Journal of Medical Genetics (1975). **12**, 152.

Population studies on Gilbert's syndrome

D. OWENS and J. EVANS

Nuffield Unit of Medical Genetics, University of Liverpool, Crown Street, Liverpool L69 3BX

Summary. Total serum bilirubin concentration was measured by an Auto-analyzer technique in 197 normal males and 102 normal females. The mean bilirubin concentration was significantly lower in the females than in the males. Total bilirubin concentration in the males showed a bimodal distribution with an antimode at 24 μmol/l (1.4 mg/100 ml). Individuals with bilirubin concentration above this value had unconjugated hyperbilirubinaemia and probable Gilbert's syndrome. Total bilirubin concentration in the females again showed a bimodal distribution with an antimode at 12 μmol/l (0.7 mg/100 ml). It is conceivable that females with bilirubin levels above this also have Gilbert's syndrome. This suggests that the population incidence of Gilbert's syndrome could be as high as 6% and that the sex incidence is approximately equal.

Gilbert's syndrome, first described in 1901 (Gilbert and Lereboullet, 1901), is a benign condition in which unconjugated hyperbilirubinaemia occurs in the absence of structural liver disease and overt haemolysis. The plasma concentration of conjugated bilirubin is normal. Unconjugated hyperbilirubinaemia occurs as a result of decreased hepatic bilirubin clearance (Billing, Williams, and Richards, 1964; Berk *et al*, 1970) which is probably secondary to decreased hepatic activity of bilirubin uridine diphosphate glucuronyl transferase, the microsomal enzyme which catalyses the conjugation of bilirubin with glucuronic acid (Black and Billing, 1969). Studies of hepatic bilirubin clearance also suggest that the hepatic uptake of unconjugated bilirubin may be defective in this condition (Biling *et al*, 1964; Berk *et al*, 1970).

Powell, Billing, and Williams (1967a) have shown that approximately half of the patients with Gilbert's syndrome have a slightly reduced red cell life span as measured by both diisopropylfluorophosphate and radiochromium techniques. However, they calculated that although this contributed towards the unconjugated hyperbilirubinaemia it was insufficient to explain the degree of hyperbilirubinaemia encountered. These findings have since been confirmed by Berk *et al* (1970).

More recently, it has been shown that some patients with Gilbert's syndrome have abnormal hepatic clearance of the dye bromsulphthalein. The reason for this is not yet clear (Berk, Blaschke, and Waggoner, 1972).

The familial nature of this condition was first recognized by Gilbert and Lereboullet (1901) who observed mild jaundice without bilirubinuria in individual members of the families of propositi. Powell *et al* (1967b) in their study of the families of 42 propositi with Gilbert's syndrome suggested that the condition was inherited as an autosomal dominant trait with incomplete penetrance. Only 27.5% of the sibs and 16.2% of the parents had Gilbert's syndrome. The ratio of affected males to females in this study was approximately 4 to 1.

The population incidence of this condition is unknown but Billing (1970) states that it may affect as many as 1 in 200 males. Using an abnormal bilirubin loading test as his diagnostic criterion, Kornberg (1942), however, found seven probable but previously unsuspected cases among 100 medical students.

In order to determine the population incidence of Gilbert's syndrome, serum bilirubin concentration was measured in 299 normal subjects (197 males and 102 females).

Subjects and methods

The total serum bilirubin concentration was measured on one occasion in 252 healthy National Blood Transfusion Service donors and in 47 healthy medical student

Received 3 June 1974.

volunteers. Bilirubin concentration was measured using an Autoanalyzer technique (Simmons, 1968). When the bilirubin concentration was above 20.5 μmol/l (1.2 mg/100 ml) the levels of unconjugated and conjugated bilirubin were estimated manually using the method of Michaëlsson, Nosslin, and Sjölin (1965). Bilirubin standards, purchased from Wellcome Reagents, Ltd or made up according to Billing, Haslam, and Wald (1971), were estimated with each batch of unknowns.

None of the subjects studied gave a history of previous jaundice or viral hepatitis and none were anaemic. Each donor was asked to return after eating if he or she had presented in the fasting state. It is known that bilirubin concentration tends to be higher in fasting individuals or when the caloric intake is reduced (Owens and Sherlock, 1973).

It has been claimed that the rise in unconjugated bilirubin concentration on reducing the caloric intake distinguishes Gilbert's syndrome from other causes of unconjugated hyperbilirubinaemia (Owens and Sherlock, 1973). For this reason, the serum bilirubin concentration was also estimated after a 12-hour fast in the 47 medical students.

Results

Figure 1 shows the distribution of total serum bilirubin concentration in the 299 subjects studied (252 blood donors and 47 medical student volunteers). It can be seen that the histogram shows skew distribution with a suggestion that it is bimodal. The bimodal distribution is more obvious when the logarithms of the bilirubin concentrations are used to construct the histogram (Fig. 2). The antimode corresponds to a total bilirubin concentration of 24 μmol/l (1.4 μg/100 ml). It can be seen that several individuals have bilirubin concentrations above the usually accepted upper limit of normal of 13.7 μmol/l (0.8 mg/100 ml). The bimodal distribution of total serum bilirubin concentration was confirmed by plotting the percentage cumulative frequency distribution on probability paper (Fig. 3). This shows that two populations are

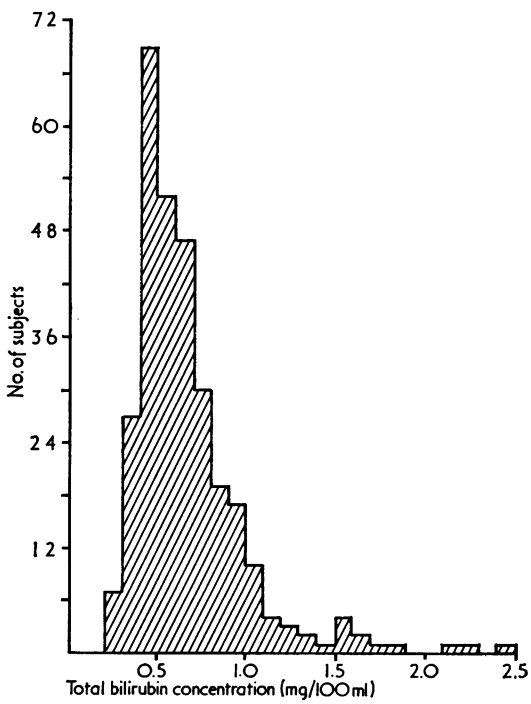

FIG. 1. Distribution of total serum bilirubin concentration in the 299 subjects studied. **Conversion**: traditional to SI units. Total serum bilirubin: 1 mg/100 ml ≃ 17.1 μmol/l.

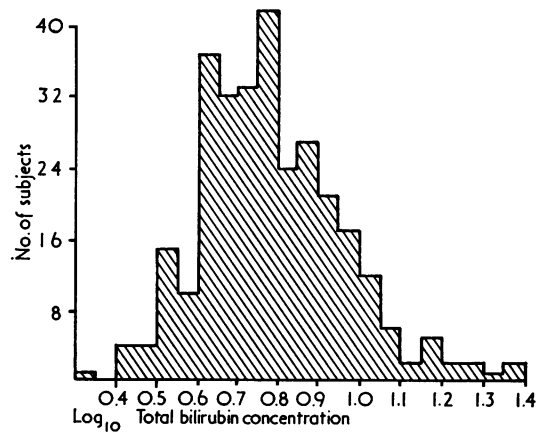

FIG. 2. Distribution of the logarithms of the total serum bilirubin concentration in the 299 subjects studied.

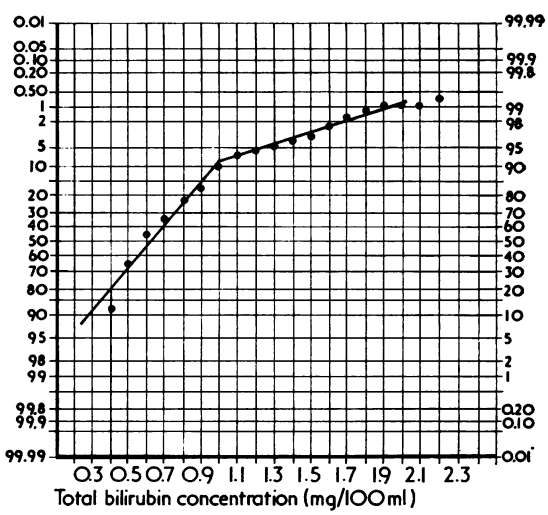

FIG. 3. Percentage cumulative frequency distribution of total serum bilirubin concentration in the 299 subjects studied.

present indicated by the unequivocal inflection in the line. The minor population with bilirubin concentration above 24 μmol/l accounts for approximately 3% of the total population. With one exception, all these subjects were male, and all had unconjugated hyperbilirubinaemia with normal conjugated bilirubin concentrations.

Figure 4 shows the distribution of total bilirubin concentration in the 197 male and 102 female subjects analysed separately. The mean concentration in the females (8.9 ± 3.8 μmol/l [0.52 ± 0.22 mg/100 ml], mean ± SD) was significantly lower than in the males 12.5 ± 5.8 μmol/l [0.73 ± 0.34 mg/100 ml], $t = 5.888$; $p < 0.001$). The distribution of total bilirubin concentration was again bimodal in males and females (Fig. 5).

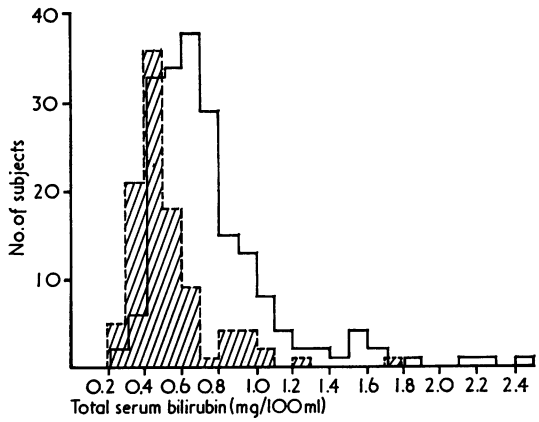

FIG. 4. Distribution of total serum bilirubin concentration in males and females separately. Females are represented by the hatched columns and males by the open columns.

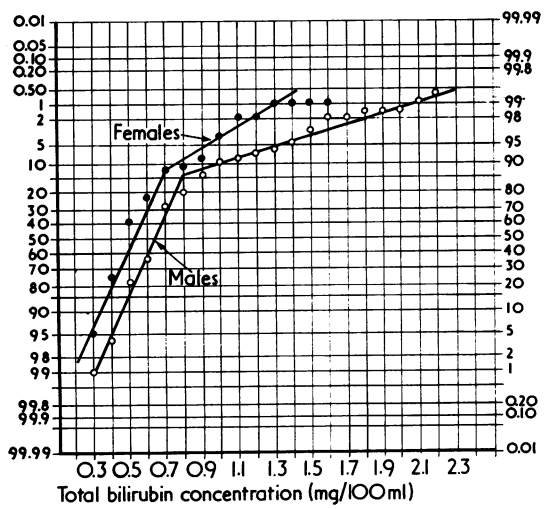

FIG. 5. Percentage cumulative frequency distribution of total serum bilirubin concentration in males and females separately.

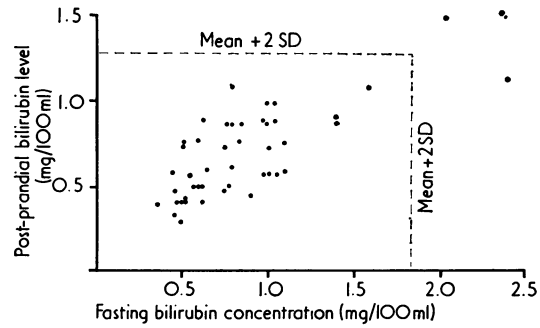

FIG. 6. The relationship between total bilirubin concentrations in the fasting and non-fasting state in 47 medical students. $y = a + bx$, $a = 0.25$, $b = 0.52$, $r = 0.80$.

Figure 6 shows the relationship between bilirubin concentrations measured before and after a 12-hour fast in the 47 medical students. It can be seen that the bilirubin concentration tends to be higher in the fasting state. The mean total bilirubin concentration in the non-fasting state was 12.0 ± 5.1 μmol/l (0.70 ± 0.30 mg/100 ml) and in the fasting state 14.9 ± 7.7 μmol/l (0.87 ± 0.45 mg/100 ml, $t = 3.973$; $p < 0.001$). Moreover, whereas only two subjects had bilirubin concentrations above the mean + 2 SD in the non-fasting state, three had values above this after fasting for 12 hours.

Discussion

Total serum bilirubin concentration showed a bimodal distribution in the population studied. This suggests that two populations exist with reference to bilirubin concentration. These two populations are separated by the antimode, shown best in Fig. 2, which corresponds to a bilirubin concentration of 24 μmol/l. Individuals whose bilirubin concentrations exceeded this value had unconjugated hyperbilirubinaemia. Haemolysis is unlikely to be the cause of this as none of the subjects were anaemic. It is suggested that these individuals have Gilbert's syndrome indicating that the population incidence of the condition is about 3%. The actual incidence may be higher than this because individuals with a previous history of jaundice do not present to donate blood.

This incidence of 3% is higher than the figure of 1 in 200 males quoted by Billing (1970), but is lower than the 7% incidence suggested by Kornberg (1942).

The majority of the population (97%) had bilirubin concentrations below 24 μmol/l. This suggests that this value should be taken as the upper

limit of the 'normal' total bilirubin concentration. Up to the present time lower values are given: 13.7 to 17.1 μmol/l (0.8 to 1.0 mg/100 ml). The one exception is Nosslin (1960) who gives the upper limit of the normal bilirubin concentration as 24 μmol/l.

The lower mean total bilirubin concentration in the females compared to the males is unexplained. It could be due to greater bilirubin production or to lower hepatic bilirubin clearance in males. Berk *et al* (1969) using ^{14}C-labelled unconjugated bilirubin found significantly lower hepatic bilirubin clearance per kg body weight in males compared to females. However, there was no significant difference in the bilirubin production rate per kg body weight between the two sexes. Why this is so has not been explained.

It has been claimed by Powell *et al* (1967b) that Gilbert's syndrome is inherited as an autosomal dominant trait. However, these workers found a low incidence of the condition in parents and sibs of propositi. Moreover, the incidence of the condition appeared to be about four times greater in males than females. These observations do not correspond well with those expected for simple dominant inheritance. If, however, we assume the condition to be inherited in this manner, the above discrepancies could be accounted for by the following observations. Firstly, it is known that the bilirubin concentration fluctuates in Gilbert's syndrome and may occasionally be normal (Foulk *et al*, 1959). Powell *et al* (1967b) measured bilirubin concentration in the relatives of the propositi only once. This could have been at a time when the bilirubin level was normal in affected relatives. Second, some of their propositi had bilirubin concentrations in the range 13.7 to 24 μmol/l (0.8 to 1.4 mg/100 ml). The present study suggests that the males with bilirubin levels in this range are normal and not individuals with Gilbert's syndrome.

When the distribution of total serum bilirubin concentration in the females is analysed separately from the males, it can be seen that this is bimodal (Figs. 4 and 5). However, the bimodal distribution in the population as a whole seems to be due to the distribution of total bilirubin concentration in the male subjects. The antimode separating the females into two populations corresponds to 12 μmol/l compared to 24 μmol/l in the male population. It is conceivable that females with bilirubin levels above 12 μmol/l also have Gilbert's syndrome. These individuals are not normally recognized as having Gilbert's syndrome because their bilirubin concentrations are within the range of the normal bilirubin concentration in the male subjects. If these conclusions are correct the incidence of Gilbert's syndrome now becomes approximately 6% with an almost equal incidence in both sexes (10 females and nine males). These findings suggest that the diagnosis of Gilbert's syndrome should be made with reference to the distribution of bilirubin concentration in the two sexes separately.

Liver function tests apart from bilirubin estimations were not carried out in the subjects with Gilbert's syndrome in the blood donor population. These tests were normal, however, in the three fasting medical students who had total bilirubin concentrations exceeding the mean + 2 SD. The findings in these medical students alone again suggests a population incidence of Gilbert's syndrome of about 6%.

The mean bilirubin concentration in these 47 medical students was higher in the fasting state. This may be due to a decrease in hepatic uridine diphosphate glucuronyl transferase activity as a result of a reduction in caloric intake (Owens and Sherlock, 1973). Estimating the bilirubin concentration in the fasting state may enable a diagnosis of Gilbert's syndrome to be made when the non-fasting bilirubin concentration is normal. Fasting also appears to separate more distinctly the normal population from those with Gilbert's syndrome (Fig. 6).

Gilbert's syndrome is an important condition which appears to be more common than was hitherto realized. Most subjects with this condition have no symptoms but some complain of lethargy, upper abdominal pain, and indigestion. The prognosis is excellent and hyperbilirubinaemia can be treated with enzyme-inducing drugs such as phenobarbitone (Black and Sherlock, 1970). It is important to be aware of this condition so that extensive investigation of suspected liver disease can be avoided and so that the patient can be reassured of the excellent prognosis and normal life expectancy.

We are grateful to Dr D. Lehane, Regional Blood Transfusion Centre, for his cooperation and to Professor D. A. Price Evans, Dr E. A. Jones, and Miss H. Wright for assistance in the preparation of the paper.

References

Berk, P. D., Blaschke, T. F., and Waggoner, J. G. (1972). Defective bromosulfophalein clearance in patients with constitutional hepatic dysfunction (Gilbert's syndrome). *Gastroenterology*, **63**, 472–481.

Berk, P. D., Bloomer, J. R., Howe, R. B., and Berlin, N. I. (1970). Constitutional hepatic dysfunction (Gilbert's syndrome). A new definition based on kinetic studies with unconjugated radiobilirubin. *American Journal of Medicine*, **49**, 296–305.

Berk, P. D., Howe, R. B., Bloomer, J. R., and Berlin, N. I. (1969). Studies of bilirubin kinetics in normal adults. *Journal of Clinical Investigation*, **48**, 2176–2190.

Billing, B. H. (1970). Bilirubin metabolism and jaundice with special reference to unconjugated hyperbilirubinaemia. *Annals of Clinical Biochemistry*, **7**, 69–74.

Billing, B. H., Haslam, R., and Wald, N. (1971). Bilirubin standards and the determination of bilirubin by manual and Technicon Autoanalyser methods. *Annals of Clinical Biochemistry*, **8**, 21–30.

Billing, B. H., Williams, R., and Richards, T. G. (1964). Defects in hepatic transport of bilirubin in congenital hyperbilirubinaemia: an analysis of plasma bilirubin disappearance curves. *Clinical Science*, **27**, 245–257.

Black, M. and Billing, B. H. (1969). Hepatic bilirubin UDP-glucuronyl transferase activity in liver disease and Gilbert's syndrome. *New England Journal of Medicine*, **280**, 1266–1271.

Black, M. and Sherlock, S. (1970). Treatment of Gilbert's syndrome with phenobarbitone. *Lancet*, **1**, 1359–1362.

Foulk, W. T., Butt, H. R., Owen, C. A., Jr., Whitcomb, F. F., Jr., and Mason, H. L. (1959). Constitutional hepatic dysfunction (Gilbert's disease): its natural history and related syndromes. *Medicine*, **38**, 25–46.

Gilbert, A. and Lereboullet, P. (1901). La cholémie simple familiale. *Semaine Médicale*, **21**, 241–243.

Kornberg, A. (1942). Latent liver disease in persons recovered from catarrhal jaundice and in otherwise normal medical students as revealed by the bilirubin excretion test. *Journal of Clinical Investigation*, **21**, 299–308.

Michaëlsson, M., Nosslin, B., and Sjölin, S. (1965). Plasma bilirubin determination in the newborn infant. *Pediatrics*, **35**, 925–931.

Nosslin, B. (1960). The direct diazo reaction of bile pigments in serum. Experimental and clinical studies. *Scandinavian Journal of Clinical and Laboratory Investigation*, **12**, Suppl. No. 49.

Owens, D. and Sherlock, S. (1973). Diagnosis of Gilbert's syndrome: role of the reduced caloric intake test. *British Medical Journal*, **3**, 559–563.

Powell, L. W., Billing, B. H., and Williams, H. S. (1967a). An assessment of red cell survival in idiopathic unconjugated hyperbilirubinaemia (Gilbert's syndrome) by the use of radioactive diisopropylfluorophosphate and chromium. *Australasian Annals of Medicine*, **16**, 221–225.

Powell, L. W., Hemingway, E., Billing, B. H., and Sherlock, S. (1967b). Idiopathic unconjugated hyperbilirubinemia (Gilbert's syndrome). A study of 42 families. *New England Journal of Medicine*, **277**, 1108–1112.

Simmons, N. A. (1968). An automated method for serum bilirubin determination. *Journal of Clinical Pathology*, **21**, 196–201.