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DRUGS AND THE LIVER—I

EFFECTS OF GLUTETHIMIDE AND PHENOBARBITAL ON HEPATIC BILIRUBIN CLEARANCE, PLASMA BILIRUBIN TURNOVER AND CARBON MONOXIDE PRODUCTION IN MAN* †

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Abstract—The effects of glutethimide and phenobarbital on the plasma unconjugated bilirubin concentration, hepatic bilirubin clearance (C_{BR}) and plasma bilirubin turnover (BRT) were determined in 19 patients with mild chronic unconjugated hyperbilirubinemia (Gilbert's syndrome) and 11 normal volunteers. C_{BR} and BRT were calculated from plasma radio-bilirubin disappearance curves obtained both before and during drug administration. The response to both drugs was essentially identical. During drug administration, the patients with Gilbert's syndrome attained a new steady state in which the plasma unconjugated bilirubin concentration ($\overline{BR}$) was $30 \pm 2\%$ (mean $\pm$ S. E. M.) and C_{BR} $314 \pm 25\%$ of baseline. In the normal volunteers, $\overline{BR}$ fell to $65 \pm 6\%$, while C_{BR} increased to $135 \pm 9\%$ of baseline during the period of drug administration. Although neither total red cell volume nor the half-life of ^{51}Cr-labeled erythrocytes was altered by either agent, daily plasma bilirubin turnover fell significantly ($P < 0.05$) to $87 \pm 4\%$ of baseline in the 30 subjects studied, and endogenous carbon monoxide production, which provides an independent estimate of the rate of heme degradation and bilirubin formation, was $93 \pm 5\%$ of control ($0.2 > P > 0.1$). These studies indicate that both accelerated hepatic bilirubin clearance and reduced plasma bilirubin turnover contribute to the reduction in bilirubin concentration observed during administration of phenobarbital and glutethimide. The methods employed provided no evidence that these agents produce an increase in the rates of heme catabolism, bilirubin production or carbon monoxide formation.

THE ADMINISTRATION of a variety of drugs metabolized by the microsomal enzyme systems of the liver, including phenobarbital, glutethimide and dicophane (DDT), has been shown in man to cause a reduction in the concentration of unconjugated bilirubin in the plasma,[1–5] as well as other alterations of hepatic physiology[6] and ultrastructure.[7] The plasma unconjugated bilirubin concentration is determined by

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a balance between two processes: the rate at which newly synthesized bilirubin enters the plasma pool, and the rate at which the liver clears bilirubin from this pool. Changes in the rates of either of these processes, i.e. bilirubin formation or elimination, can produce changes in the plasma unconjugated bilirubin concentration. We have examined the effects of phenobarbital and glutethimide administration on each of these two aspects of bilirubin metabolism in man by mathematical analysis of plasma radio-bilirubin disappearance curves obtained both before and during administration of these agents. Since the formation of bilirubin during heme catabolism has been shown to be accompanied by the equimolar production of carbon monoxide,[8,9] measurements of endogenous carbon monoxide production were obtained simultaneously with each radio-bilirubin study in most of the participants in order to provide an independent estimate of the effects of both agents on the rate of heme catabolism. A preliminary report of some of our findings has appeared previously.[10]

MATERIALS AND METHODS

Subjects

Nineteen patients with Gilbert's syndrome (14 male, 5 female) and 11 healthy normal volunteers (6 male, 5 female) admitted to the Metabolism Service of the National Cancer Institute between July, 1970 and July, 1972 are included in these studies. All volunteers met strict, previously described criteria for "normality".[11] The diagnosis of Gilbert's syndrome was based on: (a) the characteristic clinical picture of chronic unconjugated hyperbilirubinemia in the absence of abnormalities of other routine tests of liver function (normal SGOT, SGPT, alkaline phosphatase and plasma protein electrophoresis) or of overt hemolysis; and (b) the absence of inflammation, hepatocellular necrosis, scarring or distortion of lobular architecture in percutaneous liver biopsy specimens, which were obtained in 12 of the 19 patients. Diagnosis was confirmed during the control period by the use of plasma radio-bilirubin disappearance curves, which demonstrated a characteristic reduction of hepatic bilirubin clearance (C_{BR}) in all 19 patients.[12] Six of the 19 patients were found to have occult hemolysis, with increased rates of plasma bilirubin turnover and moderately shortened ^{51}Cr-red cell half-lives ($T_{\frac{1}{2}}$ = 18–24 days; normal $\geqslant$ 26 days). Because these patients met the above criteria in all other respects, and because the plasma concentration of unconjugated bilirubin was elevated in these patients beyond the levels predicted from studies in other patients with comparable rates of hemolysis,[12,13] the finding of occult hemolysis was not felt to be incompatible with the diagnosis of Gilbert's syndrome.[12,14]

Radio-bilirubin clearance studies

Radio-bilirubin clearance studies were performed as previously described,[11] using tracer doses of unconjugated bilirubin-$^{14}C^{15}$ or $-^{3}H$.[16] In order to minimize any possible effects of restricted caloric intake on heme[17] or bilirubin metabolism,[18] all studies were performed in the fed state, as previously indicated.[19] The experimental plasma radio-bilirubin disappearance curves were analyzed on a Univac 1108 digital computer, using the Simulation, Analysis and Modeling (SAAM) program of Berman and Weiss,[20] in order to calculate: (a) hepatic bilirubin clearance, the volume of plasma irreversibly cleared of bilirubin/min; (C_{BR}; ml/min/kg) and (b) plasma bilirubin turnover, the mass of bilirubin entering and being removed from the plasma

each day (BRT; μmoles/kg/day). The basis for these calculations, which are entirely independent of any hypothetical compartmental model of bilirubin metabolism, has been described previously.[11] The mean plasma concentration of unconjugated bilirubin ($\overline{BR}$) was calculated from 8 to 14 measurements obtained during the course of each radio-bilirubin clearance study. Plasma unconjugated bilirubin concentrations were measured by the method of Weber and Schalm.[21]

Carbon monoxide production

Carbon monoxide production (COP, μmoles/kg/day) was determined by means of a closed rebreathing system similar to that described by Coburn *et al.*[22] Minor modifications in the Coburn technique, employed in these studies, have been reported previously.[9] The blood carboxyhemoglobin per cent saturation (COHb %) was determined in duplicate on 0·1-ml blood samples by the gas chromatographic method of Collison *et al.*,[23] as modified by Rodkey and Collison.[24] The coefficient of variation for this method is ±1 per cent. The CO space (ml/kg), a measure of the readily accessible heme-protein content of the body, and carbon monoxide production (μmoles/kg/day) were calculated as previously described from the time/concentration curves of blood COHb %.[9,22]

Total red cell volume and ^{51}Cr-red cell half-life

The total red cell volume (TRCV) and ^{51}Cr-red cell half-life were both determined by standard methods[25,26] from the same injection of autotransfused ^{51}Cr-labeled erythrocytes.

Experimental design

During a baseline period of observation, all 30 participants underwent an initial radio-bilirubin clearance study and measurement of total red cell volume. After completion of these baseline studies, participants were begun on either glutethimide, 500 mg by mouth at bedtime, or phenobarbital, 60 mg by mouth thrice daily. Ten patients with Gilbert's syndrome and 5 normal volunteers received glutethimide; 9 patients and 6 normal volunteers received phenobarbital. In two of the normal volunteers, the phenobarbital dose was reduced to 120 mg/day after 1 week due to excessive sedation at the higher dose. Plasma bilirubin levels were measured daily during drug administration. After 12–17 days of treatment (average: 14 days), when a stable new level for the plasma concentration of unconjugated bilirubin had been evident for at least 4 days, the radio-bilirubin and chromium blood volume studies were repeated. In eight additional subjects, including 5 normal volunteers and three patients with Gilbert's syndrome, plasma radio-bilirubin studies were performed on two occasions, in the absence of any therapy, in order to determine the reproducibility of values for $\overline{BR}$, C_{BR} and BRT derived from this technique.

The half-life of ^{51}Cr-labeled erythrocytes was determined in every study participant after at least one of the two injections of radio-chromium, usually during the period of drug therapy which followed the first injection. In 15 individuals, a second chromium survival was determined after the cessation of therapy.

Measurements of endogenous carbon monoxide production were made in 25 of the study participants during both control and treatment periods. In general, these studies were performed simultaneously with or within 24 hr of the corresponding

studies employing radio-bilirubin. Twelve glutethimide-treated individuals (7 with Gilbert's syndrome; 5 normal volunteers) and 13 treated with phenobarbital (7 with Gilbert's syndrome; 6 normal volunteers) underwent the CO measurements.

Because Coburn[27] has reported that phenobarbital produced a large increase in CO production in man after 7 days of treatment, whereas most of our studies were performed after at least 14 days of drug administration, the effect of phenobarbital on CO production was determined in one additional individual, who did not participate in the remainder of the protocol. After an initial baseline CO study, phenobarbital was begun at the usual dose of 180 mg/day. CO studies were repeated on days 3, 7 and 14 of administration after which phenobarbital was stopped. Six weeks after the cessation of phenobarbital, two additional control studies were obtained on consecutive days. In this one individual, in order to minimize any possible effects of variation in caloric intake on CO production,[28,29] an identical menu, consisting of three balanced meals and a bedtime snack, was served on the day preceding and the day of each of the six studies.

The protocol for these studies was approved by the Clinical Research Committee and the Medical Board of the National Institutes of Health. All studies were performed with the fully informed consent and cooperation of each participant.

RESULTS

Experimental values for the plasma concentration of $\overline{BR}$, hepatic C_{BR} and plasma BRT during both control periods and periods of glutethimide or phenobarbital administration are summarized in Table 1. During administration of either agent, there was a fall in the plasma unconjugated bilirubin concentration in 28 of the 30 individuals studied. Only two normal volunteers, both given phenobarbital, whose mean initial bilirubin concentrations were the lowest of all the study participants (0·25 and 0·29 mg/100 ml, respectively), failed to achieve a statistically significant reduction in bilirubin concentration during drug administration. In the patients with Gilbert's syndrome, the downward trend became apparent as early as 24–48 hr, and all patients had achieved values within the normal range (< 1·0 mg/100 ml) by

TABLE 1. EFFECTS OF PHENOBARBITAL AND GLUTETHIMIDE ON THE PLASMA UNCONJUGATED BILIRUBIN CONCENTRATION ($\overline{BR}$), HEPATIC BILIRUBIN CLEARANCE (C_{BR}) AND PLASMA BILIRUBIN TURNOVER (BRT) IN NORMAL VOLUNTEERS AND PATIENTS WITH GILBERT'S SYNDROME*

	$\overline{BR}$ (mg/100 ml)		C_{BR} (ml/min/kg)		BRT (μmoles/kg/day)	
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment
Gilbert's syndrome						
Glutethimide group	1·93 ± 0·21	0·57 ± 0·06	0·19 ± 0·02	0·54 ± 0·06	8·2 ± 0·7	6·9 ± 0·5
Phenobarbital group	2·03 ± 0·28	0·55 ± 0·06	0·16 ± 0·01	0·49 ± 0·05	7·4 ± 0·8	6·1 ± 0·5
Normal volunteers						
Glutethimide group	0·53 ± 0·03	0·33 ± 0·05	0·53 ± 0·04	0·60 ± 0·01†	6·8 ± 0·6	4·9 ± 0·6
Phenobarbital group	0·43 ± 0·06	0·27 ± 0·02	0·60 ± 0·04·	0·87 ± 0·04†	6·1 ± 0·6	5·7 ± 0·3

* All values represent mean ± S. E. M.

† C_{BR} in normal volunteers receiving phenobarbital was significantly greater than in volunteers given glutethimide ($P < 0·01$). However, when each individual's treatment data were expressed relative to his own baseline values, the percentage increment in the two groups of volunteers was not significantly different (see text).

day 7. In the normal volunteers, in whom the magnitude of the changes was smaller, it frequently took several days before a downward trend could be clearly distinguished from random fluctuations in the data. Nevertheless, with the exception of the two individuals noted above, the mean concentration of $\overline{BR}$ during the second radio-bilirubin study was significantly lower than during the initial study (Student's t-test: $P < 0{\cdot}01$ for each of the 19 patients with Gilbert's syndrome, 4 phenobarbital-treated normal controls and 3 glutethimide-treated control subjects; $P < 0{\cdot}05$ for the remaining 2 glutethimide-treated controls). During the drug study, $\overline{BR}$ averaged 30 ± 2 per cent (mean $\pm$ S. E. M.) of baseline in the 19 patients with Gilbert's syndrome, and 65 ± 6 per cent of baseline in the 11 normal volunteers (Fig. 1). In contrast, $\overline{BR}$ was 96 ± 4 per cent of baseline during the second of the replicate studies in the group of 8 untreated control subjects. When the data obtained during drug administration were expressed as a per cent of the control values, there was no significant difference in the effects of glutethimide, as compared with phenobarbital, on $\overline{BR}$ ($P > 0{\cdot}8$) or any of the other physiologic parameters determined during the study.

The effects of drug administration on plasma radio-bilirubin disappearance curves in four typical subjects are illustrated in Fig. 2. Both phenobarbital and glutethimide produced alterations in the shape of the plasma radio-bilirubin disappearance curves. These alterations were reflected in changes in the slopes and intercepts of the computer-fitted radio-bilirubin disappearance functions from which plasma BRT and

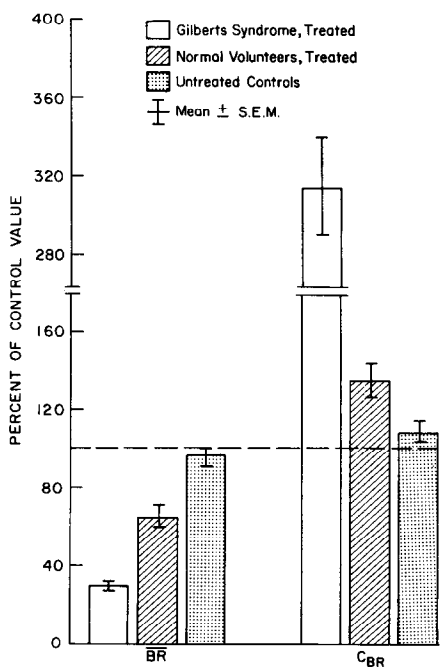

FIG. 1. Effects of phenobarbital and glutethimide administration on the plasma unconjugated bilirubin concentration ($\overline{BR}$) and bilirubin clearance (C_{BR}) in normal volunteers and patients with Gilbert's syndrome. Results obtained during drug administration are expressed as a per cent of the baseline values. Results of replicate studies in 8 untreated individuals indicate the reproducibility of the method.

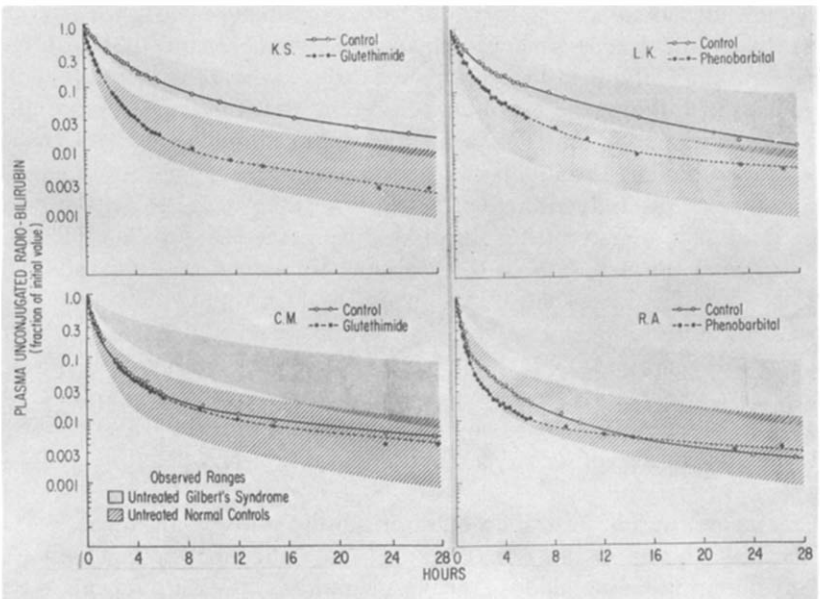

FIG. 2. Effects of phenobarbital and glutethimide administration on plasma radio-bilirubin disappearance curves in patients with Gilbert's syndrome (upper panels) and normal volunteers (lower panels). Stippled area represents observed range in untreated patients with Gilbert's syndrome; diagonal shading indicates the observed range in untreated normal volunteers.

hepatic C_{BR} are calculated.[11] The effects of drug administration on calculated values for C_{BR} and BRT are illustrated in Figs. 1 and 3.

Experimental values for C_{BR} both before and during drug administration are presented in Table 1. In the patients with Gilbert's syndrome, in whom a reduction in bilirubin clearance is a characteristic abnormality,[12] C_{BR} increased to 314 ± 25 per cent of baseline during drug administration (phenobarbital 325 ± 30 per cent; glutethimide 305 ± 33 per cent; $p > 0.6$) (Fig. 1), achieving values within the normal range in 18 of 19 individuals. In normal volunteers, C_{BR} during drug administration increased to 135 ± 9 per cent of baseline (phenobarbital 151 ± 19 per cent; glutethimide 116 ± 13 per cent; $P > 0.1$). Not only were the effects of drug administration greater in the Gilbert's syndrome group than in the normal volunteers, but among the normal volunteers alone there was a significant negative correlation between the baseline value for C_{BR} and the percentage change observed during drug treatment (glutethimide: $r = -0.95$, $0.05 > P > 0.01$; phenobarbital: $r = -0.77$, $0.1 > P > 0.05$). The mean value for C_{BR} in all of the patients with Gilbert's syndrome during phenobarbital and glutethimide administration (0·51 ± 0·04 ml/min/kg) was similar to the baseline value in the normal volunteers (0·57 ± 0·03 ml/min/kg). This value in normal subjects increased to 0·75 ± 0·05 ml/min/kg during drug administration.

Control values for BRT in the 19 patients with Gilbert's syndrome (7·8 ± 0·5 μmoles/kg/day) were significantly greater than the control value in the 11 normal volunteers (6·4 ± 0·4 μmoles/kg/day; $P < 0.05$), reflecting the presence of patients with mild hemolysis in the Gilbert's syndrome group (Table 1). Values for BRT fell by approximately the same proportion during drug administration in both the patient group and

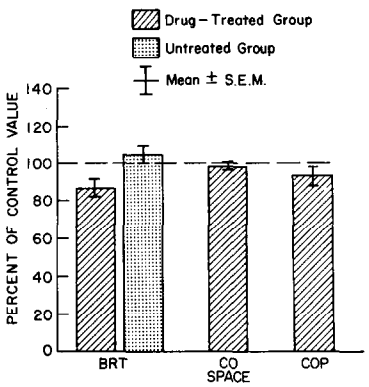

FIG. 3. Effects of phenobarbital and glutethimide administration on plasma bilirubin turnover (BRT), the CO space, and endogenous carbon monoxide production (COP). Data include both normal volunteers and patients with Gilbert's syndrome.

the normal volunteers, but the value during treatment in the patients with Gilbert's syndrome remained significantly greater than in the volunteers (6·5 ± 0·4 vs 5·3 ± 0·3 μmoles/kg/day, respectively; $P < 0.05$), again reflecting the presence of continued hemolysis in some members of the Gilbert's syndrome groups. When considered relative to baseline values, BRT had fallen to an average of 87 ± 4 per cent of baseline in the total population of 30 treated individuals when studied at their new steady state bilirubin concentration (Gilbert's syndrome: 86 ± 5 per cent; normal volunteers: 87 ± 10 per cent; $P > 0.9$) (Fig. 3). This value is significantly less than 100 per cent ($P < 0.05$) and is also significantly less than the 104 ± 5 per cent of baseline ($P < 0.05$) observed in a group of replicate studies in 8 untreated individuals previously performed in our laboratory to determine the reproducibility of the method. Evidence that the fall in BRT does not result from drug-induced alterations in the rate of destruction of circulating red cells is suggested by the fact that: (1) the total red cell volume at the time of the second radio-bilirubin turnover study averaged 97 ± 1 per cent of baseline; and (2) the ^{51}Cr-red cell half-life determined during drug administration averaged 103 ± 5 per cent of the value during a baseline period in the 15 individuals in whom this parameter was determined twice.

The CO space during drug administration averaged 98 ± 2 per cent of baseline in the 25 individuals in whom paired CO studies were performed. Similarly, carbon monoxide production (COP) at the time of the second study averaged 93 ± 5 per cent of baseline ($0.2 > P > 0.1$) (Fig. 3), falling from 9·1 ± 0·5 to 8·6 ± 0·6 μmoles/kg/day in the 19 patients with Gilbert's syndrome, and from 8·9 ± 0·6 to 8·2 ± 1·0 μmoles/kg/day in the 11 volunteers. While the data are, therefore, suggestive of a decrease in CO production during glutethimide and phenobarbital administration, the fall is not statistically significantly different from baseline, and is not as large as the 14 per cent fall seen in BRT. Nevertheless, our results are significantly different from those of Coburn,[27] who reported an increase in COP to 174 per cent of baseline after 7 days of phenobarbital administration. When values were expressed as percentage change during drug administration, there were no significant differences in the effects of drug on either CO space or COP when phenobarbital was compared with glutethimide, or when normal volunteers were compared with the Gilbert's syndrome group.

The results of serial determinations of CO space and COP in a single individual during both control and phenobarbital administration periods are presented in Table 2. Phenobarbital produced no changes in the CO space. After 3 days of phenobarbital, COP was increased by 5 per cent above the mean of the three control studies, but this increase is within the range of cumulative analytical error for the procedure employed. In contrast to previously reported data,[27] COP had decreased, rather than increased, after 7 days of phenobarbital administration. Results after 7 days were, in fact, similar to those observed after 14 days of phenobarbital treatment.

DISCUSSION

The size of the plasma pool of unconjugated bilirubin—and hence, the plasma unconjugated bilirubin concentration—reflects the balance between two processes. One of these is the rate of hepatic C_{BR}. The other is the rate at which newly synthesized bilirubin enters the plasma pool. In the steady state, the latter is equivalent to plasma BRT. The methodology employed in this study is unique in that the use of radiobilirubin tracer techniques permits simultaneous determination of both C_{BR} and BRT in the intact individual.

Effects of phenobarbital and glutethimide on hepatic bilirubin clearance

Our results indicate that the principal factor responsible for the reduction in plasma unconjugated bilirubin concentration during phenobarbital or glutethimide administration is the acceleration of hepatic bilirubin clearance. This accounts for more than 95 per cent of the reduction in bilirubin concentration observed in patients with Gilbert's syndrome, and an average of 70 per cent of the reduction observed in normal volunteers. Although it has been generally assumed that acceleration of hepatic extraction of bilirubin is the cause of the reduced plasma bilirubin concentrations observed during phenobarbital administration, data *in vivo* in support of this supposition are limited to studies in a single child with type II congenital nonhemolytic jaundice[30] and to preliminary reports of our own data[10,31] and those of Black *et al.*[32] Thus, the present study is the first to report in detail on the quantitative alterations in hepatic bilirubin clearance produced by drugs in a large number of individuals.

The observation of an inverse relationship between baseline values for bilirubin

TABLE 2. EFFECTS OF PHENOBARBITAL ADMINISTRATION ON REPLICATE MEASUREMENTS OF CO SPACE AND CO PRODUCTION IN A SINGLE SUBJECT*

Study	Date	Days of phenobarbital administration	CO space (ml/kg)	Carbon monoxide production (μmoles/kg/day)
Control 1	10/2/72		16·8	12·5
Treatment 1	10/6/72	3	16·4	13·2
Treatment 2	10/10/72	7	16·7	9·3
Treatment 3	10/17/72	14	17·0	8·8
Control 2	11/28/72		16·6	12·2
Control 3	11/29/72		16·8	12·9

* Phenobarbital was administered from 10/3/72 to 10/17/72. Control studies 2 and 3 were performed 6 weeks after cessation of phenobarbital administration.

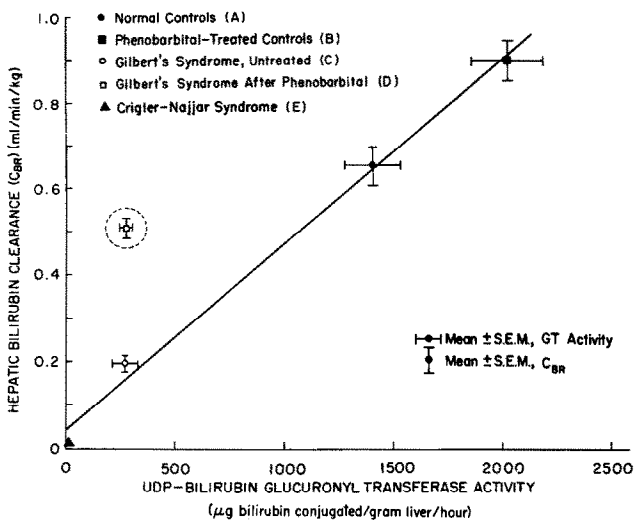

FIG. 4. Comparison of mean values for hepatic bilirubin clearance (C_{BR}) and bilirubin UDP-glucuronyl transferase activity in patients with type I Crigler–Najjar syndrome, Gilbert's syndrome and normal controls. For the latter two categories, data are presented for both untreated and phenobarbital-treated subjects. All values for C_{BR}, and the glucuronyl transferase values in Crigler–Najjar syndrome are from the National Institutes of Health. The remaining glucuronyl transferase values are from the published data of others.[2,41–44] Line represents a least squares fit to the mean values for groups A, B, C and E (see figure).

clearance and the percentage increase in this parameter achieved during drug administration suggests that the drug effects are related to induction of a protein whose level is under genetic control.[33] Because a number of different proteins are involved in the over-all bilirubin transport process, several possible mechanisms are compatible with this hypothesis. In experimental animals, administration of phenobarbital at high doses increases total liver weight,[6] stimulates bile flow,[34,35] increases the level of the Y intrahepatic organic anion binding protein,[36] and markedly augments hepatic UDP–bilirubin glucuronyl transferase activity,[37–39] all of which would be expected to increase hepatic bilirubin clearance. Phenobarbital therapy also increases the relative hepatic storage capacity for organic anions,[36] which might lower the plasma bilirubin concentration by altering the distribution of bilirubin between the plasma and intrahepatic unconjugated bilirubin pools in favor of the liver. The influence of glutethimide on all of these parameters has not been studied; however, its effect on UDP–glucuronyl transferase is similar to that of phenobarbital.[39]

The data presented above do not, in and of themselves, indicate which of these possible mechanisms contribute to the increased bilirubin clearance produced by these agents. Useful information may be obtained, however, by comparing values for bilirubin clearance with independent measurements of specific aspects of hepatic bilirubin transport. In Fig. 4, values for C_{BR}, determined isotopically in our laboratory in various groups of untreated and phenobarbital-treated individuals, are plotted against measurements *in vitro* of hepatic bilirubin UDP-glucuronyl transferase (UDPGT) activity. The UDPGT data were obtained in analogous populations in our own laboratory and from the published data of Black and Billing,[40] Billing and Black,[41] Black *et al.*[42] and Felsher *et al.*[43] The linear relationship observed between C_{BR} and UDPGT activity in untreated patients with type I Crigler–Najjar syndrome

(congenital non-hemolytic jaundice due to complete glucuronyl transferase deficiency) and Gilbert's syndrome, untreated normal controls and phenobarbital-treated normal individuals suggests that differences in the activity of UDPGT are related to the differences in C_{BR} observed among these four groups. However, it is unlikely that drug-induced changes in UDPGT provide a complete explanation in all instances for the increases in C_{BR} observed during drug administration. In patients with Gilbert's syndrome, in particular, phenobarbital administration produced a more than 3-fold increase in bilirubin clearance, whereas measurements of UDPGT revealed little[2] or no[43] increase in enzyme activity during drug administration (Fig. 4). Although the duration of drug administration prior to the UDPGT determination was as little as 3 days in some of these patients,[43] this was a sufficient time to produce clear-cut reductions in bilirubin concentration in the present study, and an appreciable augmentation of UDPGT activity in experimental animals.[39] Hence, these observations suggest that the large increases in C_{BR} observed during drug administration reflect, at least in part, changes in other aspects of the hepatic organic anion transport system. An alternate explanation, which can by no means be excluded at this time, is that the UDPGT assay correlates poorly with enzyme activity, *in vivo*, possibly because it fails to simulate physiologic conditions such as substrate, cofactor and metabolite concentrations.[44]

Effects of phenobarbital and glutethimide on bilirubin turnover and carbon monoxide production

Phenobarbital treatment of experimental animals has been shown to increase the incorporation of precursors into hepatic microsomal heme and heme proteins,[45] to augment the activities of certain heme enzymes such as cytochrome P_{450},[45–47] and to increase the incorporation of glycine-2-^{14}C into early labeled bilirubin and CO.[47,48] These data suggested the possibility that drug therapy would be associated with an increase in the molar production of bilirubin and CO. This possibility appeared to be substantiated by a reported 2-fold increase in bile bilirubin excretion in phenobarbital-treated bile fistula rats,[47] and significant increases in both the CO space and CO production in human subjects treated with either phenobarbital or dilantin for 7 days.[27]

Our own data provide no support for the hypothesis that phenobarbital or glutethimide administration is associated with an increase in bilirubin or carbon monoxide production. In our studies, both plasma bilirubin turnover and CO production actually fell during drug administration, and the CO space was unchanged. The fall in BRT was appreciable enough to account for an average of 30 per cent (range 0–100 per cent) of the observed reduction in bilirubin concentration in normal volunteers. The possibility that a fall in the load of bilirubin presented to the liver (BRT) might contribute to the reduction in plasma bilirubin concentration during phenobarbital administration has not previously been considered and represents an important new observation. The reason for the discrepancy between our own observations on CO production and those of Coburn[27] is unclear. Coburn employed a phenobarbital dose of 100 mg/day, whereas 180 mg/day were employed in the current study. In addition, he used an infra-red, rather than a gas chromatographic method for measuring CO in blood samples. It seems unlikely that either of these factors is responsible for the conflicting results obtained in the two investigations.

Furthermore, the data in Table 2 clearly indicate that the difference in the duration of therapy employed in the two studies does not account for the difference in results.

The doses of phenobarbital employed in our studies in man (2–4 mg/kg/day) are far below those used to induce alterations in hepatic heme metabolism in rats (75–120 mg/kg/day), although they have, in fact, been shown to be adequate to produce a significant increase in hepatic cytochrome P_{450} in a surgical population.[42] Nevertheless, this dose difference does not appear to account for our failure to detect increased bilirubin or CO formation. More recent studies of bile bilirubin excretion[46,49] or radio-bilirubin turnover[50] in rats given phenobarbital have failed to confirm the earlier observations and have indicated that phenobarbital administration is not, in fact, associated with an increase in the molar production of bilirubin. The mechanism by which phenobarbital increases the incorporation of labeled precursors into hepatic microsomal heme and heme proteins, as well as early labeled bilirubin and CO, without producing an increase in the molar production of these two products remains to be determined. A number of plausible explanations for this observation have recently been reviewed by Schmid.[51]

One further aspect of the data is of interest. It has previously been reported that, in the absence of drug therapy, paired measurements of COP and BRT are highly correlated ($r = 0.99$), and are linked by the relationship COP = BRT + constant.[9] In 37 untreated subjects, the ratio of COP/BRT was 1.14 ± 0.03. The small discrepancy between COP and BRT was postulated to result from the direct biliary excretion of some of the bilirubin formed in the hepatocytes from the catabolism of microsomal heme enzymes. To the extent that some of this bilirubin does not enter the plasma prior to biliary excretion, measurements of plasma bilirubin turnover will underestimate total bilirubin production and hence, heme catabolism.

In the present study, administration of glutethimide and phenobarbital was associated with a greater reduction in BRT (87 ± 4 per cent of baseline) than in COP (93 ± 5 per cent of baseline) further widening the difference between these two measurements of heme turnover. While the ratio of COP/BRT during drug administration is not significantly different than the baseline ratio, the data suggest that these agents may increase the efficiency of the liver in excreting the bilirubin of hepatocellular origin directly into the bile without prior reflux to the plasma. This hypothesis is presently being tested in our laboratory using a recently reported double isotope procedure[52] to investigate the effect of these two agents on the incorporation of radioactive δ-aminolevulinic acid into both fecal bile pigment and the plasma unconjugated bilirubin pool.

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Note added in proof—Subsequent to the acceptance of this manuscript for publication, similar observations on the effects of phenobarbital on plasma radiobilirubin disappearance curves were published by Black *et al.*[53]