Augmentation of Bilirubin UDP Glucuronyltransferase Activity in Rat Liver Homogenates by Glutethimide (36665)

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Conjugation of bilirubin with various substrates to form a polar, water-soluble molecule has been shown to be an essential process for normal bilirubin metabolism in many mammalian species (1). The enzyme uridine diphosphate glucuronyltransferase (UDP–GT) is thought to play a critical role in bilirubin conjugation, although this enzyme itself has not been completely purified, and there is still some question as to whether it exists in single or multiple forms (2, 3).

Treatment with phenobarbital has been shown to reduce the plasma concentration of unconjugated bilirubin by accelerating hepatic bilirubin clearance in normal man and in patients with unconjugated hyperbilirubinemia due to several types of hepatic dysfunction (4–6). Since phenobarbital produces augmented levels of hepatic UDP–GT activity both *in vivo* (5, 7, 8) and *in vitro* (9), it has been suggested that accelerated bilirubin conjugation was the basis for the reduction in plasma bilirubin concentration observed during treatment (7). However, a steadily increasing number of additional alterations in hepatic physiology and cellular composition attributable to phenobarbital provide possible alternative explanations (10, 11). Recently, it has been shown that the nightly administration of glutethimide—a hypnotic bearing a structural similarity to phenobarbital, but with a different metabolic fate—also accelerates hepatic bilirubin clearance in man at doses which produce no daytime sedation (8, 12). This agent therefore may have advantages over phenobarbital for outpatient clinical studies. As part of a broader investigation of the mechanisms by which drugs influence bilibrubin metabolism, we have examined the effects of glutethimide on bilirubin-UDP glucuronyltransferase activity on rat liver homogenates.

Methods. Crystalline phenobarbital (Merck and Co., Rahway, N.J.) and glutethimide (kindly provided by CIBA Pharmaceutical Co., Summit, N.J.) were dissolved in polyethylene glycol 400 solvent (CIBA) and given by intraperitoneal injection to either adult male Sprague–Dawley, heterozygous (J/j) or homozygous (j/j) Gunn rats. Treated animals received either glutethimide, 50 or 75 mg/kg/day, or phenobarbital, 90 mg/kg/day. Matched control rats received daily intraperitoneal injections of solvent. Except where specified, treatment was carried out for 9–14 days. Animals were permitted free access to food and water at all times. On the day of sacrifice, the rats were killed by decapitation, and their livers immediately excised and weighed. Hepatic bilirubin UDP–GT activity was then assayed on liver homogenates by the method of Black, Billing, and Heirwegh (13). All assays were performed in duplicate. The coefficient of variation among identical samples or between separate homogenates prepared from different areas of the same liver was 4%.

Results. The data are summarized in Table I. At the dose levels and duration of therapy employed, glutethimide produced a significant increase in bilirubin-UDP glucuronyltransferase activity (expressed per gram liver tissue) in the Sprague–Dawley rats. Values during treatment averaged 145 ± 5% (mean ± SEM) of the values in matched controls, and were similar to those observed in the phenobarbital treated animals (144 ± 7% of control). The effects of glu-

TABLE I. Effect of Phenobarbital and Glutethimide on Glucuronyltransferase Activity in Rat Liver Homogenates.

t	Average weight[a]	No.	Group	Drug dose mg/kg/day	Length of therapy (days)	Liver weight as % body weight[a]	Liver protein mg/gm liver[a]	Bilirubin UDP-Glu transferase activ: Mg bilirubin conjugated/g liver/hr[a]	M conj
e–Dawley	258 ± 7	14	Control[b]	—	9–14	3.75 ± .15	208 ± 2	2.059 ± .091	7
	259 ± 15	10	Glutethimide	50–75	9–14	4.18 ± .16	211 ± 8	2.892 ± .111[c]	11
	248 ± 7	12	Phenobarbital	90	9–14	4.94 ± .20[c]	207 ± 7	2.968 ± .143[c]	14
ygous	257 ± 15	3	Control[b]	—	10–11	3.83 ± .10	211 ± 12	0	
Gunn	230 ± 9	4	Glutethimide	75	10–11	3.92 ± .19	226 ± 8	0	
	258	1	Phenobarbital	90	9	5.50	205	0	
zygous	247 ± 15	6	Control[b]	—	9–11	3.42 ± .13	221 ± 4	1.735 ± .090	5
Gunn	272 ± 11	7	Glutethimide	50–75	9–11	3.60 ± .09	218 ± 3	2.110 ± .074[d]	7
	271 ± 10	5	Phenobarbital	90	9–11	4.33 ± .14[c]	233 ± 4	2.389 ± .174[d]	10

an ± 1 SEM.

Control rats received intraperitoneal injections of solvent.

Significantly different from matched control group ($p < 0.001$).

Significantly different from matched control group ($p < 0.01$).

tethimide at a dose of 50 mg/kg/day were identical to those produced by 75 mg/kg/day, and these data are therefore pooled in the table. Neither agent produced detectable changes in total liver protein content (14), so that alterations in enzyme activity are identical whether expressed per gram liver tissue or per milligram liver protein. Although the effect on enzyme specific activity produced by both agents is comparable (Student's t test, $p > 0.6$), the increase in liver size produced by phenobarbital (132 ± 5% of control) was appreciably greater than that produced by glutethimide (111 ± 4% of control), as previously reported (15). Hence, the increase in enzyme activity produced by glutethimide was significantly less than that produced by phenobarbital ($p < 0.001$) when the data are expressed as milligrams bilirubin conjugated/hr/100 g body weight.

In a study involving a smaller number of animals treated for only 4 days with 50–75 mg/kg/day of glutethimide, the increase in glucuronyl transferase activity (144 ± 7% of control) was equivalent to that produced by 9–14 days of therapy. However, a dose of 25 mg/kg/day of glutethimide given for 12–14 days produced only a 9% increase in UDP–GT activity ($p > 0.1$).

There was no detectable UDP–GT activity in liver homogenates from the untreated homozygous, jaundiced (j/j) Gunn rats. Neither glutethimide nor phenobarbital stimulated measurable enzyme activity in these animals, although the effects of both agents on liver size seemed to parallel those seen in the Sprague–Dawley and heterozygous (J/j) animals.

UDP–GT activity in untreated heterozygous (J/j) animals was significantly lower than that in the corresponding Sprague–Dawley controls ($p < 0.05$). Both glutethimide and phenobarbital produced significant increases in UDP–GT activity (per gram liver) in those animals (122 ± 4%, and 138 ± 10% of control, respectively). As with the Sprague–Dawley animals, the larger increase in liver size produced by phenobarbital therapy resulted in a greater total increase in UDP–GT activity per 100 g body weight from this agent as compared to glutethimide.

When glutethimide and phenobarbital were added directly to liver homogenates in amounts in excess of what might be present *in vivo* at the time of homogenization (1.7 mg phenobarbital/g liver and 1.0 mg glutethimide/g liver) enzyme activity was identical to that seen in an aliquot of the homogenate removed before the addition of drug. Therefore direct activation of bilirubin UDP glucuronyltransferase in the assay system or interference with the spectrophotometric measurement of conjugated bilirubin by these agents does not account for the increase in enzyme activity seen in these experiments.

Discussion. The elimination of bilirubin from the circulation involves a number of distinct but interrelated steps, including entry into the hepatic cell; "storage" as a bilirubin–protein complex within the hepatocyte; conjugation; and biliary excretion of conjugated bilirubin (16). In the presence of a normal liver, when large quantities of bilirubin are administered by intravenous infusion, saturation of the transport system for the excretion of conjugated bilirubin limits the overall rate of transport from blood to bile (17). Although biliary excretion is, therefore, the rate-limiting step under these circumstances, it is less clear which process determines the rate of hepatic clearance of endogenous bilirubin under basal conditions. In the absence of increased bilirubin production, unconjugated hyperbilirubinemia may result from either decreased hepatic uptake or defective conjugation. Conversely, acceleration of either of these processes may reduce the plasma bilirubin concentration. In addition, since the intrahepatic pool of unconjugated bilirubin, which exceeds the plasma pool in mass, is in equilibrium with the plasma, alteration in the equilibrium distribution of unconjugated bilirubin between the intrahepatic and intravascular pools (*i.e.*, changes in relative storage capacity) will also influence the plasma concentration of unconjugated bilirubin. It is well known that phenobarbital produces an increase in the capacity of the liver to form glucuronides (5, 7–9), and the present study confirms that glutethimide has a similar effect on bilirubin conjugation.

However, unless conjugation is rate-limiting, it is not clear that these data explain the increase in hepatic bilirubin clearance resulting from therapy with either glutethimide or phenobarbital. Other documented effects, such as an increase in functional hepatic mass (10, 15), and an increase in intrahepatic binding proteins (11) and relative storage capacity may equally well be responsible for the observed reductions in plasma bilirubin concentration. Further information about the kinetics of the individual steps in the bilirubin transport process is necessary before the precise influence of phenobarbital and glutethimide can be elucidated.

Summary. This study demonstrates that glutethimide is as effective as phenobarbital in increasing the specific activity of hepatic bilirubin UDP glucuronyltransferase activity in Sprague–Dawley rats. The data also indicate that the effect is dose-dependent and can be elicited by as little as 4 days of therapy. Both glutethimide and phenobarbital increase enzyme activity to normal levels in heterozygous Gunn rats, but fail to stimulate any activity in homozygous Gunn rats, who presumably lack the genetic capacity to synthesize this enzyme.

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