced with increasing levels of bilirubin, and hepatic capacity for conjugation and excretion have yet to be "tested." It is not surprising that 20% (or more) of newborn children develop a transient physiological jaundice as the enzymatic machinery becomes functional.

Bilirubin travels in plasma bound to albumin. When taken up by the liver, bilirubin is transformed from lipid soluble to water soluble, facilitating excretion into the biliary system. When bound to albumin, or conjugated with glucuronic acid, bilirubin cannot enter the brain. In the "free" state, lipid soluble bilirubin can enter cerebral tissue, causing brain damage. Why bilirubin enters selective cerebral areas is a fascinating and unanswered question.

Many animal models of kernicterus have been used, but one stands out. A Wistar rat mutant described by C. K. Gunn had a genetic deficiency of the liver bilirubin-conjugating enzyme glucuronyl transferase. This defect correlates with the human inborn error, the Crigler–Najjar syndrome. This exciting finding permitted investigators to study a nearly perfect animal model of kernicterus. The Gunn rat model permitted studies on energy metabolism in neurologically-compromised animals,

and defects in ATP metabolism were, not surprisingly, found. Early in vitro studies predicted these definitive results in that they showed conclusively that bilirubin uncoupled oxidative phosphorylation. Electron microscopy reinforced these findings, showing selective changes in cerebellar Purkinje cells and their mitochondria. More refined studies directly measuring ATP in nanogram layers of Purkinje cell-rich layers of the cerebellum of symptomatic Gunn rats showed similar effects on energy metabolites, but not in adjacent molecular and granular cell layers (Reynolds, S., and Blass, J. 1976).

Early neuropathological studies in humans noted the selective anatomical staining. The underlying cause of human kernicterus is frequently erythro-blastosis foetalis, or a deficiency of the liver bilirubin conjugation system. Human brains of kernicteric infants often show yellow staining in the basal ganglia, cerebellum, and brainstem. The focal staining is marked when the patient dies during the acute phase; if the patient dies later, the staining has largely vanished. Before phototherapy, many cerebral palsy patients were the result of hyperbilirubinemia.

One long-standing treatment for newborn jaundice is phototherapy. The benefits of light for ameliorating jaundice were serendipitously discovered by a pediatric nurse who noticed jaundiced newborns experienced fading of the yellow skin pigmentation when exposed to sunlight. Further studies on this important observation showed that phototherapy decreased serum unconjugated serum bilirubin levels. Widespread use of this therapy in the USA lagged behind its use in Europe. Bilirubin is structurally similar to the pigment phycobilin, which captures light energy, and so bilirubin is isomerized into compounds which are water soluble, not toxic, and easily excreted.

The wavelength of light used during phototherapy impacts the extent of bilirubin isomerization. Various studies have demonstrated that turquoise light (490 nm peak with 65 nm spectral width) is considered more effective than blue light (452 nm peak with 55 nm spectral width). Side effects to phototherapy are minor, and include epidermal cell death, ultraviolet light burn, and headaches and vertigo in hospital staff. Because of the limited side effects and strong benefits, phototherapy has continued as an effective treatment for hyperbilirubinemia in the newborn.

The present volume is designed to examine the biochemistry and physiology of bilirubin. Its formation, metabolism, transport, and excretion will be examined in detail. Moreover, the deposition of unconjugated bilirubin into highly specific newborn brain regions will be described in terms of mechanisms. Treatment modalities before and after bilirubin stains brain regions will be considered. The very important concept that kernicterus can cause subtle changes in mentation and behavior will be examined. This area of sequelae to mild degrees of bilirubin entry into brain tissue is critical and largely overlooked. Chapters detailing hyperbilirubinemia in older patients and examining albumin and blood transfusion treatment paradigms are included.

Kernicterus is an excellent example of a metabolic encephalopathy. It clearly produces a biochemical lesion early when the pigment first enters the brain. If initial staining can be minimized, or reversed, permanent damage can be reduced to a minimum. This takes strict attention to detail, and a keen ability for timely diagnosis.

The realization that the early diagnosis of any disorder which might have a biochemical (metabolic) basis has the potential for rapid reversal/improvement, if not outright cure, argues strongly for increased awareness by all health care workers. Kernicterus has a potential advantage in that hyperbilirubinemia usually appears in the newborn nursery when it can be recognized by nurses and by physicians. Another huge advantage for the study of kernicterus is the availability of the Gunn rat model. Hyperbilirubinemia and resultant kernicterus is quite another issue in Third World countries. These world health concerns are of such importance that they constitute the first chapter in this volume.

Chicago, Illinois David W. McCandless

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Contents

Prologue: World Health Concerns

The enormous number of cases of hyperbilirubinemia worldwide can hardly be estimated. The World Health Organization estimates the number of people worldwide who have glucose-6-phosphate dehydrogenase (G-6-PDH) deficiency at 400 million. G-6-PDH deficiency is the disorder that contributes the largest number of jaundiced newborn infants. Endemic areas for G-6-PDH deficiency include sub-Saharan Africa, Asia, India, South America, and portions of the Middle East. These areas are also endemic for malaria. Rates of G-6-PDH deficiency include Nigeria, 28%, Cambodia, 15%, China, 8%, Myanmar, 11%, Thailand, 9.4%, Iran, 11.6%, etc. (Beutler, E., and Duparc, S. and the G-6-PDH working group 2007).

G-6-PDH deficiency results in severe hemolytic anemia, especially when triggered by some seemingly inoculus incident such as eating fava beans. Estimates place hyperbilirubinemia (greater than 15 mg/100 ml) at an incidence of 30% in G-6-PDH-deficient patients during a hemolytic crisis. Conversely, in severe neonatal jaundice, it has been shown that G-6-PDH is the most important factor in the hyperbilirubinemia (Bienzle, U., et al. 2008). In a similar vein, (Gibbs, W., et al. 2008), a total of 23 Jamaican infants with moderate/severe jaundice were examined for the presence of G-6-PDH. Results showed that the deficiency was present in 70% of the hyperbilirubinemic neonates. This was far greater than the 9.4% deficiency noted in nonmoderate/severely jaundiced newborn infants. As regards treatment in this group of neonates, phenobarbitol and phototherapy helped most, but still eight required exchange transfusion. Even with this treatment, two became kernicteric, and one of these died.

In a study of neonatal morbidity and mortality in newborn infants in a tertiary hospital in Ilesa Nigeria, 7,225 infants were included for study. The highest rate of indications for hospital admission was neonatal jaundice at 45%. In this study, jaundice was listed as the second highest cause of death in this group, at 14% (Owa, J., and Osinaike, A. 1998). Jaundice as a cause of death means the newborn infant died of kernicterus. The incidence of survivors with cerebral palsy is not mentioned. If this kind of morbidity/mortality occurs in a town with hospitals, etc., imagine what the mortality rate would be in rural areas in countries without any medical superstructure. In Zimbabwe, for example, several years ago there was only one neurologist in the country. The number of newborn dying each year of kernicterus

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must be in the thousands. We tend to become complacent given the low occurrence of kernicterus in developed countries.

One major problem regarding G-6-PDH deficiency is that in newborn infants, it cannot be diagnosed by clinical observation alone. Therefore, even in infants born under some level of medical supervision in third world countries, the mother/infant may be discharged before any "triggering" mechanism occurs. The result is the possibility for rapid onset of hemolytic anemia, and rapidly rising hyperbilirubinemia. This may leave the newborn brain damaged from unconjugated bilirubin staining of select brain areas.

One question is what medical treatment is available even if this rapid onset of hemolytic anemia occurs in a third world hospital setting? The answer would seem to be that such a setting would have few to none of the blue light photo therapy units deemed necessary to effectively reduce elevated serum bilirubin, as has been demonstrated in the USA. A recent paper from T. Hansen's group (De Carvalho, M., et al. 2007) suggests an alternative plan. They were able to demonstrate that seven ordinary day light fluorescent tubes placed close to the jaundiced infant effectively reduces unconjugated hyperbilirubinemia. This was achieved by placing the array of lights underneath a clear plexiglass bottomed crib. This treatment was compared to the conventional blue treatment.

Results showed that in 51 jaundiced newborn infants, decreases in serum bilirubin levels were greater in the two photo therapy methods than regular overhead lights (no photo therapy). The increased rate of bilirubin elimination by the two photo therapy was measured at 12 and 24 h after initiation of the photo therapy. The authors state that

> "lack of access to expensive imported special blue lamps does not preclude delivery of effective photo therapy in developing countries."

As a side note, a student at Duke University has developed a simplified photo therapy device which uses blue lights at a reported cost of $500 per unit as compared to $4,000 for the commercially available unit. Prototypes are being tested in Tanzania.

In terms of world health concerns regarding hyperbilirubinemia and kernicterus, the major disorder producing morbidity and mortality is G-6-PDH deficiency. This genetic mutation is responsible for over 400 million cases of G-6-PDH deficiency. The number of newborn infants with the disorder, who are subsequently subjected to a precipitating event, which ends in kernicterus, is probably beyond estimation, but certainly runs to thousands of cases per annum. Many cases go unreported in developing countries due to political reasons and lack of knowledge about hyperbilirubinemia and kernicterus. Education and a closer attention to health issues is the first step to ameliorating this tragic and indefensible situation.

G-6-PDH deficiency was first described (Carson, P., et al. 1956) in patients undergoing treatment for malaria when primaquine was administered. The "primaquine sensitivity" was observed in ethnic groups, suggesting a genetic component. Later observations in several ethnic groups showed similar sensitivities to other precipitators such as fava beans. The disorder is one of severe hemolytic anemia accompanied

with severe hyperbilirubinemia, sufficient to result in kernicterus and death. The World Health Organization estimates as many as 400 million people worldwide with G-6-PDH deficiency.

Glucose-6-phosphate dehydrogenase catalyzes the first (entry) step in the hexose monophosphate shunt (pentose phosphate pathway). This shunt, with the enzyme transketolase as the rate-limiting step, serves at least two functions. First, the shunt provides reducing power in the form of NADPH. This, for example, maintains glutathione in the reduced form, GSH. GSH in turn acts to detoxify H_2O_2, thereby protecting cells from the oxidizing effects of H_2O_2. Another function of the hexose monophosphate shunt is to produce ribose phosphate essential for the formation of the ribose used in RNA (see Fig. 1).

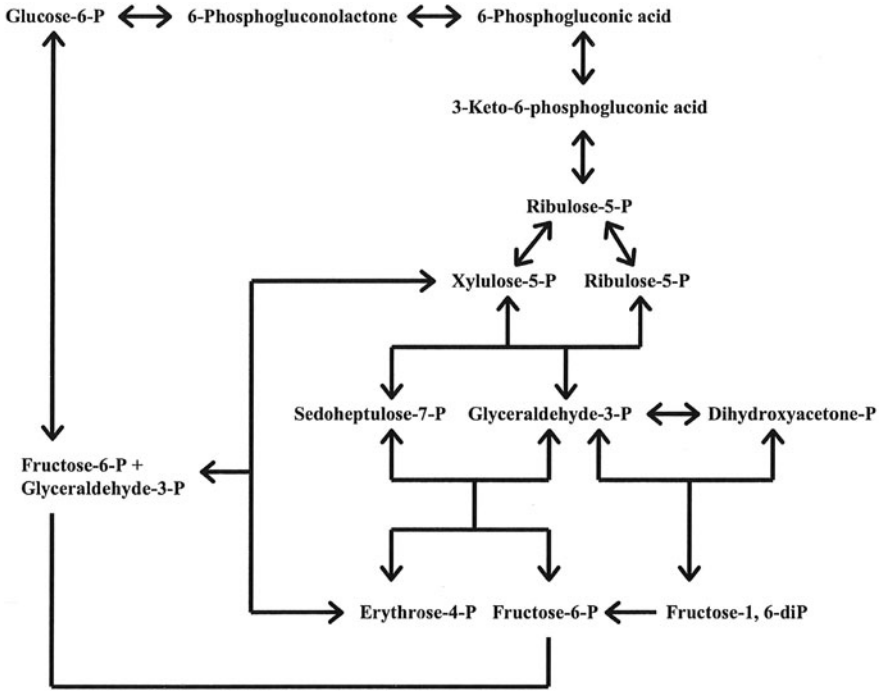

Fig. 1 Schematic representation of the hexose monophosphate shunt

As stated above, deficiencies of G-6-PDH are the most commonly inherited enzyme deficiencies. This genetic deficiency may affect as many as 25% of the population in endemic areas such as the Mediterranean, sub-Saharan Africa, and subtropical Asia. The inheritance of G-6-PDH deficiency is classified clinically as an x-linked recessive disorder. In spite of this, heterozygous females can develop symptoms. The genetic diversity of G-6-PDH deficiency is vast, with over 499 variants of the deficiency, each with unique biochemical features.

Many individuals who have G-6-PDH enzyme deficiency never show any significant symptoms. When symptomatic, the key feature is acute hemolytic anemia

and accompanying jaundice. Several compounds may precipitate the symptoms of G-6PDH deficiency such as fava beans, primaquine, and toxins from infection. Primaquine-induced hemolytic anemia in blacks first led to the discovery of G-6-PDH deficiency. The association of fava bean ingestion and sickness (anemia) dates back as far as early Greece.

There is a relatively simple screening test for G-6-PDH deficiency based on fluorescence (Beutler, E., and Mitchel, M. 1968). Given the distribution of G-6-PDH deficiencies, as well as the thalassemias, it has been suggested that these two entities provide some protection from malaria (Luzzatto, L., Usanga, E., and Reddy, S. 1969). This has been borne out in females deficient in G-6-PDH, but not in males.

Clinically, once hemolytic anemia starts in a G-6-PDH deficient patient, within 2 days, fever starts, jaundice increases, and urine turns brown/black. There is a significant risk of renal tubular necrosis. Exchange transfusion can serve to remove damaged red blood cells (hemighost cells) which compromise microcirculation in the kidney (Chan, T., Chan, W., and Weed, R. 1982). see Fig. 2.

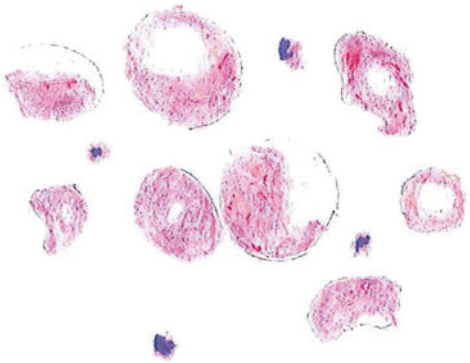

Fig. 2 Smear of G-6-PDH deficiency patient's blood. Note hemighost red blood cells

Several other illnesses, when present in G-6-PDH patients may precipitate a severe bout of hemolysis. Typhoid fever is one example (Chan, T., et al. 1971). This was demonstrated by transfusing G-6-PDH deficiency cells into non-G-6-PDH deficient patients just developing typhoid fever. Viral hepatitis is another disease which is worsened in patients with G-6-PDH deficiency.

It is clear that in G-6-PDH–prevalent areas in the world, there is a significantly higher incidence of neonatal jaundice and bilirubin encephalopathy/kernicterus. The jaundice usually starts to rise around the end of the first week, and many extend into the third or fourth postnatal weeks. A variety of factors may precipitate including various drugs and infections (Panizon, F. 1960; Yeung, C. 1992). Where possible, newborn infants should be screened for G-6-PDH deficiency, and bilirubin values monitored closely. When photo therapy is quickly and aggressively administered, requirements for exchange transfusions are reduced. Unfortunately, many areas, for example, rural sub-Saharan Africa, do not have access to G-6-PDH deficiency screening tests, no access to photo therapy, and little knowledge of the risks involved (see Table 1).

Table 1 Drugs which may precipitate hemolysis in G-6-PDH patients

Drugs with the potential to precipitate hemolysis in G-6-PD patients	
Furazolidone	Phenazopyridine
Glibenclanide	Phenylhydrazine
Isobutyl nitrate	Primaquine
Nalidixic acid	Sulfacetamide
Naphathalene	Sulfanilamide
Niridazole	Sulfapyridine
Nitro gurantoin	Urate oxidase

It is certain that if screening tests for G-6-PDH deficiency are available, and the means to implement photo therapy, and even transfusions, then the ravages of kernicterus (cerebral palsy) can probably be averted in most cases. It is imperative for these methods and education regarding newborn jaundice to be made available to areas which have many hundreds of cases of kernicterus per year, such as Africa and India.

The severity of the G-6-PDH deficiency in terms of enzyme inactivity may not always correlate with the degree of hemolytic anemia (Yoshida, A. 1973). Some variants of G-6-PDH deficiency have severe enzyme deficiencies like the Union and Markham varieties, but have little hemolytic activity. Other variations, such as the Alhambra and Triple varieties have a less pronounced loss of enzyme activity but have severe chronic hemolytic anemia.

The incidence of G-6-PDH deficiency in Egypt has been examined. In the first such study (Rageb, A., El-Alfi, O., and Abboud, M. 1966), 500 Egyptian men were treated for G-6-PDH deficiency, and the incidence was placed at slightly over 26%. In a later study (McCaffrey, R., and Awny, A. 1970), 650 males were tested for the deficiency. They were randomly selected from blood donors at a Cairo hospital. The tests administered were the ascorbate cyanide test and the methemaglobin reduction test for G-6PDH deficiency.

Results showed an excellent correlation between the two tests when both were used on the same patient. The incidence in this study was only 4.9% positive for the deficiency. This result is a striking difference from an earlier study showing a 26% incidence. The authors speculate that methods of testing may have produced false positives in the earlier study.

Another study examined the incidence, severity, and association with Gilberts syndrome in Sephardic Jews who were descendents of families who immigrated from Asia Minor (Kaplan, M., et al. 1997). The authors state that the worst consequence of G-6-PDH deficiency is neonatal hyperbilirubinemia, which can easily result in kernicterus and death. The exact cause of hyperbilirubinemia in these cases is uncertain, and could be related to a lack of glucuronyl transferase (glucuronosyl transferase) or increased bilirubin production due to massive hemolysis, or most likely by a combination of both. The rate of hemolysis is higher in Sephardic Jewish patients than others, but a precipitating agent is not always discernible. In G-6-PDH deficiency, the level of hyperbilirubinemia is not always related to the

severity of hemolysis. Decreased bilirubin elimination is thought to be a factor in hyperbilirubinemic patients in this ethnic group. The possibility exists that the Gilberts disease (UDPGT1 deficiency) gene may be present in G-6-PDH deficiency thereby increasing the incidence for neonatal hyperbilirubinemia.

Results showed that of 371 patients in the study, 131 (35%) were found to be G-6-PDH deficient. Of these neonates, 23% developed hyperbilirubinemia, much higher than the control group. Hyperbilirubinemia was defined as values over 15 mg/100 ml. When the incidence of hyperbilirubinemia was compared to the combination of G-6-PDH and each of the three UDPGT1 genotypes, the incidence of elevated bilirubin levels increased. These data strongly suggest that a variant UDPGT1 promoter is playing a role in the neonatal hyperbilirubinemia associated with G-6-PDH deficiency.

The authors suggest that while G-6-PDH deficiency is thought to be precipitated by some compounding factor such as fever or drugs, these data suggest that in addition to G-6-PDH deficiency, hyperbilirubinemia requires an additional factor – a mutation in UDPGT1. The mutation by itself is innocuous, but in combination with G-6-PDH deficiency in newborn infants, may result in bilirubin values high enough to result in kernicterus. The authors note that not too many gene–gene interactions have been described, but that this type of phenomenon is present in Sephardic Jewish families.

In another study (Medina, M., et al. 1997), the molecular genetics of G-6-PDH deficiency was studied in Mexico. The incidence of G-6-PDH deficiency had been quoted as between 0.4 and 4.1% depending on the region analyzed. The highest rate occurred in the Mestizos area, while the lowest rate was from several Indian groups (Lisker, R., Cordova, M., and Zarate, G. 1969; Gonzalez-Quiroga, G., et al. 1994).

The results examining the molecular genetics of G-6-PDH deficiency showed that the G-6-PDH-A phenotype was common in Mexico. In addition, five different genotypes were seen among the patients studied. The authors note that their data also showed that in Mexico, G-6-PDH deficiency is heterogeneous at the DNA level.

Another study (Santhi, A., and Sachdeva, M. 2004) looked at the G-6-PDH deficiency incidence in Jats and Brahmins of Sampla, Haryana. These are two geographic areas in the northern portion of India. Results from this study were from 288 total individuals. Of these, 136 were Jats, and 152 were Brahmins. Screening for G-6-PDH deficiency was accomplished by the methemoglobin test. Results showed that the incidence for G-6-PDH deficiency in Jats was 11.5% and for Brahmins, 10.5%. This was a nonstatistically significant difference. Analysis by sex showed a statistically significant higher rate of the deficiency among males in each group. The authors note that these results are in general agreement with previous reports of a higher rate of incidence in northern and western portions of India (Bhasin, M., and Walter, H. 2001).

The incidence of G-6-PDH deficiency has been examined recently in Northeast Iran (Mohammadzadeh, A., et al. 2009). This was a prospective study to determine the prevalence of G-6-PDH deficiency in Mashhad city in Iran. Over a period of 8 months in 2006, all newborns were screened for G-6-PDH deficiency. Blood samples were tested by the fluorescent spot method.

Results showed a low percent (0.8%) of positive tests for G-6-PDH in a total of 2,570 neonates. This is compared to the WHO estimate of 2.9% of the world's population as being deficient in G-6-PDH (WHO working group 1989). Results from other areas of Iran ranged from 1.2% in Tehran to over 15% in the northern parts (Mazandaran) and southern portions (Shiraz) of the country. The overall incidence of G-6-PDH in Iran is estimated to be about 11.5%.

Another study looked at the prevalence of G-6-PDH deficiency in an area of the Brazilian Amazon which is endemic for malaria (Santana, M., et al. 2009). Two hundred patients' blood was tested for G-6-PDH deficiency, and 6 (3%) positive patients were identified. Testing for malaria disclosed an incidence of 1.5% in the 200, and half of the six G-6-PDH-deficient group.

The authors point out that in a hemolytic crisis, there may possibly be increased numbers of reticulocytes, which could lead to false negative values of G-6-PDH deficiency. The increased numbers of peripheral reticulocytes may themselves have normal levels of G-6-PDH. Chactacterization of the G-6-PDH isoenzymes showed high frequency of the phenotype A- G6PDH deficiency, verifying findings in Brazil.

The authors state that they observed a statistically significant protection in their patients with G-6-PDH deficiency against malaria. This also is in agreement with earlier reports of a protective action of the deficiency against malaria (Guindo, A., et al. 2007); Roth, E., et al. 1983). One outcome of the simultaneous occurrence of G-6-PDH deficiency and malaria (usually *Plasmodium vivax*) is a significantly higher level of hyperbilirubinemia requiring an increased frequency of blood transfusions. This study, the authors note, is of benefit because it shows the incidence of having both G-6-PDH deficiency and malaria (3%). This will serve to help health care workers to develop treatment policies for G-6-PDH deficiency in endemic malaria areas. These studies showing the decrease of hyperbilirubinemia in cases in which patients have both malaria and G-6-PDH deficiency is an alert to the need for vigilance in order to initiate therapy to lower bilirubin levels. This is especially true of afflicted newborn infants in order to prevent kernicterus.

Another review paper recently published (Beutler, E., Duparc, S., and the G-6-PDH deficiency working group 2007) the association of G-6-PDH deficiency and malaria, and malaria treatment regimes were studied. G-6-PDH deficiency is a hemolytic challenge in severe cases, and the possibility of augmented hemolysis from anti-malarial drugs should be avoided. The authors state that G-6-PDH deficiency in children has been shown to be associated with an approximate 50% reduction in the risk for malaria (Cappadoro, M., et al. 1998).

The present chapter focuses on the combination of G-6-PDH deficiency and malaria. In the presence of G-6-PDH deficiency, hemolytic anemia is the key symptom. Indeed, G-6-PDH deficiency was discovered based on investigations of primaquine-related hemolytic anemia. Decreased hemoglobin levels is an easily followed clinical sign of hemolytic anemia. The authors note that neonatal jaundice is the most catastrophic result of G-6-PDH deficiency, and can lead to severe kernicterus. A variety of drugs should be avoided in patients with G-6-PDH deficiency as they may precipitate hemolysis. Many of these drugs are listed in Table 1.

The authors state that it is difficult to predict the effects of anti-malarial drugs as regards hemolytic anemia in G-6-PDH-deficient patients. They suggest some sort of a classification which "ranked" the possibility for hemolytic anemia in G-6-PDH-deficient patients would be invaluable. While some animal (mouse) studies have been directed toward this problem, there are some differences in results as compared to humans. The authors suggest that human studies be utilized in early clinical trials, and to start by giving newly developed anti-malarial drugs to normal subjects and assessing hemolysis.

For patient screening for G-6-PDH deficiency, the fluorescence test (qualitative) is excellent. It, however, is only capable of reliably detecting G-6-PDH deficiency in males and homozygous females. In clinical trials (for example, chloroproquanil, dapsone, and artesunate being tested in Africa), G-6-PDH-deficient patients are included in the study. Patients were carefully monitored for the first few days, and if hemolysis begins, it would be noted. Patients were also followed up at home. If there is a rapid drop in hemoglobin, treatment is stopped, and the need for transfusion carefully watched. In terms of children, desferrioxamine has been used to reduce the time of hemolysis, thereby decreasing the frequency of the need for transfusions (al-Rimawi, H., et al. 1999).

As regards testing women for the possibility of G-6-PDH deficiency, the cytochemical assay vs. the fluorescent spot test is discussed (Peters, A., and Van Noorden, C. 2009). Results showed that the fluorescent spot test is widely used, and is cheap and easy to perform, but not reliable for testing women. It is concluded that the cytochemical assay is best for testing women for G-6-PDH deficiency. The cytochemical assay method is more expensive and difficult to perform, but its reliability is excellent. The authors suggest a simplified kit would be a welcomed development.

Another recent paper examines hyperbilirubinemia and G-6-PDH deficiency in the Zanjan province of Iran (Koosha, A., and Rafizadeh, B. 2007). In this study, 376 newborn infants were studied. G-6-PDH deficiency was found in eight neonates (2.1%). Interestingly, the etiology was not determined in slightly over 75% of the newborns. Of the eight newborns with G-6-PDH deficiency, seven were boys and one was a female. The maximum total bilirubin value in the G-6-PDH-deficient group was 24.9 mg/100 ml.Treatment consisted of photo therapy. No G-6-PDH-deficient newborn infant received an exchange transfusion.

The authors stress, as have others, that the incidence of G-6-PDH deficiency may vary greatly in different regions of the same country. This has been described above. This may be attributed to population variations. For example, the people of Shiraz and Tehran differ from the present study in ethnicity and the inclusion of immigrants from other provinces. The authors also stress that the devastating consequence of unchecked neonatal hyperbilirubinemia is severe kernicterus, which can result in death or serious neurological sequelae.

A recent paper explores criteria for initiating national screening for G-6-PDH deficiency in Canada (Leong, A. 2007). The author suggests that several criteria should be met before starting screening. These include that early treatment will

decrease morbidity and mortality, a simple test is available, the disorder being screened is sufficiently serious to warrant testing, and knowledge of results can be communicated to patients/parents, and to health care workers.

The author notes that Singapore has had a G-6-PDH deficiency screening program in place since 1965. This has dramatically reduced the incidence of kernicterus such that there has only been one case in the last 20 years! The newborn infants who test positive are kept in the hospital for about 2 weeks after birth in order to protect them from stimuli which might precipitate a hemolytic crisis, and to educate the parents about future risks (Joseph, R., et al. 1999). In addition, asymptomatic G-6-PDH infants are identified and the parents also advised of risks. These newborn infants would be missed without testing, and be subjected to hemolytic crisis without knowledge of risk, and what to do if a crisis occurs.

In terms of the criteria for testing of newborns that there should be a high frequency of G-6-PDH deficiency, the exact numbers in Canada are unknown. No large epidemiological studies have been done in either Canada or the USA looking at the frequency of hyperbilirubinemia/G-6-PDH deficiency. It is however clear that in both the USA and Canada, rapid changes are occurring in the ethnic make up of the populations. Continued influx of peoples from all parts of the world will surely increase the incidence of G-6-PDH deficiency and associated hyperbilirubinemia. Vigilance as regards ethnicity of patients can serve to minimize the serious complications of G-6-PDH deficiency, namely hyperbilirubinemia and kernicterus. The risk, as always, is that people living away from cities might not be as aware of symptoms, and delay seeking medical advise until too late in the rapid course of rising serum bilirubin levels.

Another study emphasizes the correlation of neonatal jaundice with G-6-PDH deficiency and interaction with other risk factors (Yu, M., et al. 1992). In their report, a cohort of 333 G-6-PDH-deficient newborn infants were identified and compared to a group of 653 neonates who were G-6-PDH normal. Increased jaundice (over 15 mg/100 ml) was noted in male patients but not females, and was associated with G-6-PDH deficiency. Both hypoxia/asphyxia and maternal hepatitis B antigen were associated with an increased risk for jaundice (equal to or over 20 mg/100 ml) in G-6-PDH-deficient patients. Statistical analysis showed a significant additive synergistic effect on serum bilirubin levels. Untreated, this would be expected to result in an added morbidity and mortality rate due to the toxic effects of increased unconjugated bilirubin.

This chapter on world health concerns is placed as the prolog because of the enormous significance of these clinical disorders to human well-being. Untold thousands of children die from hyperbilirubinemia, and even more are stricken with the ravages of untreatable cerebral palsy each year. Compared to massive US federal spending on trivial items, very few dollars in expense could result in a virtual elimination of this devastating consequence of hyperbilirubinemia and kernicterus around the world.

History of Bilirubin

The history of bilirubin actually goes back many hundreds of years, when newborn infants were observed to be jaundiced. More recent accounts of jaundice in newborns seems to have begun in the late eighteenth century. One of those was written by Jean Baptiste Thimotee Baumes (Baumes, J. 1806). This description was published as a chapter in a book entitled *Traite de L'amaigrissemwnt des enfans*. The chapter relating to jaundice is entitled *Traite de L'ictere ou jaunisse des enfans de naissance.*

Before describing the Baumes chapter on jaundice and other historical landmarks relating to jaundice, a recent excellent review of the pioneers in the study of neonatal jaundice and kernicterus has been published (Hansen, T. 2002). Dr. Hansen has provided very interesting and thorough descriptions of early investigators of jaundice and kernicterus. In some cases, he has obtained information from still living grandchildren of these pioneers (Orth). Information in the Hansen manuscript cannot be found anywhere else, and the reader is referred to this outstanding paper on bilirubin history found in any other source, and the book in which resides a chapter on jaundice by Baumes is interesting in its own right. The second edition of the jaundice chapter was published in 1806, and is reproduced in a book of a collection of chapters on the maladies of infants. Other chapters in this book in which Baumes' paper on jaundice appears also include chapters on pneumonia, angina, gangrene, edema, etc.

The Baumes paper is 72 pages in length, and he is listed as a professor of pathology at the School of Medicine at Montpellier, and a member of many medical societies, so he must have been a much respected physician. The copy I have is the second edition, which Hansen says is not as clearly presented as the first edition.

Baumes work was based primarily on a description of ten newborn infants who exhibited jaundice. Baumes makes reference to the liver, so an association between the two was already known. Baumes also describes somnolence and poor feeding, symptoms of cerebral involvement in some of the patients he describes. In all cases, Baumes mentions meconium as having importance. He thought that a delay in passage of meconium might be associated with, or the cause of neonatal jaundice. The idea that meconium retention was prevalent at least as early as the 1850s, when Condie (Condie, D. 1853) stated that newborn jaundice was related to failure of free release of meconium.

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Baumes also believed that maternal breast milk, and especially colostrums were beneficial to the correction of neonatal jaundice. The very first observation of Baumes was of his own jaundiced daughter, and one wonders if this fueled his interest in neonatal jaundice. The second edition of Baumes work is in a book, "Meladies des Infants" and as stated above has other chapters, some dated as late as 1841. It would seem that advances in the jaundice field moved at a somewhat slower rate than today.

In his splendid review of the early pioneers in jaundice, Hansen states that Jaques F. E. Hervieux was a critic of Baumes. Hervieux states that Baumes' ideas on the involvement of delayed passage of meconium were "without doubt ingenious, but nevertheless only an intellectual theory." He also states that "Baumes supports his opinion with a very small number of observations, and then concludes with astonishing ease to the great majority of cases."

The first description of bilirubin staining of the brain of kernicteric newborn children is credited to Johannes Orth (Orth, J. 1875). Orth reported on the presence of yellow and red pigments and crystals in the organs of newborn infants. He presented data on 37 newborns, all of whom had evidence of pigment in their kidneys, and "most" others had small amounts of yellow pigment in all other organs. In one severe case who died only 2 days after birth, the brain was yellow, but more intense staining was noted in specific brain regions including the basal ganglia, hippocampus, cerebellum, and walls of the third and fourth ventricles. Examination of the brain microscopically by Orth, revealed that neurons in the basal ganglia contained yellow pigment whereas the surrounding glial components were not stained. This patient's skin was severely jaundiced, yet a pallor was detected, leading Orth to state that the jaundice might have had a hematologic cause. Orth was an assistant to the great pathologist Virchow in Berlin when these observations were made.

Christian Schmorl in Dresden was the first to coin the term "Kernicterus." Schmorl was chief of pathology at the University of Dresden, Medical Faculty. In the Schmorl paper (Schmorl, C. 1904), 120 jaundiced newborn infants who died were autopsied. Of these, most (114) were described as having brain icterus of a diffuse yellow nature with "fat bodies" observed in some cases. The others (six) had "core icterus" in which only particular parts of the brains were yellow. These were mostly in "central ganglia" – basal ganglia, and in the "elongated ganglia" – medulla oblongata. Microscopic study of these brains showed that the yellow pigment was selectively present in neurons in nuclei.

Schmorl also noted an absence of staining in the glial component of these nuclei. It is due to the more intense staining of nuclei in the brains of newborn infants dying from jaundice that Schmorl coined the term "kernicterus" (see Fig. 1). Schmorl correctly credited Orth with publishing similar findings on intra cerebral staining by the yellow pigment.

Schmorl may have also been the first to note that the yellow pigment in brain disappeared over time unless the tissue was preserved in formalin (see Schmorl, Fig. 1).

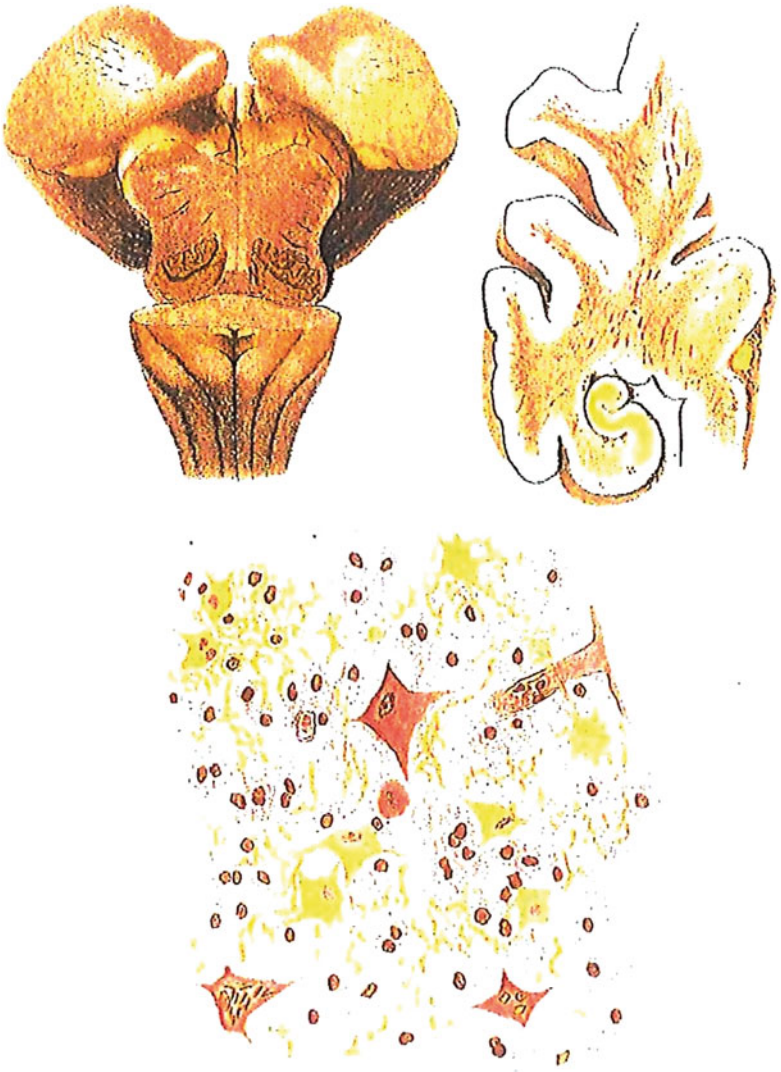

Fig. 1 Figures from Schmorl's early paper showing pigment staining in the hippocampus. These observations resulted in the term kernicterus

The concept evolved that neonatal jaundice could occur in families, and came to be called familial icterus gravis neonatorum. The understanding of the possible familial nature of jaundice was reported by several physicians, including Auden (Auden, G. 1905) and others. Interestingly, Hermann J. Pfannenstiel published a paper in 1908 (Pfannenstiel, H.J. 1908) describing a newborn case of familial icterus gravis neonatorium. This paper has been identified as the first of its kind, although

several similar papers preceded it (see above). The outcome of this is that familial icterus gravis neonatorum has come to be called Pfannenstiel's disease in some quarters.

At this time, (early 1900s) many authors published cases of multiple examples of jaundice and kernicterus in single families. Arkwright (Arkwright, J.A. 1910), for example, reported a family which had 15 children, in which 14 were jaundiced and only 4 survived.

In another paper at about the same time, Rolleston, H.D. (1910), delivered a total of four newborns who became jaundiced, and three of these died. The mother of these three newborns had herself become jaundiced late in the pregnancy. The fourth pregnancy resulted in a normal newborn infant who never became jaundiced. The cause of the jaundice was stated to be "obscure."

In spite of an increasing number of published reports of neonatal jaundice, the link between hyperbilirubinemia and brain damage was not made until 1914 (Guthrie, L. 1914). This paper describes a newborn infant with familial icterus gravis neonatorum (erythroblastosis fetalis). In this paper, the neurological features of hypotonia and chorioatetosis were described in the patient at the age of 19 months. The suggestion was made that these symptoms may represent a manifestation of brain damage by bilirubin, and Guthrie is thus credited as the first to publish a description of what is now known as kernicterus.

In 1932, a key paper was published (Diamond, L., Blackfan, K., and Baty, J. 1932) which defined and combined under one heading the clinical conditions of erythroblastosis fetalis, newborn anemia, edema of the fetus, and icterus gravis neonatorum. The exact cause of these disease entities, now under one heading was unclear since the concept of blood incompatibilities was not well understood. This, nevertheless furthered knowledge of anemia and resultant hyperbilirubinemia and kernicterus. When the pathology of hemolytic disease of the newborn was ultimately described, the techniques for exchange transfusion in newborn infants were developed. This facilitated the removal and replacement of damaged red blood cells. It also reduced levels of unconjugated bilirubin, proving to be a satisfactory treatment for the reduction of serum bilirubin levels, and a dramatic reduction of kernicterus.

As a side note, in 1944, Blackfan, K., Diamond, L., and Leister, C. published an atlas of blood disorders in children. Leister was an artist of considerable talent, and produced a series of beautiful water color paintings of blood smears which constitute most of the figures in the atlas and are used with permission in this volume (Blackfan, K., Diamond, L., and Leister, C. 1944, see chapter 5).

In 1950, Vaughan, V., Allen, F., and Diamond, L. published an important paper looking at the relation of erythroblastosis and kernicterus, They also reviewed various aspects of kernicterus as it was known at that time. Earlier studies had indicated that a Rh-positive fetus born to a sensitized Rh-negative mother was at risk, and that the chances of recovery were inversely related to the degree of anemia. Frequency of kernicterus, however, was not related to other signs and symptoms.

The incidence of reported kernicterus in early studies had a wide range, from 55% to as low as 10%. In the study by Vaughn, Allen, and Diamond, an incidence over 4 years was 12%, with 4.9% of kernicteric newborns surviving. All survivors

had some neurologic sequelae ranging from mild incoordination to severe motor disability and mental retardation.

The authors give a rather complete description of the clinical manifestations of kernicterus, which include developing jaundice on day 1, and by day 2–3 the jaundice becomes severe. This is accompanied by lethargy and poor feeding. Pathognomonic signs include a weakened or absent Moro response, and the presence of opisthotonic posturing. This includes rigidity and arm extension. It may also include brief high- pitched crying. Death in kernicterus is usually from respiratory failure, and seizures are not common, but do occur. Pulmonary hemorrhage is almost always associated with the end stages of kernicterus. Microscopically, the pathology consists of alveolar hemorrhage.

These authors found cerebral lesions in general agreement with those of early investigators. Specifically, lesions were found in the basal ganglia, hippocampus, cerebellum, olives, and medulla. These lesions were more diffuse in infants dying in 3 days or less, but were pronounced in newborns dying after 4–5 days. In infants surviving longer periods, the microscopic picture is one of neuronyl loss and glial proliferation. The location of the lesion is usually consistent with the signs and symptoms. Thus, newborn infants with motor involvement have lesions in the basal ganglia and cerebellum, whereas newborns with respiratory failure would show lesions in the medulla. (see Table 1 in the chapter "Neuropathology of Kernicterus", this volume).

The paper also describes the increase in incidence of kernicterus in newborns born prematurely, before 38 weeks of gestation, whether naturally or prematurely due to induction in sensitized women. The increased risk for kernicterus offsets the potential gains achieved in the prevention of stillbirths.

Kernicterus seemed to be a disease of neonatal life, reinforced by the findings that at birth, infants destined for kernicterus showed normal neurological reactions for a time before jaundice became severe. This indicates the brain was not damaged prior to birth. This odd course predates the findings of Schenker, who showed that unconjugated bilirubin easily crosses the placenta (Schenker, S. 1963). It is thought that death in cases of erythroblastosis fetalis is rarely from anemia, but that the greater the anemia, the higher the risk for developing kernicterus, the major cause of death in these cases. The authors downplay other hypotheses such as kernicterus acts to plug cerebral vessels leading to hypoxia, or that antibody transfer into the fetus at labor could be involved in the development of kernicterus.

Another milestone occurred when it was learned that the bilirubin in serum actually existed in two phases. The usual method for measuring bilirubin was by a spectrophotometric method in which bilirubin was coupled with diazotized sulfanilic acid-diazo reagent. It was afterward realized that there were two types of reactions occurring. One was termed direct, the other indirect – the reaction only occurred after alcohol was added to the reaction. The reason behind this peculiar phenomenon was at the time unclear.

Subsequent chromatography work showed that there was a slow moving fraction which gave the indirect diazo reaction, and a fast moving fraction which was the direct moiety. In 1956, three laboratories independently solved the mystery by

showing that the direct moiety was formed by bilirubin glucuronide (Schmid, R. 1956; Talafant, E. 1956; Billing, B., Cole, P., and Lathe, G. 1956). Bilirubin glucuronide was a water-soluble form of bilirubin, and was easily excreted by the liver, as compared to the indirect form of bilirubin which was lipid soluble.

In another classic study, the protein binding of bilirubin was examined (Odell, G., 1959). Spectrophotometric studies showed that protein (albumin)-bound bilirubin was changed when organic anions such as salicylates and sulfonamides were injected into subjects. This was indicated by a drop in absorption at 460 mμ to values of from 420 μ to 440 mμ. The lower value is that which is associated with unbound unconjugated bilirubin.

The significance of this paper was to emphasize the competitive binding characteristics of certain organic ions such as sulfonamides and salicylates for albumin-binding sites. This acts to displace unconjugated bilirubin from albumin, facilitating its entry into brain structures, producing kernicterus. This also emphasizes the idea that simple measurement of total bilirubin levels may not be a completely accurate indication of kernicterus risk. A small dissociation constant for albumin-bound bilirubin would lead to a large percentage change in unbound bilirubin whenever there was a small increase in diffusible bilirubin.

Another very important paper was published in 1968 (Lucey, J., Ferreiro, M., and Hewitt, J. 1968). Dr Lucey's studies have centered on the prevention of hyperbilirubinemia and kernicterus in newborn infants using photo therapy. Prior to 1968, photo therapy had been used successfully in several countries in South America and Europe. There was some reluctance, however, for approval to be granted in the USA possibly due to uncertainty about potential undefined risks of photo therapy per se, and questions about the safety of the photo-degraded composition of products produced by the photo therapy.

Dr. Lucey and co-workers performed a controlled study in 111 newborn infants who were less than 2,500 g at birth. This was a randomized study in which newborn infants were assigned to either a photo therapy group, or to a control group. The assignments were made before 12 h of age. Results showed that the photo therapy treatment produced a statistically significant lowering of serum bilirubin between the fourth and sixth days. Furthermore, no correlation between the levels of albumin and the ability of photo therapy to lower serum bilirubin could be found.

No toxic effects were found which could be attributable to photo-degraded bilirubin products. Other authors had shown that photo-degraded products of photo therapy were rapidly excreted in bile and urine (Ostrow, J. 1967). This implies that the lipid-soluble nature of unconjugated bilirubin is rendered, by photo therapy, to water-soluble compounds which do not require conjugation in order to be excreted.

Since as many as 10% of preterm infants at this time had serum bilirubin levels over 20 mg/100 ml with significant risk for brain damage, the institution of photo therapy had the potential to reduce the risk of kernicterus to near zero. That is indeed what happened, and as stated in the chapter in this volume on photo therapy, the significance of this study, which demonstrated beyond doubt the safety of photo therapy, is immeasurable. Photo therapy has saved thousands of newborn infants from morbidity and mortality due to unconjugated bilirubin in the USA.

The study of Schenker, et. al. mentioned earlier, regarding the placental transfer of bilirubin was key in the understanding of why fetal hyperbilirubinemia was nonexistent, and answering in part why bilirubin levels tended to rise shortly after birth (Schenker, S., Dawber, N., and Schmid, R. 1964)

Advantage was taken of the availability of biosynthesized ^{14}C radiolabeled bilirubin. Radiolabeled unconjugated bilirubin, infused into fetal guinea pigs in vivo appeared in maternal bile as radiolabeled conjugated bilirubin. This excretion of fetal injected label began to appear in maternal bile within 15 min. Less than 2% of label appeared in fetal bile.

These results helped explain why fetal tissues are not stained in utero by unconjugated bilirubin. In regards to physiological and pathological hyperbilirubinemia, the results explain jaundice appearing within hours after birth. Since the placenta is a route for transfer of potentially toxic unconjugated bilirubin, the fetal liver is not significantly challenged as regards the enzymatic machinery (glucuronyl transferase) for conjugating bilirubin. At birth, the maternal mechanism for conjugating bilirubin is removed. This places a temporary but increasing load of bilirubin on the newborn liver. This had not been previously experienced by the liver, and a certain "lag" time exists in about 60% of newborn infants. Just a few days is enough to resolve the physiological unconjugated hyperbilirubinemia. In cases of hemolysis, the correction of hyperbilirubinemia may need clinical intervention.

Biochemistry and Physiology of Bilirubin

Early work on the biochemistry of bilirubin was conducted by Nobel Laureate Hans Fischer. Hemoglobin is an important constituent of the pyrrole group of molecules, and Fisher studied hemoglobin. Hemoglobin breaks into heme and globin in its first degradation step. The combination of heme and globin possesses the unique ability to loosely bind oxygen, enabling it to be released to body tissues. Heme bound to iron is termed hemin, which has the chemical formula: C34 H32 O4 N4 FeCl. When iron is removed from hemi, porphyrins are the result.

It was known that all porphyrins contain a pyrrole ring which consists of four carbon atoms, with the presence of a nitrogen atom which serves to close the pyrrole ring. Fischer was the first to show that the synthetic substance phorphin contained four pyrrole rings linked by four methane bridges (=CH) which serve to close the circlular structure. Subsequently, Fischer showed that all porphyrins have a porphin nucleus with four pyrrole rings in which the carbon atoms are linked by hydrogen. Fischer is credited with the discovery of porphyrin synthesis, and ultimately was successful in synthesizing over 130 isomers of porphyrin. Continued work on porphyrins led to the synthesis of a synthetic hemin which was indistinguishable from the naturally occurring hemin which is obtained from hemoglobin. (see Fig. 1).

Finally, Fischer turned his attention to the structures of biliverdin and bilirubin. Fischer showed that if one carbon atom was absent, the now opened ring resembled bile pigments. He subsequently elucidated the chemical structure of both biliverdin and bilirubin (Fischer, H., and Pleininger, H. 1942). (see Figs. 2 and 3).

Bilirubin is formed as a result of the breakdown of red blood cells. This process takes place in cells of the reticuloendothelial system. The system is widespread in the body, occurring in the liver, spleen, and bone marrow. In diseased states where there may be an increased production of bilirubin, or when the "regular" sites of lysis of red blood cells are not functional, several other locations may function to lyse the red blood cells. The heme moiety of hemoglobin results in at least 80% of unconjugated bilirubin. The smaller component of unconjugated bilirubin may come from a heme which has a very rapid turnover rate. Hepatic cytochromes may be the source (Schmid, R. et al. 1966).

As stated above, bile pigments are structures with four pyrrole rings linked by –CH2 or =CH groups. These naturally synthesized pigments result from oxidative scission of ferroprotoporphyrin IX. This results in the IX alpha structure

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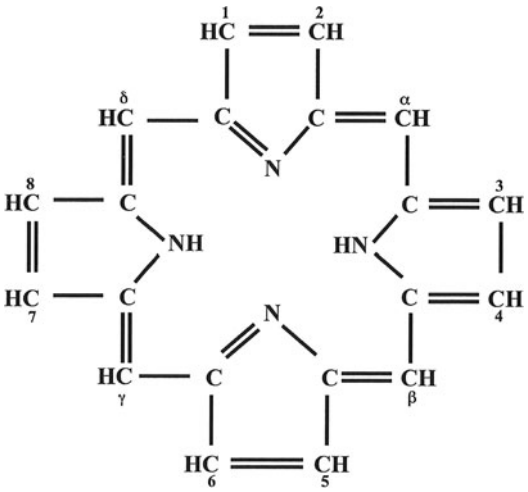

Fig. 1 Schematic representation of porphoryn

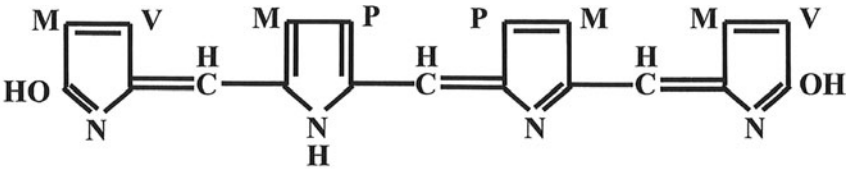

Fig. 2 Schematic representation of biliverdin

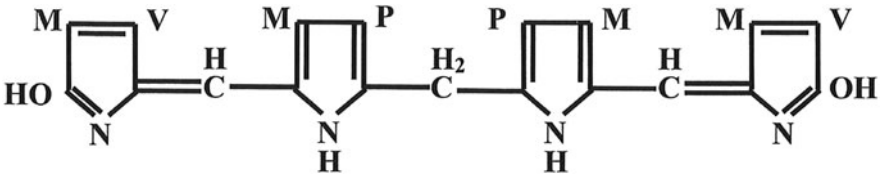

Fig. 3 Schematic representation of bilirubin

being present in the resultant bile pigments. The pigment biliverdin is very similar structurally to protoporphyrin. The central methane bridge of biliverdin is easily reduced, transferring it into bilirubin.

The bilirubin molecule is stabilized by its presence of six hydrogen bonds. These hydrogen bonds most likely represent the reason that this form of bilirubin is not water soluble. This form of unconjugated bilirubin has at least three geometric isomers, and the conversion of bilirubin to these isomers may be the mechanism by which photo therapy acts to produce forms which can be more readily eliminated. Additionally, conjugation of bilirubin IX alpha to bilirubin glucuronide produces a molecule which does not have hydrogen bonds, thereby creating a molecule which is

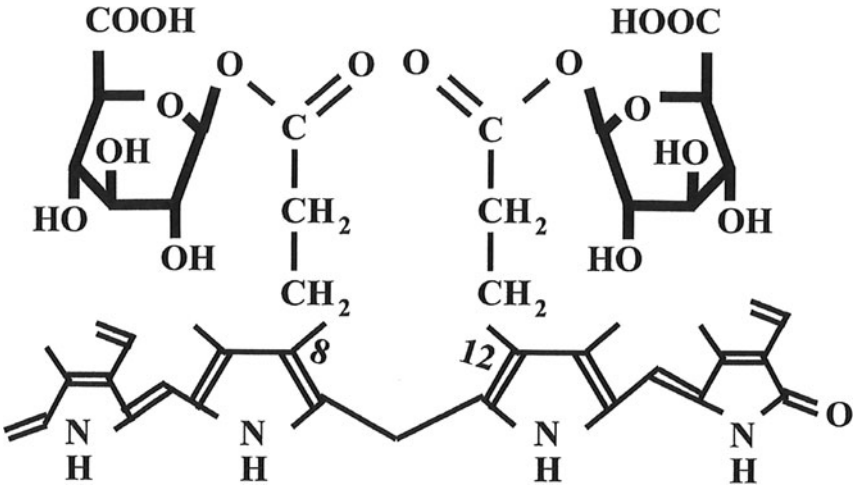

Fig. 4 Schematic representation of bilirubin glucuronide

water soluble, and can be readily excreted by liver cells into bile ducts (Grodsky, G., and Carbone, J. 1957). In aqueous solutions, bilirubin IX alpha can be cleaved and the halves may reform as isomers of bilirubin IX alpha, bilirubin III alpha, and bilirubin XIII alpha. Further bacterial or chemical reduction of bilirubin yields urobilins and urobilinogens. (see Fig. 4).

Conjugation of the lipid-soluble unconjugated bilirubin is a process of esterification of bilirubin's propionic acid with glucuronic acid. This may involve one or both of the propionic moieties. This results in a diminution of the ability of bilirubin to have strong hydrogen bonds, thereby rendering the now conjugated bilirubin water soluble. Thus, the conjugated bilirubin can be excreted in bile and urine, and the reabsorption in the digestive tract is impeded.

In normal bile, 80% of conjugated bilirubin is in the diglucuronide form, while the other 20% represents a single bilirubin/propionic bond – the monoglucuronic acid form. The enzyme catalyzing the conjugation of bilirubin is uridine diphospho-glucuronyl transferase (EC 2.4.1.17). This enzyme is located in the microsomal components of both rough and to a lesser extent, smooth endoplasmic reticulum. The enzyme uridine diphospho-glucuronyl transferase is specific for bilirubin. The interconversion of conjugated bilirubin between the monoglucuronide and diglucuronide forms is discussed in a later chapter in this volume.

The uptake and conjugation processes of metabolism of bilirubin have a greater capacity than does the excretion into bile. Therefore, the latter step is rate limiting in the entire process. When the conjugated bilirubin reaches the large intestine, it undergoes chemical changes due to bacterial action. The conjugated bilirubin is deconjugated and reduced to form several urobilins. The exact make up of these urobilins depends in part on the intestinal flora, and wide normal variation occurs. If antibiotics are administered, unconjugated bilirubin is directly excreted in feces.