Estimation of Hepatic Bilirubin UDP-Glucuronyl Transferase in Patients with Noncirrhotic Portal Fibrosis and Liver Disease: Significance and Limitations

D. V. Datta, MD, R. Nair, MD, and C.R. Nair, MD

Using a micromethod, hepatic bilirubin UDP-glucuronyl transferase has been assayed in percutaneous needle biopsy samples obtained from patients with infectious hepatitis, postnecrotic cirrhosis, Gilbert's disease, noncirrhotic portal fibrosis (NCPF), granuloma of the liver, and extrahepatic portal vein obstruction. The results were compared with those obtained from 10 control subjects. Patients with cirrhosis and infectious hepatitis revealed normal bilirubin transferase levels, whereas those with Gilbert's disease showed significantly low enzyme levels. Many patients with NCPF, some with extrahepatic portal vein obstruction, and patients with granulomatous involvement of the liver demonstrated significantly low levels. This low hepatic-enzyme activity was not associated with hyperbilirubinemia. The mechanism of such low values in NCPF and other disorders is not known. It is postulated that low hepatic-enzyme activity in noncirrhotic portal fibrosis is due to sparse smooth endoplasmic reticulum. This study also emphasizes that serum bilirubin may remain normal with very low hepatic-enzyme activity. Although induction of the microsomal enzyme bilirubin transferase was observed following phenobarbitone administration in noncirrhotic portal fibrosis, this was not apparent in patients with cirrhosis, possibly due to maximal enzyme induction having been achieved by endogenous substrate.

Details of bilirubin metabolism have come to light during the last 2 decades. At the present it is well known that the free bilirubin of plasma is rapidly taken up by the hepatocytes and conjugated with UDP-glucuronic acid (UDPGA) to form a water-soluble derivative bilirubin glucuronide which is easily excreted into the biliary system. Thus conjugation of bilirubin within the liver cell is an essential step in the clearance of this pigment from the body, and this process is catalyzed by a microsomal enzymes known as bilirubin UDP-glucuronyl transferase. Almost complete absence of this enzyme system has been demonstrated in the Criglar–Najjar syndrome and in Gunn rats, and has been said to be responsible for unconjugated hyperbilirubinemia (1, 2). Delay in the maturation of the bilirubin transferase system is known to be related to the jaundice of prematurity (3), and deficient enzyme levels have been reported in Gilbert's disease (1, 2). Studies on the alterations and significance of enzyme conjugation in different human liver disorders had been limited due to the nonavailability of a sufficiently sensitive assay techniques. By employing diazotized ethylanthranilate (4) Black and coworkers (5) had developed a technique which can estimate the enzyme content in small

From the Division of Hepatic Diseases and Division of Metabolism and Nutrition, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Address for reprint requests: Dr. D.V. Datta, division of Hepatic Diseases, Postgraduate Institute of Medical Education and Research, Chandigarh-160011, India.

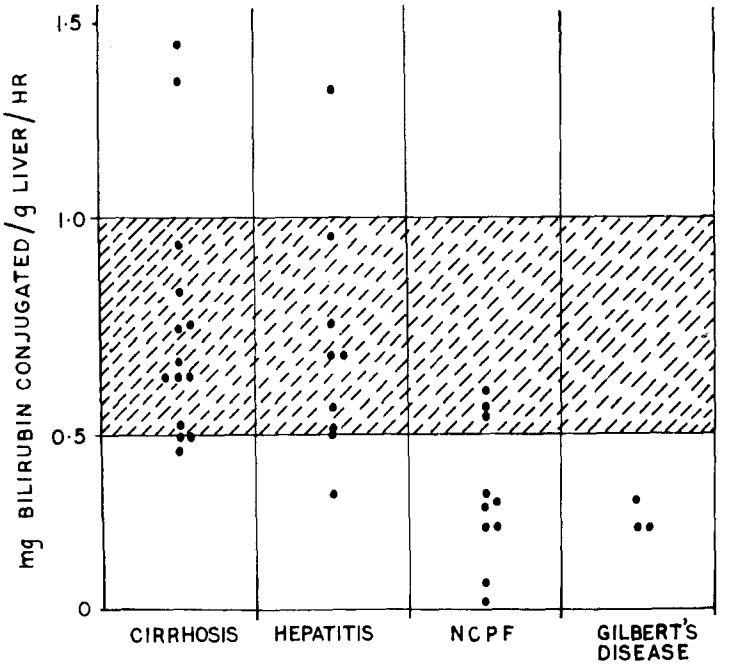

Fig 1. Bilirubin transferase activity in liver biopsy samples in patients with liver disease. Shaded area shows the normal range of activity.

amounts of tissue with accuracy. Using the method the present study reports the results of an assay of the hepatic enzyme in various liver disorders. The extent of bilirubin transferase induction following the administration of phenobarbitone sodium in cirrhosis and noncirrhotic portal fibrosis has also been studied.

MATERIALS AND METHODS

56 subjects were investigated for hepatic bilirubin UDP-glucuronyl transferase activity. Controls were investigated for a possible liver disease. Biochemical and histological investigations in the controls did not reveal any abnormality. Their prior consent was obtained to do all the relevant investigations including liver biopsy.

14 patients had postnecrotic cirrhosis; 1, alcoholic cirrhosis; 10, noncirrhotic portalfibrosis (NCPF); 9, infectious hepatitis; 5, extrahepatic portal vein obstruction; and 3, Gilbert's disease. The ages of these patients ranged from 20 to 50 years except in cases of extrahepatic portal vein obstruction where the age ranged from 20 to 30 years. The extrahepatic portal vein obstruction was of idiopathic type in all. Nutritional status of all the subjects including control was well preserved except in cases of cirrhosis and granuloma of the liver, where patients showed signs of undernourishment. None of these patients received any drug known to influence the hepatic enzyme system. None were alcoholic. The diagnosis of patients was based on liver histology coupled with suitable clinical and biochemical investigations (6).

In another group of 11 patients (6 NCPF and 5 cirrhosis) the enzyme activity was measured after the administration of phenobarbitone sodium (microsomal enzyme-inducer) in doses of 180 mg twice a day for 10 days. In another experiment, both pre- and postphenobarbitone therapy, enzyme measurement in one patient each with cirrhosis, NCPF, and Gilbert's disease was made. In patients with cirrhosis and NCPF repeat tissue samples were obtained at laparotomy for elective shunt surgery. Preliminary studies established that enzyme values for specimens obtained at laparotomy and those obtained by needle biopsy in the same patient without any treatment with inducers do not differ. Repeat tissue samples in a patient with Gilbert's disease was obtained by percutaneous needle biopsy with prior consent of the patient.

Table 1. Bilirubin UDP-Glucuronyl Transferase and Serum Bilirubin in Noncirrhotic Portal Fibrosis and Other Hepatic Disorders

Patient No.	Diagnosis	Bilirubin (mg/100 ml)		UDP-glucuronyl transferase activity (mg/bilirubin conjugated/g liver)
		Total	Conjugated	
1	Noncirrhotic portal fibrosis	1.0	0	0.222
2	Noncirrhotic portal fibrosis	1.0	0	0.300
3	Noncirrhotic portal fibrosis	1.0	0.5	0.222
4	Noncirrhotic portal fibrosis	1.5	0	0.281
5	Noncirrhotic portal fibrosis	1.0	0.5	0.540
6	Noncirrhotic portal fibrosis	1.0	1.0	0.572
7	Noncirrhotic portal fibrosis	1.5	0.50	0.370
8	Noncirrhotic portal fibrosis	1.0	0.0	0.518
9	Noncirrhotic portal fibrosis	0.75	0.5	0.100
10	Noncirrhotic portal fibrosis	1.0	0.5	0.150
11	Granuloma liver	1.5	1.0	0.259
12	Granuloma liver	0.5	1.0	0.296
13	Granuloma liver	0.5	0.0	0.296
14	Granuloma liver	3.5	2.0	0.671
15	Extrahepatic portal vein obstruction	0.75	0.00	0.74
16	Extrahepatic portal vein obstruction	1.00	0.00	0.081
17	Extrahepatic portal vein obstruction	0.75	0.75	0.890
18	Extrahepatic portal vein obstruction	0.75	0.75	0.048
19	Extrahepatic portal vein obstruction	1.0	0.5	0.540

Tissue for the enzyme assay was obtained by percutaneous needle biopsy of the liver with Menghini's needle. 10 mg of liver tissue was found to be sufficient for the enzyme estimation. Biopsy tissues were collected into dry-chilled tubes and kept at 0° C till the time of assaying which was undertaken immediately or well within half an hour. The enzyme activity was measured by the method of Black and coworkers (5), and the results were presented as bilirubin conjugated/g wet liver tissue or mg of tissue protein. A similar pattern of results was found when the results were presented by both methods. Tissue protein was measured by the method of Lowry and coworkers (7), and liver function tests were carried out by standard methods (8). Serum bilirubin was estimated repeatedly by method of Michaelsson and coworkers (9). The normal upper limit of total bilirubin in our laboratory has been found to be 1.5 mg/100 ml, with a mean of 0.9 mg/100 ml.

RESULTS

The results of the hepatic enzyme assay in different groups of patients are shown in Figure 1. The mean value for the control group was 0.712 (± 0.13 SD) mg of bilirubin conjugated/g liver/30-min incubation. As shown, there was no significant difference in the enzyme activity in control subjects and patients with cirrhosis of the liver and acute infectious hepatitis ($P > 0.05$). Patients with Gilbert's disease showed lower levels of enzyme activity.

A marked reduction of bilirubin transferase was seen in patients with NCPF ($P < 0.05$). Similarly, 3 of the 5 patients with extrahepatic

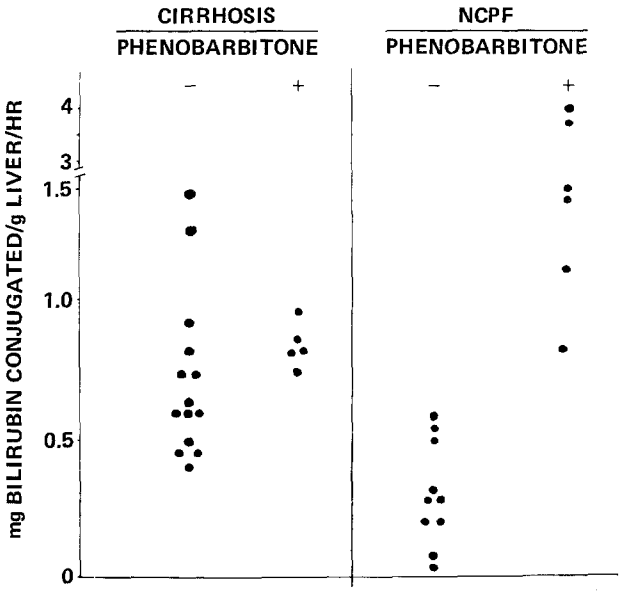

Fig 2. Effect of phenobarbitone sodium administration on hepatic bilirubin transferase in cirrhosis of the liver and noncirrhotic portal fibrosis. Untreated and treated groups are 2 different groups of patients; no patient serves as his own control.

portal vein obstruction and all 4 patients with nonspecific granulomatous hepatitis (possibly secondary to tuberculosis) revealed significantly lowered enzyme activity (Table 1). However, low levels of hepatic-enzyme activity were not associated with high plasma bilirubin levels which were estimated for several months in many patients. This study demonstrates that low levels of hepatic enzymes can coexist with normal levels of serum bilirubin.

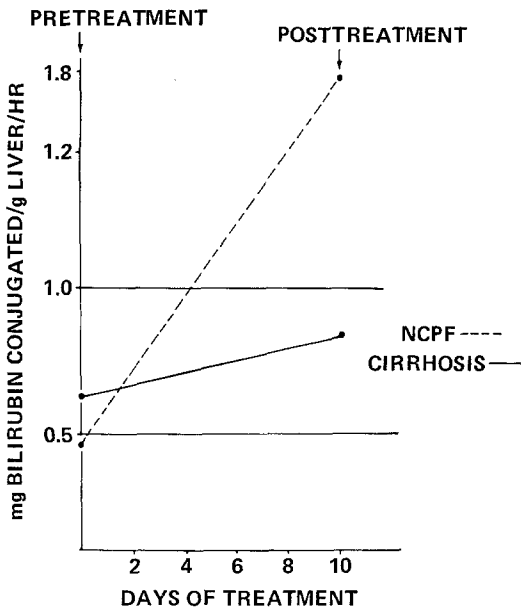

Fig 3. Effect of phenobarbitone sodium administration on hepatic bilirubin transferase in cirrhosis of the liver and noncirrhotic portal fibrosis.

Figure 2 shows the effect of phenobarbitone administration on hepatic bilirubin UDP-glucuronyl transferase activity in patients with cirrhosis of the liver and NCPF. Patients with cirrhosis of the liver who had phenobarbitone treatment did not reveal higher activity than patients receiving no treatment. However, patients with NCPF receiving phenobarbitone revealed significantly higher enzyme activity compared with those receiving no treatment. This is also very well demonstrated in Figure 3, where 1 patient with cirrhosis and 1 patient with NCPF were studied for the hepatic enzyme prior to and at the end of the therapy.

Figure 4 shows the effect of phenobarbitone administration for 24 days in a patient with Gilbert's disease. Although the enzyme activity was markedly low, it showed a rise which, however, did not exceed the normal level. Increase

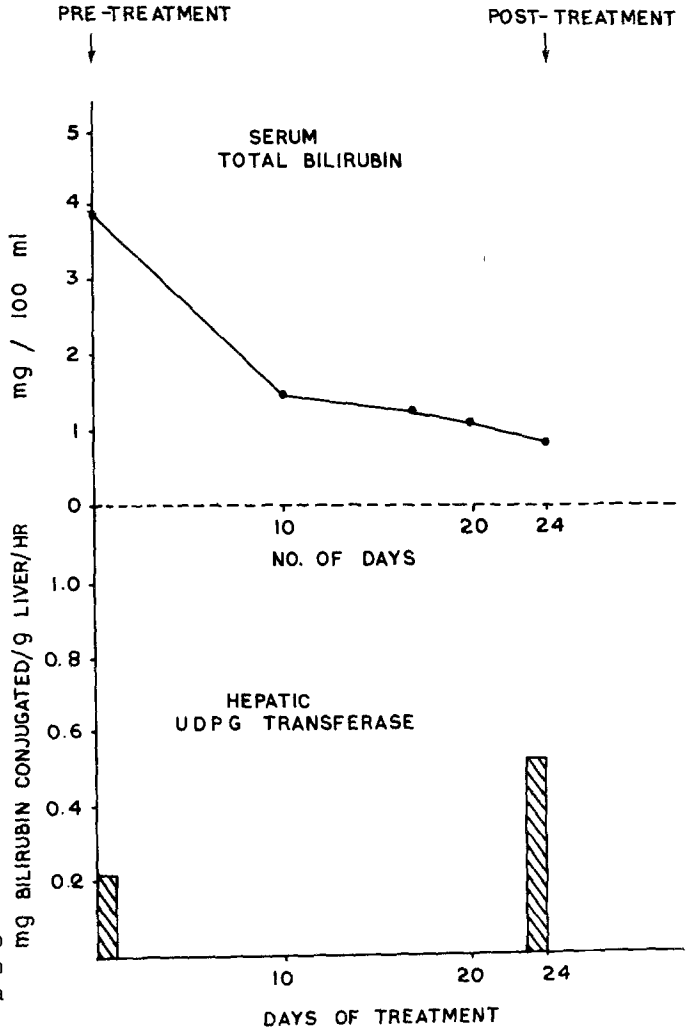

Fig 4. Effect of phenobarbitone sodium administration on hepatic bilirubin transferase and serum bilirubin in a patient with Gilbert's disease.

in enzyme activity was associated with a fall in the serum bilirubin.

DISCUSSION

The mean values for hepatic bilirubin UDP-glucuronyl transferase activity in our control subjects is relatively low compared with that reported from England (1). As an identical technique was used by the present workers, the lower values noted in this study could be due to either a racial difference or different dietary habits.

This study confirms the previous findings regarding the normal enzyme activity in cirrhosis of the liver, infectious hepatitis, and low enzyme activity in Gilbert's disease (1, 2). These observations confirm that hyperbilirubinemia ob-

served in patients with infectious hepatitis and cirrhosis is not due to a low enzyme activity. The preserved hepatic-enzyme level in these disorders has been explained on the basis of possible enzyme induction, either due to hepatic regeneration or to a substrate induction (1). That the induction, to a great extent, has occurred at its peak level is evidenced by a lack of significant effect on the hepatic-enzyme activity following administration of a known enzyme inducer, phenobarbitone sodium, in patients with cirrhosis.

Patients with NCPF showed a low hepatic-enzyme activity. It may be recalled that this entity is characterized by preservation of hepatic architecture and absence of any regeneration (6). In these patients, all liver function tests, including serum bilirubin level which was repeatedly estimated for months, were within normal limits. Similarly the findings of a low enzyme activity in various hepatic disorders such as granulomatous involvement of the liver in the absence of hyperbilirubinemia is worth noting. This is one of the important aspects of the study and may caution one not to draw any firm conclusion on the significance of low enzyme activity, as measured by this method, in the pathogensis of hyperbilirubinemia in certain clinical states.

The mechanism of low hepatic-enzyme activity in NCPF remains unexplained. Recent studies have reported a sparse smooth endoplasmic reticulum in noncirrhotic portal fibrosis (10), and this may be the major factor for a reduced hepatic-enzyme activity. Similarly, recent studies from our laboratory have shown that as little as 2 weeks of low-protein-diet intake reduces the hepatic glucuronyl-transferase activity in rats (11). It is conceivable that poor diet intake in some patients may be one of the factors causing a low enzyme level, but this is not the cause in NCPF and extrahepatic portal vein obstruction, as nutrition was well maintained in these patients. However, nutrition was not well preserved in cirrhosis where enzyme activity was high. Similarly, the role of low-grade chronic infection of the liver in absence of hepatic regeneration, as in granulomatous involvement of the liver, in the pathogenesis of low enzyme activity is not known. Further work is warranted in this direction. It must be emphasized that bilirubin UDP-glucuronyl transferase is a microsomal enzyme, and its pattern of alteration is bound to be different from a cytoplasmic nonmicrosomal enzyme, eg bromsulfathalein glutathione conjugating enzyme (12).

The pathogenesis of hyperbilirubinemia in Gilbert's disease is not clear. Low bilirubin transferase has been thought to be an important mechanism (1). The same conclusion may be drawn from the present study, as not only the enzyme activity was low in these patients, but phenobarbitone administration reduced the hyperbilirubinemia as reported by Black et al (13) and increased the hepatic-enzyme activity. However, finding the same degree of low enzyme activity in NCPF and other hepatic disorders with absence of hyperbilirubinemia suggests that low activity may not necessarily be the cause of jaundice in Gilbert's disease and that some other factors might well be operating. Felsher and coworkers (14) have reported no close correlation between the extent of jaundice and the hepatic-enzyme activity in patients with Gilbert's disease. In fact anicteric patients with reduced bilirubin transferase activity were also seen. It is to be noted that no information is available on the intrahepatic bilirubin carrier protein in Gilbert's disease. Similarly the critical level of hepatic enzyme level below which hyperbilirubinemia occurs has not been worked out. In addition to enzyme deficiency (14), an enhancement of hepatic heme-oxygenase activity incident to caloric restriction in Gilbert's disease resulting in increase bilirubin production has been suggested.

On the basis of the present study, one may doubt the usefulness of measurement of hepatic digitonin-activated bilirubin transferase activity in liver tissue, as it may not necessarily reflect the true extent of *in vivo* conjugation of bilirubin. Another limitation of the technique may be

that the *in vitro* assay system results may be influenced by the methods of preparation of homogenates and characteristics of the incubation system or alternatively that more than one system is probably operating for bilirubin conjugation *in vivo*. Moreover enzyme activity may vary from lobe to lobe. This fact warrants further investigation. Alternatively some other mechanism(s) may be operating to deal with bilirubin.

The finding of marked induction of bilirubin transferase by phenobarbitone administration in patients with NCPF is of interest. This would suggest that in this clinical state, as in normal subjects, inducible enzyme reserve is present, whereas such reserve may be low in Gilbert's disease.

ACKNOWLEDGMENTS

Our thanks are due to Dr. B. Billing of the Royal Free Hospital, London, who helped us in standardizing the technique. Prof. Inderjeet Dewan helped in editing the paper, for which the authors are particularly grateful.

REFERENCES

1. Black M, Billing BH: Hepatic bilirubin UDP-glucuronyl transferase activity in liver disease and Gilbert's syndrome. N Eng J Med 280:1266–1271, 1969
2. Fevery J: Recent development in bilirubin conjugating. Thesis for Geaggregeerde Voor Hot Hoger Onderwijs. University of Leuven, Belgium, 1972.
3. Zuelzer WW, Brown AK: Neonatal jaundice. Am J Dis Child 101:87, 1961
4. Van Roy FP, Heirwegh KPM: Determination of bilirubin glucuronyl transferase with bilirubin as acceptor. Biochem J 107:507–518, 1968
5. Black M, Billing BH, Heirwegh KPM: Determination of bilirubin UDP-glucuronyl transferase activity in needle of biopsy specimens of human liver. Clin Chim Acta 29:27–35, 1970
6. Indian Council of Medical Research: Monogram on Noncirrhotic Portal Fibrosis, 1969
7. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ: Protein measurement with the folin phenol reagent. J Biol Chem 193:255–265, 1951
8. King EJ, Wooton IDP: Micro Analysis in Medical Biochemistry, 3rd edition. London, JA Churchill, 1959
9. Michaelsson M, Nosslon B, Sjolin, S: Plasma bilirubin determination in the new born infant. Paediatrics 34:925–931, 1965
10. Tandon BN, Lakshminarayanan R, Bhargawa S, Nayak NC, Sama SK: Ultrastructure of liver in noncirrhotic portal fibrosis. Gut 11:905–910, 1970
11. Nair CR, Nair R, Datta DV: Personal observation.
12. Datta DV, Singh S, Samanta AK, Mukherjee M, Nirankari OP, Chuttani PN, CR Nair: Determination of bromsulphthalein, glutathione conjugating enzyme activity in needle biopsy specimens of human liver Clin Chim Acta 47:133–137, 1973
13. Black M, Martin, Sherlock S: Treatment of Gilbert's syndrome with phenobarbiton. Lancet 1:1359–1361, 1970
14. Felsher RP, Craig JR, Carpiol N: Hepatic bilirubin glucuronidation in Gilbert's syndrome J Lab Clin Med 81:829–837, 1973