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deference to Japanese colleagues who have asked if the original abbreviation can be retained rather than the more recently used MLNS.

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Various Bilirubin Conjugates in Pregnant and Nonpregnant Rats with and without Phenobarbital Treatment

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Extract

The relative participation of bilirubin conjugating systems other than uridine diphosphate glucuronic acid-dependent bilirubin glucuronyl transferase in 21-day pregnant (P) and nonpregnant (NP) rats with and without prior phenobarbital (PB) administration was studied. Relative enzyme activities of 74.5%, 17.6%, and 7.9% were observed for glucuronic acid, xylose, and glucose conjugates, respectively, in untreated NP rats. Pregnancy itself did not alter the relative activities. In response to PB administration, P rats increased activity less than NP animals for all three enzymes. The ratios of the three conjugates remained unchanged in both P and NP groups after PB stimulation.

Speculation

The mechanisms by which pregnancy reduces the stimulatory effect of phenobarbital on bilirubin glucuronyl transferase in the rat while not altering unstimulated enzyme activity remain unexplained.

The induced enzyme may be different from the noninduced enzyme and may, therefore, be susceptible to inhibitors present in increasing concentrations during pregnancy, inhibitors to which the noninduced enzyme is not susceptible. Alternatively, the induced enzyme may be the same as the noninduced enzyme but the reduced stimulation observed may result from either changes in phenobarbital metabolism during pregnancy or a reduced capacity to synthesize enzyme protein during pregnancy.

Phenobarbital administration to adult rats markedly increases the formation of bilirubin conjugates with glucuronic acid *in vitro*. The degree of response of maternal hepatic bilirubin glucuronide formation to PB stimulation during the second half of pregnancy has recently been found to be significantly less than in nonpregnant or early pregnant rats, despite similar hepatic phenobarbital concentrations (8). At 21 days of gestation (term) the increase in glucuronyl transferase activity was approximately one-third of that in early pregnancy (8). Although hepatic conjugation of bilirubin in mammalian species is predominantly with glucuronic acid to

form the water-soluble, excretable form of bile pigment, significant quantities of glucose and xylose conjugates have also been identified in bile (1). Therefore, it was postulated that with the advance of pregnancy, there was a shift in PB-stimulated conjugation of bilirubin from predominantly that with glucuronic acid to that with xylose and/or glucose. Because our previous studies were performed only with addition of uridine diphosphate glucuronic acid to the incubation medium, relatively greater increases in glucose and xylose conjugates would have gone undetected.

The purpose of this study, therefore, was to explore the relative participation of bilirubin conjugating systems other than uridine diphosphate glucuronic acid (UDP-GA)-dependent bilirubin glucuronyl transferase in 21-day P and NP rats with and without prior PB administration.

MATERIALS AND METHODS

Sprague-Dawley 18-day P and NP female rats weighing between 270 and 340 grams were obtained from Marland Breeding Farms, N. J. The animals were handled, housed, fed, treated with PB (100 mg/kg/24 hr s.c. for 3 days) and killed in exactly the same manner as described in our previous study (8). Livers were removed immediately after time of killing, washed in cold 0.154 M KCl, blotted, and weighed. Homogenates (8%) were prepared in cold 0.154 M KCl using a Teflon pestle. Bilirubin transferase assays were done immediately using whole liver homogenates (0.1 cc) (digitonin activated) with bilirubin as substrate and either UDP-GA (10) (6.19×10^{-2} M), uridine diphosphate xylose (UDP-X) (10) (2.49, 4.98, 7.46, 14.92, and 22.38×10^{-2} M), or uridine diphosphate glucose (UDP-G) (10) (2.19, 4.38, 6.56, 13.2, 19.7, and 26.2×10^{-2} M). Optimal concentrations were established at 7.46×10^{-2} M UDP-X and 6.56×10^{-2} M UDP-G and used in a micromodification of the previous method (3). Each sample was run in duplicate. Intensity of color formed was determined spectrophotometrically at 525 nm using quartz microcells with a 1-cm light path in a Perkin-Elmer double beam spectrophotometer with a final total volume of 0.92 cc. Uridine diphosphate glucuronyl transferase (UDP-GT), uridine diphosphate xylosyl

transferase (UDP-XT), and uridine diphosphate glucosyl transferase (UDP-GST) activities were expressed as micrograms of bilirubin conjugates per g liver per 40 min after correction for blank (no UDP-GA, UDP-X, or UDP-G). The reactions are linear throughout this incubation period. Total liver protein was measured according to the method of Lowry (6). All results are expressed as the mean value $\pm$ SE. The Student t-test was used for determination of statistically significant differences between groups at a significance level of $P < 0.05$ for a two-tailed test (7).

RESULTS

The data obtained from studies of 22 rats are presented in Figures 1 and 2. The number of animals in each group is presented in Table 1.

UDP-GT (GLUCURONIC ACID)

Untreated NP and P rats had similar mean activities: $1,271 \pm 78$ (SE) μ g bilirubin conjugated per liver per 40 min and $1,303 \pm 95$, respectively. After PB administration mean enzyme activity in the NP group increased 60.9% to $2,046 \pm 88$, whereas that of P rats increased by only 24.2% to $1,619 \pm 83$. In both groups the increase was significant ($P < 0.02$), but P animals had significantly lower activity ($P < 0.05$) than NP rats after PB treatment.

UDP-XT (XYLOSE)

Untreated animals, NP and P, also had similar mean levels of enzyme activity (NP 295 ± 31 ; P 318 ± 20). After PB treatment UDP-XT increased 25% to 369 ± 34 in the NP group and only 7%

to 339 ± 31 in the P group. Neither increase is significant ($P > 0.05$), however.

UDP-GST (GLUCOSE)

Untreated NP and P rats had similar mean enzyme activities (134 ± 16 and 142 ± 19 , respectively). After PB administration activity for NP rats increased 69% to 227 ± 27 and for P rats increased 41% to 200 ± 5 . Both increases are significant ($P < 0.02$).

RELATIVE CONJUGATING ENZYME ACTIVITIES

The percentage of total conjugating capacity attributable to each of the three enzymes studied was similar in each of the four groups of animals (Table 1). Thus, neither pregnancy nor administration of phenobarbital altered the relative capacities to form the three conjugates of bilirubin.

DISCUSSION

In 1970 Heirwegh *et al.* (4) reported a thin layer chromatographic technique for the identification of azopigments derived from conjugated bile pigments. They found that 30–40% of the bilirubin conjugates in dog bile were with glucose and xylose (2), and that microsomal preparations from rat liver conjugated bilirubin with glucuronic acid, xylose, and glucose *in vitro* after

Fig. 1. Hepatic uridine diphosphate glucuronyl transferase (UDPGT), uridine diphosphate xylosyl transferase (UDPXT), and uridine diphosphate glucosyl transferase (UDPGST) activity in untreated nonpregnant ($n = 6$) and pregnant ($n = 5$) rats expressed as micrograms of bilirubin (*bil*) conjugated per g liver per 40 min. Total conjugating capacity = sum of the three enzyme activities. Results presented as mean of each group $\pm$ SE.

Fig. 2. Increase in hepatic activity of uridine diphosphate glucuronyl transferase (UDPGT), uridine diphosphate xylosyl transferase (UDPXT), and uridine diphosphate glucosyl transferase (UDPGST) after 3 days of phenobarbital treatment of nonpregnant ($n = 6$) and pregnant ($n = 5$) rats. Final total conjugating capacity = sum of the three enzyme activities. Results presented as mean of each group $\pm$ SE.

Table 1. Percentage distribution of uridine diphosphate glucuronyl transferase (UDP-GT), uridine diphosphate xylose transferase (UDP-XT), and uridine diphosphate glucosyl transferase (UDP-GST) in pregnant and nonpregnant rats with and without phenobarbital (PB) treatment¹

	<i>n</i>	UDP-GT	UDP-XT	UDP-GST	Total
Nonpregnant untreated	(6)	74.5 $\pm$ 3.2	17.6 $\pm$ 2.3	7.9 $\pm$ 1.1	100
Nonpregnant PB	(6)	77.1 $\pm$ 2.1	14.2 $\pm$ 1.6	8.7 $\pm$ 1.1	100
Pregnant untreated	(5)	73.4 $\pm$ 3.4	18.4 $\pm$ 2.1	8.2 $\pm$ 1.5	100
Pregnant PB	(5)	75.1 $\pm$ 2.6	15.7 $\pm$ 1.1	9.2 $\pm$ 2.4	100

¹ Mean $\pm$ SE.

activation with digitonin at relative proportions of 61.3%, 23.3%, and 15.4%, respectively (5). With our somewhat different assay system and the use of whole liver homogenates after digitonin activation we found relative values of 74.5%, 17.6%, and 7.9% for UDP-GT, UDP-XT, and UDP-GST, respectively, in untreated NP rats (Table 1). Pregnancy itself did not alter the relative distribution of activities. Total conjugating capacity *in vitro* calculated by summation of the three enzyme activities was the same in untreated NP and P rats (Fig. 1).

In response to PB administration, activity for term P rats increased less than for NP animals (Fig. 2) for all three enzymes. Total conjugating capacity after PB treatment increased significantly in both the P and NP rats, but the increase was significantly less in the P animals (Fig. 2). The ratios of the conjugates remained unchanged in both P and NP groups after PB stimulation (Table 1).

Thus, this study has demonstrated that the relative lack of response of glucuronic acid conjugation of bilirubin to PB during late pregnancy is not compensated for by an increase in either glucose or xylose conjugation. In fact, the response of the latter two conjugates to phenobarbital stimulation was depressed by pregnancy in a manner similar to the previously demonstrated effect of pregnancy on stimulated glucuronide conjugation. Bilirubin may be conjugated with other carbohydrate moieties and with sulfate to form more polar compounds (5, 9). These other conjugates account for a relatively small proportion of total bilirubin conjugates in bile and have not yet been studied in the pregnant rat.

SUMMARY

Three different bilirubin transferase activities were measured using whole liver homogenates obtained from 18-day pregnant and nonpregnant female Sprague-Dawley rats with and without phenobarbital treatment (100 mg/kg/24 hr for 3 days) and UDP-GA, UDP-X, and UDP-G as substrates.

Total conjugating capacity obtained by summation of the three enzyme activities (UDP-GT, UDP-XT, and UDP-GST) was the same in untreated nonpregnant and pregnant rats. In response to phenobarbital treatment, the total conjugating capacity was increased significantly in both pregnant and nonpregnant rats with

the same proportion of each of the three enzyme activities as in the control animals. In response to phenobarbital, however, total conjugating capacity in pregnant rats was significantly less than in nonpregnant rats. Thus, the inhibitory effect of pregnancy on induction of glucuronic acid conjugation of bilirubin *in vitro*, as previously demonstrated, also extends to xylose and glucose conjugation.

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