Role of bilirubin overproduction in revealing Gilbert's syndrome: is dyserythropoiesis an important factor?¹

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SUMMARY Gilbert's syndrome was diagnosed in 37 patients with unconjugated hyperbilirubinaemia without overt haemolysis or structural liver abnormality, who had a marked reduction in hepatic bilirubin UDP-glucuronosyltransferase activity (B-GTA) (as compared with that of 23 normal subjects). No significant correlation existed in these patients between serum bilirubin level and the values of B-GTA, thus suggesting that factors other than a low B-GTA must influence the degree of hyperbilirubinaemia in Gilbert's syndrome. Studies of ⁵¹Cr erythrocyte survival and ⁵⁹Fe kinetics in 10 unselected patients demonstrated slight haemolysis in eight, whereas mild ineffective erythropoiesis was suggested in all from a low 24-hour incorporation of radioactive iron into circulating red cells. This overproduction of bilirubin resulting from mild haemolysis and perhaps dyserythropoiesis might reflect only an extreme degree of the normal situation. It certainly contributes to the hyperbilirubinaemia of Gilbert's syndrome and may play a major role in the manifestation of this condition.

Gilbert's syndrome is a fairly common condition characterised by a chronic, mild, familial, benign unconjugated hyperbilirubinaemia without overt haemolysis or structural liver disease (Foulk et al., 1959; Arias, 1962). Although its precise cause is still debated (Berk et al., 1974), several publications have pointed out its association with a deficiency in hepatic bilirubin UDP-glucuronosyltransferase activity (B-GTA) (Black and Billing, 1969; Black et al., 1970; Felsher et al., 1973; Felsher and Carpio, 1975; Kutz et al., 1975). In about half of the patients with Gilbert's syndrome, a mild reduction of ⁵¹Cr red cell survival has been reported (Foulk et al., 1959; Powell et al., 1967a, 1967b; Berk and Blaschke, 1972); this slight haemolysis, which is insufficient per se to yield hyperbilirubinaemia (Foulk et al., 1959; Powell et al., 1967b; Berk and Blaschke, 1972), remains unexplained, and the link between this bilirubin overproduction and the deficiency in B-GTA is still poorly understood. The aim of this study is to demonstrate that not only haemolysis but also dyserythropoiesis is quite frequent in Gilbert's syndrome; it is suggested that the ensuing bilirubin overproduction plays an important role in revealing this condition.

Methods

Patients

During the last five years, Gilbert's syndrome was found in 37 patients (mean age 32 years, range 13-65 years). There were 24 males and 13 females. Twenty-three patients were referred to our department for persistent unconjugated hyperbilirubinaemia. Five subjects were investigated for chronic abdominal pain. In the remaining nine patients, unconjugated hyperbilirubinaemia was detected by systematic screening for liver disease. Physical examination was always normal except for mild jaundice in some patients. Apart from the increase in serum unconjugated bilirubin, routine liver function tests were normal: these included serum


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Table 1  Haematological findings in 37 patients with Gilbert’s syndrome

<table>
<thead>
<tr>
<th></th>
<th>Red blood cells per µl (× 10^6) (n = 37)</th>
<th>Haemoglobin g/dl (n = 37)</th>
<th>Reticulocytes per µl (n = 37)</th>
<th>Serum haptoglobin g/l (mg/100 ml)† (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>4.55 ± 0.45</td>
<td>13.7 ± 1.8</td>
<td>71 ± 600</td>
<td>(5-60 ± 2-70) 560 ± 270</td>
</tr>
<tr>
<td>Range</td>
<td>3.30 - 5.70</td>
<td>11.7 ± 1.61</td>
<td>8000 - 214 000*</td>
<td>(3-00 ± 12-00) 300 ± 1200</td>
</tr>
</tbody>
</table>

*Only one patient had a reticulocyte count over 150 000.
†Immunoprecipitation technique: normal values: 4-00 to 16-00 g/l (400 to 1 600 mg/100 ml).

protein electrophoresis, alkaline phosphatase, alanine transaminase, and prothrombin time. Gilbert’s syndrome was diagnosed on the following basis: (1) unconjugated hyperbilirubinaemia over 1.0 mg/100 ml (17.1 μmol/l) at least once (1.8 ± 1.2 mg/100 ml (30.8 ± 20.5 μmol/l), mean ± 1 SD); (2) absence of overt haemolysis and dyserythropoiesis (no anaemia) in all patients (routine haematological investigations are shown in Table 1); (3) normal liver histology; (4) a B-GTA below 0.7 mg (1.19 μmol) of bilirubin conjugated per hour per g of liver; the mean value of B-GTA in our patients was 0.25 ± 0.17 mg (0.43 ± 0.29 μmol) the normal value of our laboratory being 1.33 ± 0.44 mg (2.27 ± 0.75 μmol).

Serum total and conjugated bilirubin concentrations were determined by the method of Jendrassik and Gröf as modified by Nosslin (Nosslin, 1960). The first slope (K₁) of the plasma disappearance rate of bromsulphalein (BSP) (5 mg per kg of body weight) was estimated in 36 patients according to the technique described by Fauvert (1959), from blood samples taken at four, eight, 12, and 16 minutes after the BSP injection. In nine of these patients, the plasma concentration of BSP was also determined 45 minutes after the injection.

Percutaneous needle liver biopsy specimens were obtained from all patients after a 12-hour fast. Liver B-GTA was determined according to the method of Black et al. (1970). The hepatic protein concentration was measured by the procedure of Lowry (Lowry et al., 1951). B-GTA was also determined from hepatic tissue obtained by percutaneous needle liver biopsies in 23 control subjects who were investigated for possible liver disease, but in whom a normal liver was found on clinical, biochemical, and histological grounds. Neither the controls nor the patients with Gilbert’s syndrome took any drug known to be a microsomal enzyme inducer (Conney, 1967). Finally, B-GTA was measured in eight patients having overt overproduction of bilirubin, five with haemolysis (spherocytosis in one, thalassaemia minor in three, ovalocytosis in one), and three with acquired dyserythropoiesis of unknown cause.

In 10 unselected patients with Gilbert’s syndrome out of our group of 37 the following investigations were performed: (1) Bone marrow was examined. (2) The red cell survival was estimated according to the method of Gray and Sterling (1950), using the patient’s own erythrocytes labelled in vitro with 51Cr. (3) Iron kinetics were studied after an intravenous injection of 0.3 μCi per kg of body weight of 59Fe bound to transferrin, according to the method of Najean et al. (1965), without any modification. Blood samples were taken every hour during the first eight hours, then every day for 15 days after the injection of the labelled iron. The complete iron plasma disappearance curve comprises three exponentials. The 59Fe plasma T 1/2 was calculated from the first exponential after curve stripping (Najean et al., 1969). From this value, that of plasma iron, the iron plasma turnover was calculated. The rate of incorporation of iron into circulating red cells was also determined. Finally, the release of the radioactive iron from the sacrum was estimated by external counting during the hour after the injection, then every day for two weeks. Radioactivity over heart, liver, and spleen was also determined during this period.

The statistical calculations were made according to standard methods (Snedecor and Cochran, 1967) including Student’s t test. The results are all expressed as mean ± 1 standard deviation (SD).

Results

No significant correlation was found between B-GTA and serum total bilirubin (r = 0.29) (Figure) in the 37 patients with Gilbert’s syndrome, even when a semilogarithmic plot was used. The values of B-GTA in the eight patients with overt haemolysis or dyserythropoiesis were within normal limits, except for one patient with thalassaemia minor and another one with ovalocytosis (Table 2).

The mean value for BSP K₁ in the patients with Gilbert’s syndrome was 0.133 ± 0.032 which did not differ significantly from the normal value of 0.145 ± 0.017 (Fauvert, 1959) or 0.14 ± 0.02 (Berk et al., 1972) reported in the literature. A slight reduction in BSP K₁ was observed in only two patients whose values were 0.085 and 0.095. The 45-minute retention was 3.3 ± 0.9% (range 2.2 to 4.5%), which is within the normal value of
Table 2  Bilirubin UDP-glucuronosyltransferase activities in eight patients with overt bilirubin overproduction

<table>
<thead>
<tr>
<th></th>
<th>mg bilirubin conjugated/g liver/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassaemia minor</td>
<td>2.57</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
</tr>
<tr>
<td>Spherocytosis</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>1.17</td>
</tr>
<tr>
<td>Ovalocytosis</td>
<td>0.09</td>
</tr>
<tr>
<td>Normal values</td>
<td>1.33 ± 0.44</td>
</tr>
</tbody>
</table>

Table 3  Red cell half-life and percentage of erythroblasts in bone marrow in 10 unselected patients with Gilbert's syndrome

<table>
<thead>
<tr>
<th>Case no.</th>
<th>&quot;^1&quot;Cr red cell half-life (days)</th>
<th>Erythroblasts in bone marrow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>Normal*</td>
<td>Values 25 – 33</td>
<td>6 – 30</td>
</tr>
</tbody>
</table>

*From Wintrobe et al. (1974).

less than 5% (Combes and Schenker, 1969; Blaschke et al., 1974).

The red cell survival and the percentage of erythroblasts in bone marrow from the 10 individuals with Gilbert's syndrome who were carefully investigated for haematological disorders are shown in Table 3. Two patients (cases 4 and 8) had a normal red cell survival, whereas the remaining eight subjects had a shortened erythrocyte half-life, with an increased destruction in the spleen and/or the liver. In addition, the number of erythroblasts in bone marrow was increased in six of the 10 patients. None had any of the cytological abnormalities sometimes reported in dyserythropoiesis (Verwilghen et al., 1973). The results of labelled iron kinetics are shown in Table 4: the plasma iron turnover was increased in eight patients and normal in two (cases 2 and 5); serum iron was normal in every case. The maximum rate of incorporation of radioactive iron into circulating red cells was always normal; however, the erythrocyte incorporation measured at 24 hours was low in all patients including cases 2 and 5 (Table 4); in addition, the half red cell incorporation time was increased: 3.5 ± 0.3 days (normal 2.8 ± 0.3 according to Faille et al., 1972) (p < 0.001). The release of radioactive iron over the sacrum was slow and incomplete in every patient. In our patients sacrum activity decreased by 50% in a mean of four days instead of three days in normal subjects (Najean

Figure  Relation between serum total bilirubin and hepatic bilirubin UDP-glucuronosyltransferase activity (B-GTA) in 37 patients with Gilbert's syndrome. Serum bilirubin values are those which were measured on the same day as B-GTA; all the five patients having on that day ≤ 10 mg/100 ml had higher concentrations on other occasions. (SI units for serum bilirubin: × 17.1.)
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Table 4  Radioactive iron kinetics in 10 unselected patients with Gilbert’s syndrome

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Serum iron (mg/dl)</th>
<th>Iron turnover (mg/kg/day)</th>
<th>**Fe incorporation in circulating red cells (% of injected activity)</th>
<th>Half red cell incorporation time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rate 24 h after injection</td>
<td>Maximum rate (11-15 days)</td>
</tr>
<tr>
<td>1</td>
<td>0.85</td>
<td>0.61</td>
<td>5.7</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>1.05</td>
<td>0.34</td>
<td>1.7</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>0.54</td>
<td>4.4</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>0.61</td>
<td>6.3</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>1.60</td>
<td>0.31</td>
<td>0.9</td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>0.95</td>
<td>1.53</td>
<td>2.7</td>
<td>76</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>1.02</td>
<td>2.8</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>1.05</td>
<td>0.65</td>
<td>2.2</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>1.00</td>
<td>0.78</td>
<td>3.0</td>
<td>86</td>
</tr>
<tr>
<td>10</td>
<td>1.05</td>
<td>0.61</td>
<td>3.4</td>
<td>85</td>
</tr>
</tbody>
</table>

Mean ± SD 1.07 ± 0.13 0.70 ± 0.34 3.4 ± 1.6 87 ± 7 3.5 ± 0.3
Normal values 0.80 – 1.00 0.34 – 0.48† > 10;‡ 81 ± 5† 2.8 ± 0.3†

Individual values of plasma iron turnover, iron incorporation rate into circulating red cells 24 hours after the injection, maximum rate of iron incorporation and half red cell incorporation time
†From Najean et al. (1969).
‡From Faille et al. (1972).

et al., 1969). There was no iron storage into liver or spleen.

Discussion

Although Gilbert’s syndrome was described more than 70 years ago (Gilbert and Lereboullet, 1901), its primary mechanism remains obscure. Since it became possible to determine B-GTA from a liver needle biopsy specimen, using bilirubin as substrate (Black and Billing, 1969; Black et al., 1970), this enzymatic activity, when assayed, was extremely low in every case of Gilbert’s syndrome (Black and Billing, 1969; Black et al., 1970; Felsher et al., 1973; Felsher and Carpio, 1975; Kutz et al., 1975). In our experience, such a deficiency was a constant finding in any adult patient having unconjugated hyperbiliarubinaemia, a normal BSP test and no overt haemolysis or dyserythropoiesis. The absence of significant negative correlation between B-GTA and serum bilirubin, as found in this study, also existed in two smaller series of respectively 11 (Black and Billing, 1969) and 16 patients (Felsher et al., 1973). This result strongly suggests that the low B-GTA is not the sole mechanism of jaundice in Gilbert’s syndrome and that one or more other factor(s) could contribute to the hyperbiliarubinaemia.

Theoretically, two additional factors could be involved: (1) an abnormal uptake of unconjugated bilirubin by the liver cell; and (2) a hyperproduction of endogenous bilirubin. As regards the first possibility, several authors postulated that the jaundice in Gilbert’s syndrome was partly the consequence of an impairment in bilirubin uptake (Schmid and Hammaker, 1959; Billing et al., 1964; Berk et al., 1970; Martin et al., 1976). Such a hypothesis was derived from compartmental analysis of bilirubin disposition (Billing et al., 1964; Berk et al., 1970) in which the reduced hepatic clearance of the pigment (Billing et al., 1964; Berk and Blaschke, 1972; Berk et al., 1970, 1974; Black et al., 1974; Frezza et al., 1973; Martin et al., 1976) resulted in part from a decrease of the fractional transfer rate of bilirubin from the plasma to the liver cell (Billing et al., 1964; Berk et al., 1970). However, the precise disposition of bilirubin within the hepatocyte, in particular the transport of the pigment between the plasma liver membrane and the endoplasmic reticulum, remains unknown. Obviously, links must exist between the various steps which are involved and, conceivably, as suggested by Black et al. (1974) an impairment of the conjugation of bilirubin could reduce the rate of its uptake. Such an uptake defect in Gilbert’s syndrome has also been suggested by abnormalities of the kinetics of cholephilic anions other than bilirubin. However, to the best of our knowledge, such abnormalities have been reported by only one group (Martin et al., 1976), which found an abnormal BSP test in 11 out of the 26 patients. In our series, BSP K1 was normal in all the patients but two. A similar finding was previously reported (Dameshek and Singer, 1941).

If other factors than a low B-GTA contribute to unconjugated hyperbiliarubinaemia in Gilbert’s syndrome, the role of bilirubin overproduction must be considered. In all our 37 patients, overt haemolysis and dyserythropoiesis were excluded. However, when 10 unselected patients of this series were studied in detail, diminution of erythrocyte half-life was found in eight of them, together with an increase in plasma iron turnover. Such mild haemoly-
osis was associated, in all the 10 patients, with a defect in the 24-hour incorporation of iron into the erythrocytes and a slow and incomplete release of radioactive iron from the sacrum. This would suggest that some dyserythropoiesis existed in every case in spite of the normal maximum rate of incorporation of labelled iron into circulating red cells. Several reports already mentioned a slight reduction in red cell span in about half of the patients with Gilbert’s syndrome (Foulk et al., 1959; Powell et al., 1967a, b; Berk et al., 1972), but, even if the mechanism of this abnormality has not been elucidated yet, it has been clearly shown that this haemolysis alone was never sufficient to explain the degree of unconjugated hyperbilirubinaemia (Foulk et al., 1959; Powell et al., 1967b; Berk et al., 1972).

In the present work, we also suggest the frequency of some dyserythropoiesis, another cause of bilirubin overproduction, which has not yet been reported in Gilbert’s syndrome.

Our results are not in agreement with those of Berk et al. (1976), who found by an indirect method that the proportion of bilirubin derived from sources other than senescent circulating red cells was not different in patients with Gilbert’s syndrome and in patients with normal hepatic bilirubin transport (Berk et al., 1976). However, the proportion of the bilirubin derived from these sources is very small and it is questionable if the method used by Berk et al. would be sensitive enough to detect the mild overproduction of bilirubin.

The cause of the association between Gilbert’s syndrome and bilirubin overproduction is still obscure. It is unlikely that unconjugated hyperbilirubinaemia per se might be responsible for the haematological disorders: no haemolysis has been found in Crigler-Najjar syndrome, a most severe unconjugated hyperbilirubinaemia (Arias et al., 1969; Blaschke et al., 1974). Similarly, it is not conceivable that haemolysis and/or dyserythropoiesis might result in B-GTA deficiency for the following reasons: the mean values of B-GTA were not lower, but were actually higher in 11 patients with sickle cell anaemia than in the control subjects (Maddrey et al., 1974). Although Auclair et al. (1976) found a marked deficiency of B-GTA in 14 out of 20 patients with haemolytic anaemia, such a defect was found in only two out of 11 patients by Felsher and Carpio (1975); in addition, in the present work, out of eight individuals with overt haemolysis or dyserythropoiesis, only two had a low B-GTA (Table 2). Consequently, the most likely explanation is that the low B-GTA and bilirubin overproduction in Gilbert’s syndrome are associated but there is no causal relationship. However, it can be postulated that bilirubin overproduction is a determinant in revealing Gilbert’s syndrome which, in its absence, would remain latent. This hypothesis is supported by the following finding: among 26 patients presumed to have normal liver, systematic determination of B-GTA in our laboratory revealed normal activity in 23, whereas in three of them the values were 0·27, 0·34, and 0·49 mg bilirubin conjugated/g liver/h—that is, of the same order of magnitude as those found in Gilbert’s syndrome. A similar proportion of very low values for B-GTA was found in a surgical population of otherwise normal individuals (Black et al., 1973). This indicates that B-GTA deficiency occurs relatively frequently in the general population and shows that this disorder can remain undetected.

Dyserythropoiesis, which accounts for approximately 10% of bilirubin production (Berk et al., 1974), is a physiological phenomenon, the importance of which is probably quite variable from one subject to another. In fact, the dyserythropoiesis in our patients was never severe, thus suggesting that it should not be considered as an additional disease, but is more likely to be an extreme degree of the normal situation. The association, however, of any kind of bilirubin overproduction with low B-GTA must be an important condition in the manifestation of Gilbert’s syndrome.

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