Twenty-five years of progress in bilirubin metabolism (1952-77)

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SUMMARY This review deals with the development of our understanding of the chemistry of bilirubin and its glucuronide derivatives during the years 1952–77. It examines the relation between haem metabolism and bilirubin formation and our present knowledge of hepatic transport of bilirubin. The heterogeneity of familial hyperbilirubinaemia is discussed.

The progress made in our understanding of bilirubin metabolism during the last 25 years is due to the combined activities of the biochemist, organic chemist, mass spectroscopist, electron microscopist, and cell biologist, as well as the hepatologist, surgeon, and haematologist. This multi-disciplinary approach has resulted in a better appreciation of the mechanisms involved in the formation and excretion of bilirubin. It is, therefore, now possible to describe in biochemical terms some of the abnormalities responsible for hyperbilirubinaemia and in some circumstances to introduce appropriate therapeutic measures to modify the degree of jaundice.

Chemistry of bilirubin

Although bilirubin was isolated from bile by Stadler as early as 1864 it was not until 1942 that Fischer and Pleninger (1942) demonstrated that it had an open chain structure consisting of four pyrrole rings joined by three carbon bridges (Fig. 1). The sequence of the four methyl, two vinyl, and two propionic side chains was identical with that found in the protoporphyrin IX ring of haem after removal of carbon at the α methene bridge, so it was designated bilirubin IXα. It has now been established that the terminal pyrrole rings exist predominantly in the lactam form (Hutchinson et al., 1971). The linear structure of the molecule would favour a compound with polar properties but this is not the case at a physiological pH and an explanation for the lipid solubility of bilirubin has long been sought. It was first postulated by Fog and Jellum (1963) that there was strong intramolecular hydrogen bonding and recently Bonnett et al. (1976), using x-ray analysis, have demonstrated that the molecule is stabilised by six intramolecular hydrogen bonds (Fig. 2). The other three bilirubin isomers (β, γ, and δ) have significantly different physical properties so that, unlike the naturally occurring IXα isomer, they can be excreted directly into bile, without requiring modifications to their structure (Blanckaert et al., 1977).

With chemical treatment it is possible to cleave the unsymmetrical bilirubin IXα molecule into pairs of dipyrrrole methene units which then undergo random recombination to give mixtures of the isomers bilirubin IIIα, bilirubin 1Xα, and bilirubin XIIIα (McDonagh and Assisi, 1972). Although bilirubin IIIα and bilirubin XIIIα are rarely found in nature, it is important to appreciate that, under certain conditions, dipyrrrole exchange occurs, as this can influence the interpretation of data obtained when determining the structure of bile pigments (Jansen, 1973; Heirwegh et al., 1975).

When a solution of bilirubin is exposed to visible light with wavelengths in the region of 430 to 470 nm it undergoes photo-oxidation, due to the formation of singlet oxygen (McDonagh, 1971); this results in the formation of colourless products which are soluble in water. A similar reaction is thought to occur in the skin of jaundiced infants during phototherapy. In addition, experiments in the Gunn rat suggest that, during treatment, bilirubin may be temporarily converted from the 'trans' (Z) to the 'cis' (E) form (Bonnett et al., 1976), which is free of hydrogen bonding and therefore capable of being excreted directly into the bile (Ostrow, 1971; Zenone et al. 1977).

Identification of bilirubin derivatives

'INDIRECT' AND 'DIRECT' BILIRUBIN

With the use of the Van den Bergh reaction for the measurement of bilirubin (Van den Bergh and Muller,
1916) in plasma it became evident that there were at least two different types of bilirubin. The bilirubin in the plasma of patients with haemolytic disease required the presence of alcohol to give a positive reaction with diazotised sulphanilic acid and was considered to give an 'indirect' reaction. In contrast, bile and plasma or urine from a patient with obstructive jaundice gave a 'direct' reaction in the test. It was therefore concluded that the liver produced a change in the bilirubin molecule in the course of its passage through the organ into the bile ducts. Whether this involved alterations in the chemical structure of the molecule or was associated with differences in protein binding was not established until two bile pigment fractions were obtained free of protein and other biliary constituents from obstructive jaundice serum (Cole and Lathe, 1953).

**BILIRUBIN GLUCURONIDES**

Using reverse phase chromatography it was shown that 'direct' bilirubin was a mixture of two pigments, arbitrarily called pigments I and II (Cole et al., 1954). Identification of these pigments was made possible using the stable azo pigments formed in the van den Bergh reaction as the result of an electrophilic ion attacking the bilirubin at either side of the methylene bridge so that two dipyroles are produced, which react covalently with the diazonium salt. It was shown that, whereas 'indirect' bilirubin gave rise to two molecules of azo-pyrrole A, the major 'direct
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reacting’ pigment of bile (pigment II) formed two molecules of a more polar azo-pyrrrole B. Purification of azo-pyrrrole B by chromatography (Billing et al., 1957) revealed that it was the glucuronide of azopyrrole A. As the result of this observation and similar findings by other investigators (Talefant, 1956; Schmid, 1957) it was concluded that bilirubin was converted by the liver to a diglucuronide ester.

As pigment I yielded equimolar amounts of azopyrroles A and B, it was postulated (Billing et al., 1957) that bilirubin monoglucuronide was also formed by the liver. Its existence as a chemical entity was questioned for some years, as it was somewhat unstable and, moreover, a complex of bilirubin and bilirubin diglucuronide would also yield equimolar amounts of azo pigments A and B in the diazo reaction. The matter was finally resolved when it was shown that two mono-amide derivatives of bilirubin (Jansen and Billing, 1971) could be formed from bile, which would be possible only if two isomers of bilirubin monoglucuronide were present.

OTHER BILIRUBIN CONJUGATES

With the development of a thin layer chromatographic procedure with a high resolving power for the analysis of azo pyrrrole derivatives, Heirwegh and associates were able to show that the bile pigment pattern was considerably more complex (Heirwegh et al., 1970) than had previously been appreciated and that there were significant species differences (Fevry et al., 1977). With their techniques the azo-pigments formed in a ‘direct’ reaction can be divided into four main groups \( \alpha, \beta, \gamma, \) and \( \delta \), the \( \delta \) group consisting essentially of the glucuronide moiety (Fig. 1). As azo-pigment \( \alpha \) can result only from the monoconjugates, this technique can be used to assess the relative amounts of mono and diconjugates.

From analysis of the azo-pigments formed with dog bile it has been possible to recognise more than 20 diazo positive tetra-pyrrolic bile pigments (Heirwegh et al., 1975), whose conjugating groups are linked as esters to the aglycone bilirubin 1\( \alpha \). Glucuronic acid appears to be the dominant conjugating group in all the animals so far tested, apart from the chicken, (Fevry et al., 1977); in most animals the monoconjugates predominate but, in man, diconjugates are usually present in greater amounts. Significant concentrations of xylose and glucose conjugates are present in dog bile (Fevry et al., 1971; Gordon et al., 1974) and may also be found in human bile in obstructive jaundice (Fevry et al., 1972). Although thin layer chromatographic systems have now been developed for the separation of the tetra pyrroles (Noir, 1976; Heirwegh et al., 1975; Gordon et al., 1976) into mono and diconjugates most structural information has had to be derived from the analysis of the dipyrrolic azo-pigments, as these are more stable and therefore more suitable for such analytical techniques as mass spectrometry.

It has been difficult to reconcile the findings of the above investigators with those of Kuenzle (1970) who, using reverse phase chromatography on silicone treated columns for the isolation of azo-pigments from 15 litres of human bile and very precise analytical techniques, was unable to detect the presence of bilirubin glucuronide. Instead, Kuenzle proposed that the major bilirubin conjugates in man were acidic disaccharides. New light has, however, recently been shed on this problem by the observation that in aqueous solution above pH7 bilirubin 1\( \alpha \)-1-O-acyl glucuronide rapidly isomerises to non-C-1 glucuronides by sequential migration of the bilirubin acyl group from position 1 to positions 2, 3, and 4 of the sugar moiety (Compernolle et al., 1978). These non-C-1 glucuronides are not attacked by \( \beta \)-glucuronidase. When normal bile from man and rats is collected at 0\( ^\circ \)C and then immediately analysed it has been found that bilirubin 1\( \alpha \)-mono- and diglucuronides consist exclusively of 1-O-acyl isomers, and that \( \delta \) azo-dipyrrrole is the major azopigment formed on diazotisation. If, however, the bile is incubated at 37\( ^\circ \)C or is obtained from rats or patients with cholestasis (Fevry et al., 1972), then relatively large amounts of \( \beta \) and \( \gamma \) azopigments, which are derived from 2-, 3-, and 4-O-acyl glucuronides (Blanckaert et al., 1978), are detected, thereby indicating that sequential migration of the bilirubin acyl group can occur in vivo. The bile used by Kuenzle was obtained after cholecystectomy and then stored in a deep freeze so that it is likely that acyl migration could also have occurred in his pigments. If this is true, then his azopigments \( B_4 \), \( B_3 \), and \( B_2 \) might be mixtures of 2-, 3-, and 4-O acylglucuronides instead of disaccharides as originally proposed; contamination could possibly account for the glucose moiety (Gordon et al., 1976; Compernolle et al., 1978).

The ease with which the transformation of the 1-O-acyl glucuronide occurs probably accounts for the fact that bilirubin glucuronide has not yet been synthesised, except in a form which is not attacked by \( \beta \)-glucuronidase (Thompson and Hofmann, 1970). It is to be hoped that, now that there is awareness of this problem of transformation, synthesis of both the natural bilirubin monoglucuronide and bilirubin diglucuronide will be possible so that a better understanding of the factors influencing their metabolism can be attained.
ENZYMATIC FORMATION OF BILIRUBIN GLUCURONIDES

With the identification of 'direct' bilirubin as bilirubin glucuronide it was quickly established that, in common with other glucuronides, the main site of synthesis of bilirubin monoglucuronide is in the endoplasmic reticulum with uridine diphosphate glucuronic acid as the glucuronyl donor (Schmid et al., 1957; Lathe and Walker, 1958). Two models have been proposed to explain the mechanism of UDP-glucuronyl transferase activation. In the 'compartmental' model, it is postulated that the UDPGA is synthesised in the cytoplasm and gains access to the enzyme, which is situated in the lumen of the cisternae of the microsomal membrane, as the result of the action of a permease (Berry and Hallinan, 1976). In contrast, in the 'conformational' model (Zakim and Vessey, 1976), it is assumed that activation of the enzyme involves release of the constraint of the enzyme by the phospholipids in the membrane with the breakdown of the permeability barrier. Probably both hypotheses contain elements of truth and may involve UDP-N-acetylglucosamine.

Although bilirubin glucuronyl transferase has not been purified, it is generally accepted that there is a specific enzyme for the glucuronidation of bilirubin. Bilirubin glucoside and xyloside conjugates are also formed in the endoplasmic reticulum (Feverly et al., 1971). It has been shown in vitro that, if the constraint of microsomal UDP glucuronyl transferase activity is released by the addition of deoxycholate or bile, then if both UDPGA and UDP-glucose are added bilirubin glucosidation is increased preferentially (Bock and Remmer, 1977). It is possible that this observation could provide an explanation for the rise in glucose and xylose conjugate formation in obstructive jaundice (Feverly et al., 1972).

There is no clear evidence that the diglucuronide is also formed in the endoplasmic reticulum and recently Jansen et al. (1977) presented data which indicated that the conversion of bilirubin monoglucuronide to bilirubin diglucuronide may occur at the surface membrane of the liver cell. (2 mol bilirubin monoglucuronide → 1 mol bilirubin diglucuronide and 1 mol bilirubin). Support for this hypothesis comes from a recent publication by Wolkoff and colleagues (1978) who found that the intrahepatic pool of conjugated bilirubin was almost exclusively bilirubin monoglucuronide, which accumulated as a ligand bound to several glutathione-S-transferases, including ligandin. Their failure to find the diglucuronide can be understood if one accepts that its formation occurs at the canalicular membrane in a site or configuration which facilitates its immediate biliary excretion.

The fate of the unconjugated bilirubin formed in the trans-glucuronidation reaction is unknown; it could either mix with the pigment formed from the haemoproteins or free haem, or else gain direct access to the microsomal membrane for reconjugation. It certainly seems to be a rather complicated system, especially as bilirubin monoglucuronide is sufficiently polar to enable it to be excreted directly into the bile. One looks forward to further information on this aspect of glucuronidation.

Relation between haem metabolism and bilirubin formation

It has long been known that the main source of bilirubin is the breakdown of haemoglobin. The senescent red cells are removed from the circulation either by intravascular lysis and release of free haemoglobin or as intact red cells, thereby involving two different cell populations (Bissell et al., 1972). The intact erythrocytes are sequestered by the reticuloendothelial cells in the liver or spleen while the circulating haemoglobin, whether haptoglobin bound or free, is taken up by the hepatic parenchymal cells (Bissell et al., 1972). If the haptoglobin binding capacity is exceeded, then the free haemoglobin may be filtered by the renal glomeruli and reabsorbed by the epithelial cells of the proximal convoluted tubules (Bunn and Jandl, 1969); thus, in intravascular haemolysis both the liver and the kidney can become important sites of bilirubin production (Pimstone et al., 1971). It is not known what proportion of erythrocyte catabolism in normal subjects involves intravascular lysis. If severe haemolysis occurs, then the haem will be transported bound to a variety of carrier proteins to form haem-haemopexin, methaemalbumin, and methaemoglobin (Müller-Erberhard and Liem, 1974). It is of interest that, whereas oxyhaemoglobin and haem-haemopexin enter the hepatocyte as an intact haem-protein complex (Müller-Erberhard and Liem, 1974), the haem of methaemalbumin is separated from its protein moiety before its uptake by the liver (Bissell et al., 1972).

'EARLY BILIRUBIN'

Studies with isotopic precursors of haem have established that 10-20% of the circulating bilirubin comes from haem compounds which have a short half life. This so-called 'early labelled bilirubin' has two major components (Yamamoto et al., 1965), one of which is erythropoietic in origin and is probably formed in the bone marrow from reticulocytes and normoblasts which fail to reach maturation; it is significantly increased in disease associated with
ineffective erythropoiesis (Israels, 1970). The presence of the non-erythropoietic component can be demonstrated by the administration of $\delta$-amino-oxovaleric acid (ALA), as it labels hepatic haem almost exclusively and only penetrates erythropoietic cells to a limited extent (Robinson, 1972). It is generally considered that at least two haemoproteins are involved, one of which is associated with cytochrome p450, which has a half life of eight hours, while the other unknown compound has an estimated half life of less than one hour and could be free haem. Recent studies (Berk et al., 1976) indicate that in normal individuals 'early bilirubin' synthesis, assessed by determining the difference between plasma bilirubin turnover and bilirubin derived from red blood cell degradation, constitutes approximately 25% of the total bilirubin turnover. There appears to be a constant hepatic component of approximately 22% so that the contribution from ineffective erythropoiesis is normally very small.

**MECHANISM OF BILIRUBIN FORMATION**

The classical studies of Lemberg and Legge (1949) had shown that the degradation of haem compounds to bile pigments can be achieved in vitro by treatment with oxygen in the presence of a mild reducing agent such as ascorbic acid and it has been postulated that coupled oxidation might also occur in vivo. The observation that when the reaction is performed with haemin (O’Carra and Colleran, 1969), a mixture of four biliverdin isomers is produced would argue against this hypothesis. However, if the haem is bound to a suitable apoprotein (O’Carra and Colleran, 1970), stereospecificity is conferred on the reaction and biliverdin 1Xα is formed. There has been much controversy as to whether haem catabolism is an enzymatic or non-enzymatic process (Gray et al., 1972) and it would now seem that the two mechanisms are not necessarily mutually exclusive (Schmid and McDonagh, 1975, O’Carra and Colleran, 1976).

Of the several enzyme systems which convert haem to bile pigments, it is the microsomal haem oxygenase of Tenhunen et al. (1969) which has received most attention, as its specific activity is highest in the spleen, bone-marrow, and liver where haem degradation is known to occur. The microsomal haem oxygenase system has an absolute and stoichiometric requirement for NADPH and requires 3 mol of oxygen for the conversion of methaemalbumin, and other haem compounds, to equimolar amounts of bilirubin 1Xα and CO. Haemoglobin is not a substrate in this reaction, so it has been concluded that in vivo haem is separated from globin and transferred to another protein before degradation occurs. There is now good evidence that cytochrome p450 is not directly involved and it has been postulated that the apoprotein of this cytochrome, which is located in the membrane of the endoplasmic reticulum, may serve as the binding protein for haem (Bissell, 1975; Schmid and McDonagh, 1975; O’Carra and Colleran, 1976). The NADPH and the microsomal electron transport system are then used to reduce the iron of the bound haem, and to generate a reactive oxygen radical which attacks the haem at its α-bridge to form α-oxo-haem (Jackson, 1974). The intermediates formed between oxyhaem and biliverdin are not known but the process certainly involves loss of the α-meso carbon atom as CO as well as oxidation at the two adjacent carbon atoms. Experiments with O$^{18}$ labelling suggest that these terminal lactam atoms are derived from different oxygen molecules (Brown and King, 1978) (Fig. 3).

![Fig. 3 Pathway of haem catabolism (Brown et al., 1978)](M, CH$_3$; V, CH=CH$_2$: P, CH$_3$CH$_2$H).
explanation is that biliverdin does not cross the placenta and can be removed from the mammalian foetus only if first converted to bilirubin (Palma et al., 1977).

Hepatic transport of bilirubin

UPTAKE
The bilirubin, which is derived from senescent erythrocytes sequestered in phagocytic cells, is transported in the plasma tightly bound to albumin while approximately 10% may be bound to red cells (Barnhart and Clarenberg, 1973). Compounds, which compete with bilirubin for common binding sites on the albumin molecule, such as fatty acids and a large number of organic anions, drugs, and antibiotics, will facilitate the diffusion of bilirubin, in the unbound form, into the liver and other tissues by non-ionic diffusion (Brodersen, 1974). The administration of drugs such as sulphonamides and salicylates to neonates may therefore increase the risk of kernicterus, as their blood-brain barrier is more permeable to 'unbound' bilirubin than that of the adult (Diamond and Schmid, 1966).

The process of hepatic uptake follows detachment from albumin at the sinusoidal plasma membrane; it appears to be carrier-mediated and is shared by a variety of organic anions, excluding bile acids (Goresky, 1975; Scharschmidt et al., 1975; Paumgartner and Reichen, 1976). The flux of bilirubin across the plasma membrane is bidirectional and in congenital non-haemolytic jaundice the reflux of the bilirubin taken up by the hepatocyte into the plasma may be significantly increased (Billing et al., 1964; Berk et al., 1970). It has been well established that there are several non-specific binding proteins (aryl-glutathione transferases) in the hepatic cytosol which bind bilirubin reversibly—for example, Y and Z protein (Levi et al., 1969). There has been considerable debate as to whether these proteins, and in particular Y protein (ligandin), play an essential role in the uptake and transport of bilirubin or merely serve to increase the solubility of the pigment in the cell so that it can be stored in a non-toxic form (Fleischner and Aras, 1976). The major problem has been that in vitro, plasma albumin appears to have a greater affinity for 'unbound' bilirubin than purified ligandin, which may be due to technical artefacts. However, if the cytosol is submitted to moving boundary sedimentation then the high bilirubin binding affinity of the ligandin can be maintained (Meuwissen et al., 1977). Using data obtained with this technique it has been claimed that the binding proteins are concerned with the facilitated diffusion of bilirubin between the plasma membrane and the endoplasmic reticulum. Meuwissen and his colleagues (1977) suggest that the binding proteins act as a 'passive rotary carrier system in an aqueous barrier' and that the metabolic rate of bilirubin is regulated by the concentration of these carriers. On the other hand, others (Wolkoff et al., 1977) consider that ligandin acts as a determinant of net hepatic uptake of bilirubin by controlling its efflux from the liver into the plasma.

COMPARTMENTATION (see future research p. 487)

SECRETION
Unless conjugation is defective, the rate-limiting factor in the transport of bilirubin from plasma to bile appears to be the biliary excretion of bilirubin glucuronide, as the uptake process is normally not saturated. Secretion of the conjugated pigments probably involves a carrier-mediated active transport system which is shared by many other organic anions. Maximal bilirubin excretion can be enhanced by taurocholate in common with other substances excreted in bile at high concentrations relative to plasma (Goresky et al., 1974). A high proportion of the conjugated bilirubin formed is incorporated into mixed micelles (Goresky et al., 1974; Goresky, 1975; Scharschmidt and Schmid, 1977). Goresky has postulated that the bile salts form a 'micellar sink' in the bile canalculus which removes the conjugated bilirubin from the non-micellar phase so that a net concentration gradient is formed which enhances the flux of pigment from the liver cell into the bile. The role of the Golgi apparatus and microfilaments in the intrahepatic transport of conjugated bilirubin remains to be defined.

ELIMINATION OF BILE PIGMENTS
Conjugated bilirubin is not significantly reabsorbed in the intestine. In the terminal ileum and large intestine it is hydrolysed by bacterial β-glucuronidase and reduced to a complex mixture of urobilinogens, their structure depending on the gut flora (Watson, 1969). They are then excreted in the faeces as oxidised urobilins, the measurement of which does not give a valid assessment of bilirubin production (Bloomer et al., 1970). A small fraction of the urobilinogens is reabsorbed and then excreted in the bile; in the presence of excess bilirubin production or liver disease renal excretion of urobilinogen may be observed (Bourke et al., 1965).

Only in cholestasis, when the pigment accumulating in plasma is almost entirely bilirubin glucuronide, are bile pigments excreted in the urine. The 'unbound' conjugated bilirubin is freely filtered by the glomeruli and then largely reabsorbed in the proximal tubules.
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Gollan et al., (1978). Studies in an isolated kidney preparation support the view that, in contrast to the liver, conjugated bilirubin and bile acids are reabsorbed by a similar mechanism (Barnes et al., 1967) so that bilirubin glucuronide may inhibit the reabsorption of bile acids.

Some areas for future research in bilirubin metabolism

Considerable progress has been made in the last 25 years in our understanding of bilirubin metabolism at a molecular level. An enzymatic mechanism for the formation of biliverdin has been described, but to what extent it plays a physiological role has still to be assessed. It is not known whether all haem proteins are catabolised in the same way or, indeed, whether a proportion of the haem liberated from senescent red cells gets reutilised. There is indirect evidence that bilirubin may not necessarily be the only end product of haem catabolism (Landaw et al., 1970). Also, in the Gunn rat and patients with the Crigler-Najjar syndrome, who have an absolute deficiency of bilirubin glucuronyl transferase activity, it is obvious that there must be an alternative route for the disposal of bilirubin which does not involve glucuronidation (Schmid and Hamburger, 1963).

The role of intracellular compartmentation in the regulation of haem and bilirubin metabolism is of increasing interest to many investigators. It is known that the sinusoidal cells are able to sequester and degrade intact erythrocytes (Bissell et al., 1972) but their functional relationship with the parenchymal cells is uncertain. Moreover, there is good evidence that the parenchymal cells are not homogeneous and may undergo differentiation on a lobular basis so that the centrolobular and periportal cells may have different functions (Drochmans et al., 1975; Jones et al., 1976). According to the lobular gradient hypothesis of Goresky (1975), there is a concentration gradient for bile acids from the portal to the central zones in each lobule. This would suggest that the bilirubin excretion related to bile acid dependent flow is greatest in the centrolobular zone.

Comparisons of bilirubin production obtained by measuring the rate of disappearance from the circulation of a tracer dose of radioactive bilirubin with CO production have shown that plasma bilirubin turnover is less than total bilirubin production (Landaw et al., 1970). This observation, together with studies by Jones and colleagues (1972) on the measurement of 'early bilirubin' production in man, suggested that a fraction of the bilirubin which entered the bile directly was non-haemoglobin in origin. From the computer-derived data of Kirshenbaum and colleagues (1976) in a patient with Gilbert's syndrome it has been postulated that two independent hepatic bilirubin pools are involved; on the basis of their size and kinetics these have been tentatively assigned to cytochrome P450, and cytochrome b5 together with other mitochondrial cytochromes. As the haem of haemoproteins is bound to the microsomal membrane it could be cleaved and converted to conjugated bilirubin in situ and then transported into the bile by the tubular system of the endoplasmic reticulum without entering the cytosol. A third very rapidly turning over bi-

The exact role of the plasma membranes and ligandin in the uptake of both unconjugated and conjugated bilirubin still requires to be defined so that a better understanding of what is meant by the term 'hepatic clearance' can be obtained. There is also uncertainty about the mechanism and site of concentration of bilirubin before its excretion in bile and further information is needed about the factors controlling the formation of bilirubin diglucuronide.

Although it has been known for 20 years that bilirubin is mainly excreted as mono- and digluco-

Familial hyperbilirubinaemia

It has become increasingly apparent that jaundice is rarely associated with a single abnormality in bilirubin metabolism even in familial hyperbili-

rubaemia. Nevertheless, there is usually a domin-

ant defect which is responsible for the patient
becoming icteric.

The capacity of the liver to eliminate bilirubin is greatly in excess of normal total haem catabolism, so that haemolysis rarely produces gross jaundice unless there are concomitant defects in biliary and urinary excretion (Fulop et al., 1971). Mild defects in glucuronyl transferase activity have been documented in patients with chronic haemolytic disease (Auclair et al., 1976) but it is unlikely that this would be sufficient to cause jaundice if it were not for the marked increase in bilirubin production. Similarly, the shortened erythrocyte life span seen in 50% of patients with Gilbert's syndrome (Powell et al., 1967; Berk and Blaschke, 1972) would not be sufficient to cause jaundice if it were not for a significant reduction in their ability to synthesise bilirubin monoglucuronide.

Analysis of the bile of patients with Gilbert's syndrome and the Crigler-Najjar syndrome has shown that, unlike that found in the normal subject, bilirubin monoglucuronide is the dominant pigment (Feverv et al., 1977; Gordon et al., 1977), which suggests that there may be a second enzyme defect relating to the conversion of monoglucuronide to diglucuronide. The rate of disappearance of a tracer dose of radioactive bilirubin from the plasma is always significantly reduced in patients with unconjugated hyperbilirubinaemia (Berk et al., 1970; Black et al., 1974) and compartmental analysis has indicated that this is due to a defect in hepatic clearance. As the concentration of ligandin in these patients appears to be normal, the possibility exists that there are also abnormalities at the level of the plasma membrane. Further evidence of the heterogeneity of this condition is apparent from the observation that a small proportion of patients have a mild impairment in their ability to eliminate a standard dose of BSP (Berk et al., 1972). A reduced clearance of tolbutamide (Carulli et al., 1976), which does not undergo glucuronidation, has also been reported. The multiplicity of these abnormalities would argue against the suggestion that Gilbert's syndrome is not a real disease (Bailey et al., 1977) and that the hyperbilirubinaemia merely constitutes the upper end of the normal range.

As bilirubin glucuronyl transferase is a microsomal enzyme it is possible to treat patients with a partial deficiency of this enzyme (Crigler-Najjar type II and Gilbert's syndromes) by enzyme induction using drugs such as phenobarbitone and glutethimide (Black et al., 1974; Blaschke et al., 1974) which will cause a significant decrease in their jaundice. Phototherapy also has a similar effect in some patients by degrading the bilirubin in their skin but this is obviously not a practical long-term therapeutic proposition. Another treatment worthy of consideration is enzyme replacement; this has been attempted in Gunn rats with the transplantation of hepatocytes (Matas et al., 1976) and tissue grafting (Foliot et al., 1975) but would present considerable problems in man.

The hyperbilirubinaemia of the Dubin-Johnson and Rotor syndromes is essentially of the conjugated type. Excretory defects for several organic anions, but not for bile acids, have been demonstrated and for some time it was accepted that the two syndromes were variants of the same condition (Aras, 1961), the only significant difference being that, in the Dubin-Johnson syndrome, the liver appeared to be black due to retention of a melanin-like substance. Recent studies have, however, revealed further differences. The abnormality causing BSP retention in the Rotor syndrome appears to be related to a defect in hepatic uptake rather than secretion as originally demonstrated in the Dubin-Johnson syndrome (Abe and Okuda, 1975). Both syndromes have abnormalities in urinary coproporphyrin excretion (Wolff et al., 1976); in the Dubin-Johnson syndrome the total coproporphyrin excretion is slightly reduced or normal, 90% being in the form of isomer I, whereas in the Rotor syndrome the total coproporphyrin is greater than normal but the percentage of coproporphyrin I is only about 60% of the total. It is not yet understood whether there is any relation between canaliculx secretion and porphyrin metabolism or if they are just two independent abnormalities in these syndromes.

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