Liver ultrastructure in Gilbert’s syndrome

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SUMMARY Electron microscopy of hepatic tissue obtained by percutaneous needle biopsy from nine patients with Gilbert’s syndrome has revealed in every case gross hypertrophy of hepatocyte agranular endoplasmic reticulum but no other important abnormality. While this may have relevance to impairment of microsomal enzyme activity controlling bilirubin conjugation within liver cells, the serum bilirubin levels in all nine patients were below that normally associated with demonstrable UDP-glucuronyl transferase deficiency. Gross hypertrophy of agranular endoplasmic reticulum may be, therefore, a constant feature of this form of Gilbert’s syndrome and may have some diagnostic value in the investigation of unconjugated hyperbilirubinaemia.

Gilbert’s syndrome, or idiopathic unconjugated hyperbilirubinaemia, was first described by Gilbert and Lereboullet (1901). It is characterized by a defective hepatic clearance of bilirubin (Billing, Williams, and Richards, 1964) and, in some cases, decreased activity of the enzyme bilirubin uridine diphosphate glucuronyl transferase which conjugates bilirubin (Black and Billing, 1969). The diagnosis is usually made by excluding other causes of recurrent bouts of jaundice and by the finding of a raised serum level of unconjugated bilirubin in the presence of a normal level of conjugated bilirubin. Some assistance in diagnosis may be obtained from the reduced caloric intake test (Owens and Sherlock, 1973), but a specific positive diagnostic finding would be of great value.

Light microscopy of the liver reveals no structural abnormality and this is regarded as an essential component of the syndrome. It might be expected, however, that electron microscopy would reveal some cause or consequence of failure of the liver to absorb or conjugate bilirubin (Berk, Bloomer, Howe, and Berlin, 1970). There are indeed several published reports usually based on a few cases only, and presenting a variety of inconsistent findings such as prominence of pigment inclusions in pericanalicular lysosomes (Magenat and Paluello, 1967; Barth, Grimley, Berk, Bloomer, and Howe, 1971). Sagild,

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<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum Bilirubin (μ moles/l)</th>
<th>SGOT* (μl)</th>
<th>SGPT* (μl)</th>
<th>Alkaline Phosphatase (KA units)</th>
<th>Albumin (g/l)</th>
<th>Globulin (g/l)</th>
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<td>144*</td>
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</table>

Table I Results of liver function tests in nine patients with Gilbert’s syndrome at time of liver biopsy

1Serum bilirubin—upper limit of normal 20 μ moles/l.
2SGOT—upper limit of normal 42 μl (g), 38 μl (g).
3SGPT—upper limit of normal 55 μl (g), 41 μl (g).
4μl/l—upper limit of normal 250 μl/l.

*This patient had two episodes of jaundice before biopsy. During one episode (two weeks before biopsy) total serum bilirubin was 56-4 μ moles/l and conjugated bilirubin 10-2 μ moles/l; other liver function tests at that time were normal.

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Dalgaard, and Tystrup (1962) regard this as similar to the pigment which is present in great excess in the liver in the Dubin-Johnson syndrome although its nature is more likely to be non-specific (Feldman, Oudéa, Domart-Oudéa, Molas, and Fauvert, 1968). Magnenat and Paluello describe mitochondrial degenerative changes and damage to hepatocyte plasma membranes with loss of villi which might impair bilirubin absorption. Simon and Varonier (1963) likewise note damage to these structures and to the sinusoidal lining cells.

A few authors describe an increase in agranular endoplasmic reticulum (AER) sometimes associated with a reduction in the rough variety and an accumulation of glycogen (Novikoff and Essner, 1960; Simon and Varonier, 1963; Schaff, Lapis, and Sáfrány, 1969). In some other reports this feature is illustrated although not mentioned in the text. The present series consists of nine patients in all of whom excess agranular endoplasmic reticulum was the only consistent and notable feature of hepatocyte ultrastructure. While there are other well known causes of this phenomenon its presence may be a characteristic feature of Gilbert's syndrome.

Materials and Methods

All nine cases were young adults of both sexes admitted to Glasgow Royal Infirmary for investigation of jaundice during 1971-1974. Four of the patients had no symptoms in association with the episodes of jaundice. Three complained of nausea, tiredness, and headache, and two patients had abdominal discomfort. All the patients gave a history of two or more previous episodes of jaundice. The liver function tests at the time of the hepatic biopsy are shown in table 1.

Percutaneous biopsy of liver was carried out using a Menghini needle. Small portions of liver from each end of the tissue core were fixed without delay in ice-cold 2.5% glutaraldehyde and cacodylate buffer and processed for ultrastructural studies by standard methods. The remainder of each specimen was passed to paraffin wax for light microscopy. Electron microscopy was confined to the midzonal portion of the liver lobule. All parts of the ultrathin section were photographed and a rough quantitation of the amount of agranular endoplasmic reticulum obtained by a spot-counting technique. Photographic prints from at least 16 different fields from each specimen were examined, each at a magnification of 24,500. These were overlaid with a 1 cm² grid providing 396 spots per print. For comparison, liver biopsies from four patients without any clinical, biochemical, or morphological evidence of liver dysfunction were examined in a similar fashion.

Results

Light microscopy of all liver specimens revealed no abnormality. In particular there was no haemosiderin or visible bile pigment. Lipochrome pigment in centrilobular zones was rarely prominent and never more than that sometimes seen in liver biopsies from patients with no disorder of bilirubin metabolism.

The same was true of the electron microscopic findings. No case showed pigmented bodies in excessive numbers and there were none of the degenerative features in plasma membranes, mitochondria, or sinusoidal cells described in some other reports. There was no damage to rough endoplasmic reticulum although the amount was apparently reduced in some hepatocytes. In every case agranular endoplasmic reticulum was very prominent and fairly uniform between the individual parenchymal cells (figs 1 and 2). In only one of the nine cases was there any conspicuous dilatation of these vesicular structures; this was probably due to some delay in fixing the tissue for electron microscopy. The quantitative increase in agranular endoplasmic reticulum in these cases compared with the specimens of normal liver is very marked (table II) and does not require statistical analysis, there being a three- to nine-fold difference between the two groups.

Discussion

Hypertrophy of agranular endoplasmic reticulum is known to be induced by alcohol, and in our experience changes similar to those noted in our series of Gilbert's syndrome patients have been found in some but not all patients with alcoholic liver damage. None of our patients were alcoholic and none were addicted to phenobarbitone or other drugs which are also known to cause hypertrophy of agranular endoplasmic reticulum.

In conditions of severe glucuronyl transferase deficiency, such as one form of the Crigler-Najjar syndrome and in Gunn rats, hepatocytes show similar hypertrophy of agranular endoplasmic reticulum. This might be regarded as structural evidence of attempts to provide additional microsomal enzyme for bilirubin conjugation, but the reason is not clear, especially as a similar change is sometimes found in hepatocytes of cases of Dubin-Johnson syndrome which have no defect of bilirubin conjugation (Toker and Trevino, 1965; Muscatello, Mussini, and Agnolucci, 1967). A similar but less severe enzyme defect can be demonstrated in some cases of Gilbert's syndrome (Black and Billing, 1969). According to Arias (1962; 1966), these cases have unconjugated bilirubin levels in the
Fig 1  Electron micrograph of liver in Gilbert's syndrome (case 4) showing portions of the cytoplasm of five hepatocytes. The numerous small vesicles represent uniform hyperplasia of agranular endoplasmic reticulum $\times$ 8400
c, collagen; l, lipid; m, mitochondria; p, pigment bodies.
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Fig. 2a

Electron micrograph of hepatocyte cytoplasm in Gilbert's syndrome (case 3) shown in fig 2a and compared with a similar preparation from a normal control specimen at the same magnification shown in fig 2b. The latter contains glycogen granules and has much less agranular endoplasmic reticulum. a, AER; g, glycogen; m, mitochondria; p, pigment body.

Fig. 2b
<table>
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<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Agranular Endoplasmic Reticulum (points counted)</th>
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<tr>
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<tr>
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</table>

Table II. Quantity of agranular endoplasmic reticulum in hepatocytes of nine cases of Gilbert's syndrome and four normal controls assessed by point counting

1Agranular endoplasmic reticulum vesicles were counted in electron micrograph prints at a magnification of 24,500. Each figure is the mean value from 16 prints of different parts of the section examined.

Milder degrees of hyperbilirubinaemia may occur in association with a variety of infections and metabolic disorders (Levine and Klatskin, 1964), but all our patients appeared healthy apart from the episodes of jaundice. However, it would be difficult to exclude with certainty mild postviral hepatic unconjugated hyperbilirubinaemia (Foulk, Butt, Owen, Whitcomb, and Mason, 1959). It has been postulated that there is a defect in the transfer of unconjugated bilirubin from blood to liver in Gilbert’s syndrome and this occurring alone could account for the levels of serum bilirubin as were found in our patients (Billing et al, 1964). There are no ultrastructural changes in the liver which could readily support this hypothesis unless hypertrophy of agranular endoplasmic reticulum represents in these cases some compensatory mechanism responsible for bilirubin transport. Whatever the explanation it would appear that this ultrastructural feature may be part of the syndrome and could have some diagnostic significance in the investigation of doubtful cases of unconjugated hyperbilirubinaemia.

References


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