New insights into the classification and mechanisms of hereditary, chronic, non-haemolytic hyperbilirubinaemias

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SUMMARY Gilbert’s syndrome is typically associated with a deficiency in hepatic bilirubin UDP-glucuronosyltransferase activity (B-GTA). The overproduction of bilirubin that is often found in this condition could be a fortuitous coincidence that leads to the unmasking of the disease, which otherwise often remains latent. Some cases of chronic unconjugated hyperbilirubinaemia could, however, be related to a defect in hepatic uptake, as reflected by alterations in BSP kinetics. Severe deficiencies of hepatic B-GTA exist in all types of Crigler-Najjar disease. An increased proportion of bilirubin monoglucuronide is always found in bile when a B-GTA deficiency is present. This observation strongly suggests a common biochemical defect in Gilbert’s syndrome and in Crigler-Najjar disease, and thus renders the suggestion that the latter condition may be separated into two groups somewhat inappropriate. There is, however, no doubt that further knowledge of the conjugating enzyme, or enzymes, is required: such information may lead to the characterisation of several types of enzymic defects. Whereas little is new as far as the Dubin-Johnson syndrome is concerned, Rotor’s syndrome can no longer be considered to be a variant of the former. The transport defect which is involved in most cases of Rotor’s syndrome, if not in all, is an impairment of hepatic storage, thus distinguishing it from the impairment of excretion which is involved in the Dubin-Johnson syndrome. The distinct patterns of urinary coproporphyrin excretion, which were recently reported in Dubin-Johnson and Rotor’s syndromes, offer additional evidence for a clear differentiation between these two entities.

Hereditary chronic hyperbilirubinaemias are made up of several, often confusing diseases. For some of them at least, however, recent improvement in either techniques or concepts has allowed a re-appraisal of the mechanisms involved, and thus of their classification. Such a classification will be the aim of the present review, which will be restricted to those chronic hyperbilirubinaemias related specifically to the liver—that is, it will not be concerned with those cases explained solely by overproduction of bilirubin such as haemolysis.

Gilbert’s syndrome

When Gilbert first described his syndrome, at the beginning of this century (Gilbert and Lereboullet, 1901), he mixed a number of diseases together. Thus, when he referred to ‘acholuric’ patients, dark urine was present in some of them. He also included several subjects with upper gastrointestinal bleeding and enlargement of either the liver or the spleen. From his post-mortem examinations, it becomes evident that also included in his series were individuals with gallstones and with cholangitis, and so on. Subsequently, it was agreed to consider as Gilbert’s syndrome all cases of adults having chronic, mild, unconjugated hyperbilirubinaemia without overt haemolysis or structural liver disease.

The syndrome appears to be a constitutional disorder, probably inherited as an autosomal, dominant trait (Powell et al., 1967), although this is often far from evident in many cases. Its frequency seems high, as around 5% of the general population may be affected (Kornberg, 1942; Foulk et al., 1959). For obscure reasons, the jaundice usually does not appear before the age of 15 or 20 years. If one believes, however, that most cases reflect a deficiency of the enzyme which conjugates bilirubin, as will be discussed below, it seems likely that the probable regulation of this enzyme by steroid hormones may
explain the role of puberty in unmasking the disease clinically. Such a hypothesis is consistent with the inhibition that various steroids exert on the conjugating enzyme in vitro (Adlard and Lathe, 1971). The increase in jaundice during fasting (Owens and Sherlock, 1973; Kirshenbaum et al., 1976), a situation which also involves complex endocrine changes, may perhaps be subject in part to a similar mechanism; too little is known, however, of the physiological regulation of the system of the hepatic glycosyltransferases for this proposal to be fully evaluated (Fever et al., 1976). The course of this disease is essentially benign; thus attempts to influence it with phenobarbitone (Black and Sherlock, 1970) or other enzyme inducers (Orme et al., 1974) seem not justified as a long-term treatment, even though these substances have a dramatic effect on the jaundice itself.

The diagnosis of Gilbert’s syndrome has long been made on the basis of the exclusion of other diseases, and has therefore depended on the extent of the investigations performed. At present, two different approaches to diagnosis may be emphasised. The first is based on the delayed plasma disappearance rate of exogenously injected bilirubin (Billing et al., 1964), as studied in more detail with trace amounts of $^{14}$C or $^{3}$H-labelled bilirubin (Berk and Blaschke, 1972; Cobelli et al., 1975; Kirshenbaum et al., 1976). This latter procedure has also permitted the confirmation and measurement of the overproduction of bilirubin which is often associated with Gilbert’s syndrome (Foull et al., 1959; Powell et al., 1967; Berk and Blaschke, 1972; Mentre et al., 1974; Berk et al., 1976). Bilirubin kinetics are not, however, performed easily; in addition, they provide no information on the biochemical abnormalities which must be involved.

The second approach to diagnosis is related to the demonstration of markedly decreased bilirubin-UDP-glucuronosyltransferase activity in the liver of patients with Gilbert’s syndrome (Black and Billing, 1969). It apparently offers a satisfactory explanation for the unconjugated hyperbilirubinaemia. However, many difficulties persist, perhaps in part because this enzyme assay, studied in vitro (Van Roy and Heirwegh, 1968; Black et al., 1970; Heirwegh et al., 1973), may not reflect all situations encountered in vivo. There is no correlation between the activity as measured in vitro and the level of bilirubin in the serum (Black and Billing, 1969; Felsher et al., 1973; Mentre et al., 1974); because of the rather wide range in normal values, it remains somewhat artificial to fix their lowest level. Thus, for practical reasons—for example, low activities in small specimens—enzymic activity is usually assayed in a complete homogenate, which may contain inhibitors, and in the presence of detergents which can unmask enzymic sites lacking any physiological importance. Apart from the enzyme which is assayed, there may be a second enzymic system (Jansen et al., 1977) which would specifically involve the formation of bilirubin-diglucuronide. It must also be borne in mind that a deficiency in glucuronidation can be associated with other disorders. It can, for instance, be associated with a diminution in the hepatic uptake of bilirubin; such a diminution might result from the defective enzyme. It may be also associated with bilirubin overproduction, as already stated, which remains unexplained; we can offer the hypothesis that such a haemolysis or dyserythropoiesis, or both, which might represent no more than the extreme degree of the normal situation, may unmask Gilbert’s syndrome when it is associated with a rather low activity of glucuronosyltransferase (Mentre et al., 1974). Finally, Gilbert’s syndrome may even be associated with abnormalities which are apparently not related to bilirubin disposal, such as a high frequency of slow acetylators of drugs (Bircher et al., 1976), or a low hepatic clearance of tolbutamide (Carulli et al., 1976), a compound which does not undergo glucuronidation.

In spite of all these reservations, it remains clear that, when measured, bilirubin-UDP-glucuronosyltransferase activity was low in most adult patients with Gilbert’s syndrome (Black and Billing, 1969; Felsher et al., 1973; Mentre et al., 1977). Its measurement also showed that the frequency of low activity in the general population (3% in a personal series of 33 individuals) is far from that found in unconjugated hyperbilirubinaemia in the absence of overt haemolysis.

Another point which deserves comment is the handling of bromsulphalein (BSP). It is usually normal in adults with unconjugated hyperbilirubinaemia (Dameshek and Singer, 1941); in our patients (Mentre et al., 1977) only three (three brothers) of 39 had a significant reduction in their plasma disappearance rate of BSP. This is apparently at variance with the experience of the group of P. Berk (Martin et al., 1976), who reported that the kinetics of BSP removal were abnormal in 11 of 26 patients with chronic unconjugated hyperbilirubinaemia. These authors pointed out, however, that some bias probably existed in the selection of their patients. The main question which arises from these findings is whether these patients, who had quite abnormal BSP kinetics but in whom glucuronosyltransferase was not measured, were affected by a disease which was distinct from that associated with, or related to, the enzyme deficiency. In this respect, it is of interest that among the three brothers who in our series had abnormal BSP kinetics, glucuronosyl-
transferase activity could be measured in one and was normal. It is tempting to suggest that Gilbert’s syndrome includes at least two distinct entities: the most common is associated with a reduced capacity to conjugate bilirubin, while the other could primarily reflect a defect in the uptake of various cholephilic anions by the hepatocyte.

**Crigler-Najjar disease**

This is an extremely rare condition, which appears very early in life. It is usually admitted that this disease is the consequence of a severe deficiency of the liver’s ability to conjugate bilirubin. The chronic and marked resulting jaundice, due to unconjugated hyperbilirubinaemia, leads to a major potential complication in the newborn: kernicterus (bilirubin encephalopathy), causing severe cerebral damage and possible death. The reason for such a susceptibility of the ‘immature’ brain of the newborn remains unknown. Various factors which increase the risk of kernicterus can, however, be avoided, such as acidosis, hypoalbuminaemia, or the use of drugs able to increase the ratio of free/albumin-bound bilirubin in the serum. This ratio, which unfortunately remains difficult to assay precisely, seems of much greater value in predicting kernicterus than the level of unconjugated bilirubin in the serum by itself.

Arias et al. (1969) suggested that Crigler-Najjar disease should be subdivided into two groups. Patients in group I were most severely affected: the serum bilirubin was 359 to 530 μmol/l (21 to 31 mg/100 ml), the onset of jaundice was always at birth, kernicterus appeared in four out of the five patients described, and none responded to phenobarbitone therapy. In group II, the serum bilirubin ranged from 153 to 290 μmol/l (9 to 17 mg/100 ml), the onset of jaundice was at birth in only seven out of the 11 patients, kernicterus was never observed, and phenobarbitone always affected a dramatic diminution in the jaundice. When measured, UDP glucuronosyltransferase activity with bilirubin as substrate was zero or near zero in all patients, whether of group I or II. The conjugation defect seemed to be transmitted as an autosomal recessive character in group I, and as an autosomal dominant character in group II.

There are, however, at least three arguments which suggest that such separation is difficult and that links may exist between all three varieties of bilirubin conjugating defects—namely, Crigler-Najjar disease group I, Crigler-Najjar disease group II, and Gilbert’s syndrome. The first argument is that clear-cut differences in the modes of inheritance are questionable (Kreek and Sleisenger, 1968; Hunter et al., 1973). Secondly, there are a very few cases of Crigler-Najjar disease described in adults (Gollan et al., 1975), including a personal one (Fevery, Bouvry and Berthelot, unpublished observation) in whom the age, the absence of kernicterus, and a serum bilirubin level of about 256 μmol/l (15 mg/100 ml) strongly suggested that the patient belonged to group II; there was, however, no response to phenobarbitone. Thirdly, analysis of the biliary bilirubin composition (Fevery et al., 1977) showed that the proportion of bilirubin monoconjugate increased progressively from controls to patients with Gilbert’s syndrome, patients with Crigler-Najjar disease group II, and, finally, group I. This strongly suggests that a common biochemical defect, of increasing severity, may lie at the basis of all these types of jaundice.

**Dubin-Johnson syndrome**

Since it was first described (Dubin and Johnson, 1954; Sprinz and Nelson, 1954), this disorder has been shown to be a chronic, or more often intermittent, benign, familial, idiopathic jaundice which is related to a defect in canalicular excretion. This defect concerns a number of organic molecules, such as bilirubin, cholephilic dyes, cholecystographic agents, and porphyrins (Shani et al., 1970b) and has also been found in the mutant Corriedale sheep (Cornelius et al., 1965). In both sheep and man, however, the biliary transport of bile acids remains normal, thus supporting the existence of at least two distinct excretory pathways for organic anions. Serum bilirubin is predominantly conjugated; however, for reasons which are unclear, significant amounts of unconjugated bilirubin may be present. Deposits of a dark pigment, thought to be melanin (Bynum, 1957; Caroli et al., 1965), are found within the liver cell. This liver pigmentation varies in intensity from one patient to the other, and transiently disappears during and after acute viral hepatitis (Varma et al., 1970; Ware et al., 1972). In Israel, the Dubin-Johnson syndrome is associated in a considerable number of patients with a deficiency in clotting factor VII (Shani et al., 1970b); this observation has not been explained. The mode of inheritance is autosomal, recessive (Shani et al., 1970a; Wolkoff et al., 1973). The disease is slightly more common in males; in females, pregnancy or oral contraceptives can unmask the disorder by converting a mild pre-existing, conjugated hyperbilirubinaemia into overt jaundice (Colen et al., 1972).

The kinetics of BSP clearly confirm the excretory defect. Whereas the relative storage capacity (S) is always normal, the transport maximum (Tm) is...
near zero (Shani et al., 1970a). Of a greater practical value, however, is the study of the plasma disappearance curve after a single injection of the dye: (1) the first slope $K_1$ is normal, or only slightly reduced (Chevrel et al., 1969), which is quite unusual in the presence of conjugated hyperbilirubinaemia, and (2) after 45 minutes of injection, a secondary rise in concentration of the dye occurs in the plasma. This rise involves a reflux of conjugated BSP, as shown by chromatography (Charbonnier and Brisbois, 1960; Mandema et al., 1960; Abe and Okuda, 1975), and does not exist when dyes which are not conjugated by the liver, such as dibromsulphalein or indocyanine green, are used (Erlinger et al., 1973). These findings suggest that the reentry of BSP into the plasma is related to its conjugation, the conjugated BSP having less affinity than the parent compound for binding to endoplasmic proteins.

Urinary coproporphyrin abnormalities are a most interesting finding in the Dubin-Johnson syndrome (Ben-Ezzer et al., 1971; Wolkoff et al., 1973; Kondo et al., 1976). The excretion of coproporphyrin I is markedly increased, probably as a result of the defect in its biliary excretion, and is in contrast with the decrease in urinary coproporphyrin III. The consequence of both these changes is a normal, or slightly increased, urinary excretion of total coproporphyrins, with a marked increase in the ratio of isomer I to total coproporphyrins. Values intermediate between those obtained in patients and in normal subjects may be found in phenotypically normal parents or children of the patients (Wolkoff et al., 1973). There is no definite explanation for the decrease in the excretion of isomer III in urine, although it could be due to a reduced production of isomer III secondary to a defect or inhibition of the liver uroporphyrinogen III cosynthetase (Ben-Ezzer et al., 1971). Such an abnormality, if it existed, could yield a diminished synthesis of heme protein which may play a role in the excretory process in the liver (Wolkoff et al., 1973).

Administration of phenobarbitone to patients with the Dubin-Johnson syndrome generally results in a diminution of jaundice and improvement of the Tm of BSP (Shani et al., 1974). However, these effects are much too moderate to be of any therapeutic value, and their precise mechanism remains to be explained.

Rotor's syndrome: uptake and storage disease

The disorder described by Rotor et al. (1948) is a benign, familial, chronic or fluctuating, predominantly conjugated hyperbilirubinaemia without liver pigmentation. It is transmitted as an autosomal recessive character (Wolkoff et al., 1976). For a number of years, Rotor's syndrome has been considered as a variant of the Dubin-Johnson syndrome and related to an excretory defect. Among the arguments favouring this view was the report of two patients in the same family with the Dubin-Johnson syndrome, whereas a third had a Rotor's syndrome (Arias, 1961). In the recent years, however, a few patients were described who resembled those having Rotor's syndrome, but in whom jaundice was attributable to a defect in the hepatic uptake and storage of organic anions, as assessed by the kinetics of cholephilic dyes (Hachouel et al., 1971; Dhumeaux and Berthelot, 1975). As there were only scarce and conflicting data of dye kinetics (Arias, 1961; Dollinger and Brandborg, 1967; Namihisa et al., 1973) in Rotor's syndrome at that time, similarities between this syndrome and the uptake and storage disease were emphasised (Dhumeaux and Berthelot, 1975). The probable identity between both conditions was recently confirmed, when Wolpert et al. (1977) investigated the kinetics of BSP in patients who had earlier been described as suffering from Rotor's syndrome. In these patients, the first slope of the plasma disappearance curve of the dye was impaired and no secondary rise was observed; the S of BSP was dramatically reduced. Their Tm was affected to a lesser extent, possibly as a result of the uptake and storage defect. Abnormalities in $K_1$, $S$, and Tm were found for unconjugated dyes as well as for BSP (Namihisa, 1976; Delage et al., 1977). All these findings suggest that most, if not all, patients with Rotor's syndrome suffer from a primary disorder of uptake and storage of bilirubin and therefore differ from those having a Dubin-Johnson syndrome. Such a distinction is further supported by the different patterns of excretion of urinary coproporphyrins. Urinary total coproporphyrin excretion is much higher in Rotor's syndrome than in that of Dubin-Johnson, and is the result of an increased excretion of isomer I and, to a lesser extent, of isomer III (Wolkoff et al., 1976). The ratio of isomer I to total coproporphyrins is increased, but not as much as in the Dubin-Johnson syndrome (Wolkoff et al., 1976). Finally, the changes observed in urinary coproporphyrin excretion in Rotor's syndrome are similar to those observed in any hepatobiliary disease (Ben-Ezzer et al., 1971) and may be simply explained by a shift from the biliary to the urinary route of excretion.

It remains somewhat surprising that a disease affecting liver uptake and storage of bilirubin leads to a hyperbilirubinaemia which, in large part, consists of conjugated bilirubin. A probable explanation is an increase in the liver to plasma reflux related to
Table  Main features of the different types of hereditary, chronic, non-haemolytic hyperbilirubinaemias

<table>
<thead>
<tr>
<th>Current denomination</th>
<th>Uptake and storage defect*</th>
<th>Impairment of bilirubin conjugation</th>
<th>Excretory (canalicular) defect†</th>
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<tbody>
<tr>
<td>Animal model</td>
<td></td>
<td>Gingil's syndrome (GS)</td>
<td>Dubin-Johnson syndrome</td>
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<td>Mode of transmission</td>
<td></td>
<td>and Crigler-Najjar disease (CND)</td>
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<tr>
<td>Type of hyperbilirubinaemia</td>
<td></td>
<td>Gunn rat</td>
<td>Mutant Corriedale sheep</td>
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<tr>
<td>Bilirubin UDP-glucuronosyltransferase activity</td>
<td></td>
<td>Dominant for GS; uncertain for CND</td>
<td>Recessive</td>
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<tr>
<td>Bromsulphalein (BSP)</td>
<td>K₁</td>
<td>Unconjugated</td>
<td>Unconjugated</td>
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<tr>
<td></td>
<td>S</td>
<td>Normal</td>
<td>Normal or slightly</td>
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<td></td>
<td>Tm</td>
<td>Normal</td>
<td></td>
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<tr>
<td>Urinary coproporphyrins</td>
<td>Total</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isomer I over total</td>
<td>Normal</td>
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</tbody>
</table>

*Uptake and storage defects probably include several distinct entities. The most frequent type, now considered as being Rotor's syndrome, is that depicted in this table. Another one seems to exist (Métreau et al., 1977), which is characterised by entirely unconjugated hyperbilirubinaemia, normal bilirubin UDP-glucuronosyltransferase activity, and marked reduction in the K₁ and S of BSP.

†A similar disorder of canalicular excretion, not however associated with liver pigmentation and therefore differing from the Dubin-Johnson syndrome, has also been described (Arias, 1961).

the disease itself that is, a diminution in the intrahepatic binding of bilirubin. Such a reflux of conjugated bilirubin could be demonstrated with the use of 14C-labelled bilirubin in the mutant Southdown sheep, which also suffers from a similar impairment of uptake and storage (Cornelius and Gronwall, 1968; Mia et al., 1970). It is of interest that the intrahepatic content of ligandin seems to be normal in this animal (Arias, 1972), thus suggesting that this protein is not obligatorily involved in the pathogenesis of the disorder. This is also supported by the fact that phenobarbitone, which among many other actions increases the hepatic content of ligandin (Arias, 1972), influenced neither serum bilirubin, nor BSP K₁ when given to three patients (Delage and DhumEAUX, unpublished observations).

Concluding remarks

Considerable improvement has recently been achieved in defining the localisation of the hepatic defect involved in the various chronic, hereditary, non-haemolytic, hyperbilirubinaemias (Table). This has, for instance, allowed us to reconsider Rotor's syndrome, which in most cases appears as an uptake and storage disease, whereas for years it was considered to be a variant of the Dubin-Johnson syndrome—that is, an excretory defect. Furthermore, it has also allowed us to consider that a deficiency in bilirubin conjugation may explain most cases of unconjugated, chronic hyperbilirubinaemia in the adult in the absence of overt haemolysis. However, apart from this latter example, too little is known of the biochemical basis of the different types of hereditary hyperbilirubinaemias. Until more information is available, it will remain difficult to eliminate the heterogeneity which still exists among many of the various syndromes which have been described in the present review.

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