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EFFECTS OF PREGNANCY ON THE METABOLISM OF DRUGS IN THE RAT AND RABBIT

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Abstract—In rats 19–20 days pregnant, liver weight is increased by 40 per cent, cytochrome P-450 concentration is decreased by 25 per cent and the specific activities of 4-methylumbelliferone glucuronyl transferase and biphenyl-4-hydroxylase are reduced by 25 and 30 per cent, respectively; biphenyl-2-hydroxylase and *p*-nitrobenzoic acid reductase are not changed. In rats, 15–16 days pregnant, liver weight is increased by 33 per cent but the concentration of cytochrome P-450 and the specific activities of the drug microsomal enzymes are unchanged. Expressed as total amounts per whole liver, there is an increase in microsomal protein and nitro-reductase in both 15–16 and 19–20 day pregnant animals but no changes occur in cytochrome P-450, glucuronyl transferase or biphenyl hydroxylases.

Hexobarbital administered to rats at doses related to pregnant body weight increases the sleeping-time from 50 min in non-pregnant animals to 110 min at full-term, but when administered on the basis of the non-pregnant body weight the duration of anaesthesia remains unchanged.

Pretreatment of pregnant (19–20 days) and non-pregnant rats with phenobarbital leads to similar increases in microsomal protein (25 per cent) and nitroreductase activity (40 per cent); cytochrome P-450 is increased in non-pregnant animals (30 per cent) but not in the pregnant, although biphenyl-4-hydroxylase is increased in both to such extents as to annul the inhibitory effect of pregnancy. Pretreatment with methylcholanthrene gives rise to similar increases in cytochrome P-450 (30 per cent) and biphenyl-2-hydroxylase (10-fold increase) in both pregnant and non-pregnant rats and again increases biphenyl-4-hydroxylase so as to annul the effect of pregnancy.

With rabbits, no change occurs in liver weight, microsomal protein, nitro-reductase, cytochrome P-450, or biphenyl-4-hydroxylase at full-term pregnancy, but glucuronyl transferase is reduced by 20 per cent, and coumarin-7-hydroxylase by 60 per cent. Pretreatment of rabbits with phenobarbital increases microsomal protein (15, 25 per cent), nitro-reductase (70, 80 per cent), cytochrome P-450 (130, 90 per cent), biphenyl-4-hydroxylase (50, 60 per cent), coumarin-7-hydroxylase (40, 150 per cent), and glucuronyl transferase (65, 15 per cent) in both non-pregnant and pregnant animals, respectively.

The decrease during pregnancy of hepatic glucuronyl transferase is attributed to competitive inhibition by high levels of endogenous estrogenic and progestational steroids, but the decrease in the activities of the microsomal hydroxylating enzymes is attributed to the decrease in cytochrome P-450, which may result from high levels of growth factors.

THE METABOLISM of drugs may involve conjugations and biotransformation reactions such as hydroxylations,[1–3] and is known to be affected by many factors including pregnancy.[4] The conjugation of drugs and xenobiotics with glucuronic acid to form glucuronides was first shown to be inhibited during pregnancy by Cessi,[5] using experimental animals, and this was subsequently attributed to inhibition of glucuronyl transferase(s) by the high circulating levels of pregnanediol and other progestational and oestrogenic steroids that occur in experimental animals and humans during pregnancy.[6–8] Conjugation with sulphate has also been shown to be decreased during pregnancy, in the rat, and is attributed to the inhibitory effect of the high levels of oestrogens.[9] The inhibitory effects of pregnancy and progestogens on the glucuronide

conjugation of drugs were confirmed by Creaven and Parke[10] who further showed that during pregnancy in rats and rabbits the hydroxylation of certain drugs also was inhibited. A limited study in humans suggested that the metabolism of pethidine and promazine, drugs which are metabolized by hydroxylation reactions as well as by conjugations, were also impaired during pregnancy.[11]

This impairment of the biotransformation of drugs has been confirmed by other workers[12,13] and shown not to be due to inhibitors. In previous preliminary studies we[14] have shown that the inhibition of the hydroxylation reactions may be due to a decrease in the hepatic level of the hydroxylating enzyme, cytochrome P-450, and this has been confirmed by other workers.[15] In view of the importance of metabolism to the pharmacokinetics, pharmacodynamics and toxicity of drugs, and the far reaching implications that inhibition of this metabolism could have, a more detailed study of the effects of pregnancy on the activities of the hepatic enzymes concerned in drug metabolism seemed necessary. The present study deals with the effects of pregnancy on the levels of hepatic cytochrome P-450 and on the activities of a representative group of liver microsomal drug-metabolizing enzymes in the rat and rabbit.

EXPERIMENTAL

Materials

Biphenyl, and 2- and 4-hydroxybiphenyl (British Drug Houses Ltd.) were purified as described by Bridges *et al.*,[16] coumarin (B.D.H. Ltd.) and 7-hydroxycoumarin (Hopkins & Williams) were recrystallized from water (carbon); *p*-nitrobenzoic acid and *p*-aminobenzoic acid (B.D.H. Ltd.) were recrystallized from aqueous ethanol; and 4-methylumbelliferone (Koch–Light) was recrystallized as described by Mead *et al.*[17] Nicotinamide adenine dinucleotide phosphate (NADP), flavine mononucleotide (FMN) and uridine diphosphate glucuronic acid (UDPGA) (Sigma Chemical Co.) and the disodium salt of glucose-6-phosphoric acid (Koch–Light) were used without further purification. 4-Methylumbelliferone glucuronide was prepared as described by Woollen and Walker[18] from acetobromomethylglucuronate[19] and 4-methylumbelliferone.

Animals

Specific-pathogen-free, female, nulliparous (unless stated) Wistar albino rats, kept in a room with no other species, under controlled heating and lighting conditions at ground floor level on "Steralit bedding in plastic cages, were fed on Spiller's autoclaved small animal diet and water *ad lib.* Mating was carried out by placing two proven male breeders into a large cage containing four or five females (all of the same age $\pm$ 1 day, and 200–300 g body wt) during the late afternoon and leaving for 36–38 hr. The day of removal of the males was considered to be the second day of gestation in those animals that were later found to be pregnant. Pregnancy was verified by palpation of the abdomen after 7–10 days and by body weight changes. Rats that were not pregnant served as controls. By this method over half of the rats became pregnant and the time of mating was known to within one day. The effects of pregnancy were studied at 15–16 days and 19–20 days gestation, at which times the animals weighed 250–400 g.

Rabbits, New Zealand white does (Westwood Hybrid Table Rabbits Ltd., Plymouth) 3·0–4·5 kg were housed in individual cages in a room with no other species

and were fed on a diet of Dixon's rabbit pellets and water *ad lib.* Pregnant rabbits of the same strain and age, 4·0–5·0 kg were obtained from the same source, being despatched 1 week before expected parturition.

Methods

Preparation of liver fractions. Animals were killed by a blow on the head followed by cervical dislocation, always at the same time of day (10 a.m.). Livers were rapidly removed, chilled in ice-cold 1·15% w/v KCl solution, chopped and weighed, and portions homogenized in a glass Potter–Elvehjem homogeniser with a loose-fitting power-driven Teflon pestle using a total of three up and three down strokes over a period of 15 sec. The homogenate was diluted to 25% w/v with ice-cold 1·15% KCl and centrifuged at 10,000 *g* for 10 min in plastic tubes at 1–4° in an MSE High Speed 18 centrifuge. The supernatant was decanted for use for the enzyme assays, or was further centrifuged at 105,000 *g* for 1 hr at 1–4° in an MSE Superspeed 50 to obtain the microsomal fraction. The supernatant was decanted, the pellet rinsed with a few drops of 1·15% KCl solution to remove adhering supernatant and resuspended in fresh 1·15% KCl solution by gentle homogenisation, to give the microsomal fraction.

Protein concentrations were determined by a modified method of Lowry *et al.*[20]

Determination of aromatic hydroxylation. Hepatic microsomal aromatic hydroxylation activity was measured by determination of three different enzymic activities, namely, the 7-hydroxylation of coumarin, and the 2- and 4-hydroxylation of biphenyl. The 7-hydroxylation of coumarin, which is present in rabbit but not rat liver, was measured by the method of Creaven *et al.*,[21] except that nicotinamide and glucose-6-phosphate were omitted and the concentration of NADP was increased to 1·0 mM. For the determination of the hydroxylation of biphenyl in rabbit liver the method of Creaven *et al.*[22] was followed but for rat liver the incubation conditions were modified as follows: liver 10,000 *g* supernatant (0·5 ml) was incubated with 3 μmole biphenyl (in 10% Tween 80), 10 μmole $MgCl_2$, 1·5 μmole NADP, 0·05 M Tris buffer pH 8·1 (0·5 ml) in a total volume (1·15% KCl) of 2·0 ml at 37° in a shaking incubation bath for 20 min in air. The fluorescence determinations were made as previously described[22] using a Baird Atomic Spectrophotofluorimeter.

Determination of nitro-reduction. Liver nitro-reductase activity was determined in the 10,000 *g* supernatant preparations by the method of Fouts and Brodie[24] as modified by Gingell[23] using *p*-nitrobenzoic acid as substrate.

Determination of glucuronide formation. Glucuronyl transferase activity of liver preparations was measured using a method based on that of Arias *et al.*[25] in which 4-methylumbelliferone was used as the aglycone substrate. Liver 10,000 *g* supernatant preparation, diluted 1:20 with 1·15% KCl (0·2 ml, equivalent to 2·5 mg liver), was incubated with 4-methylumbelliferone (0·6 μmole); UDPGA (2·0 μmole); 0·3 ml 0·1 M Tris–HCl buffer, pH 7·6, in a total volume of 1·0 ml (1·15% KCl) for 30 min in air at 37° in a shaking incubator. The reaction was terminated by rapid cooling, addition of 2·0 ml ice-cold water and shaking with chloroform (10 ml). After centrifugation to separate the phases the chloroform layer was removed and the aqueous layer was further extracted with chloroform (10 ml) to complete the extraction of unconjugated 4-methylumbelliferone. Aliquots, (0·5 ml) of the aqueous layer were then incubated with β-glucuronidase (500 Units "Ketodase" from William R. Warner & Co. Ltd.) and 0·1 M acetate buffer, pH 5·0 (1·5 ml) for 30 min at 37° in a shaking

incubator to hydrolyse the 4-methylumbelliferone glucuronide formed during the first incubation. For controls, similar aliquots were carried through the procedure without β-glucuronidase. After incubation, 0·5 M glycine buffer, pH 10·4 (5·0 ml) was added and the fluorescence determined at $\lambda_{excit.}$ 368 nm and $\lambda_{fl.}$ 450 nm in a Baird-Atomic spectrophotofluorimeter. The increase in fluorescence following incubation with β-glucuronidase was taken as the amount of 4-methylumbelliferone conjugated with glucuronic acid in the incubation with liver preparation. Blanks, in which the substrate was added after incubation, and standards of 4-methylumbelliferone glucuronide and 4-methylumbelliferone were also carried through this procedure.

Cytochrome P-450. This hepatic microsomal cytochrome was determined by the spectral difference method of Sladek and Mannering[26] using the 100,000 *g* washed liver microsomal preparation. Cytochrome P-450 content was calculated as nanomoles/milligram of microsomal protein using the molar extinction coefficient of 91 cm^{-1} nM^{-1} as determined by Omura and Sato.[27]

Hexobarbital sleeping time. Rats, weighed immediately before dosing, were given an intraperitoneal dose of hexobarbitone (100 mg/kg) unless otherwise stated as an aqueous solution. After losing consciousness each animal was laid on its back under an electric light for warmth. During the period of anaesthesia the tail of each animal was pinched every 5 min for the first 30 min, then subsequently every 2 min until the righting reflex was restored. This was considered to be when the rat could "right" itself immediately on three consecutive occasions after being laid on its back. The difference between the time of injection and the "righting" time was taken as the hexobarbital sleeping time.

RESULTS

Expressed as enzymic activity per unit liver weight, or activity per unit weight of protein, the biphenyl-4-hydroxylase, 4-methylumbelliferone glucuronyl transferase and cytochrome P-450 are significantly decreased in the 19–20 day pregnant rats though not in the 15–16 day pregnant ones (see Table 1). *p*-Nitrobenzoic acid reductase, biphenyl-2-hydroxylase, which is very low anyway, and microsomal protein are unchanged. However, during pregnancy the liver weight of rats is markedly increased (non-pregnant, 7·7 ± 0·6 g, 15–16 days pregnant, 10·3 ± 0·9 g; 19–20 days pregnant, 10·9 ± 0·4 g) so that the enzyme activities when expressed as content per whole liver do not show similar decreases (see Table 1). The total activities of cytochrome P-450, biphenyl 4-hydroxylase and 4-methylumbelliferone glucuronyl transferase are the same or greater in the pregnant rats than in the non-pregnant controls, while the total liver content of microsomal protein and *p*-nitrobenzoic acid reductase are significantly greater in both the 15–16 day and 19–20 day pregnant rats.

In view of the observed changes in the specific activities of certain hepatic microsomal enzymes and of the general liver enlargement in pregnancy it was of interest to determine whether the effects of enzyme inducers were also changed by this condition. The effects of pretreating 19–20 day pregnant rats and non-pregnant rats with phenobarbital or methylcholanthrene are shown in Table 2. The increases in enzyme activities produced by this treatment are not great, as the animals used were sexually-mature females and were considerably older (200–300 g body wt) than those normally used (50–100 g body wt) in induction studies. Only in the case of cytochrome P-450

TABLE 1. HEPATIC MICROSOMAL ENZYME ACTIVITIES AND PROTEIN CONTENT OF PREGNANT RATS

Enzyme	15–16 days pregnant‡				19–20 days pregnant‡			
	Activity/g liver		Activity/liver		Activity/g liver		Activity/liver	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Biphenyl-4-hydroxylase (μmole/hr)	4·1 ± 0·4	3·6 ± 0·3	31 ± 6	36 ± 7	3·3 ± 0·3	2·3 ± 0·3*	23 ± 4	24 ± 3
Biphenyl-2-hydroxylase (μmole/hr)	0·14	0·12			0·14	0·13		
p-Nitrobenzoic acid reductase (μmole/hr)	1·7 ± 0·3	2·3 ± 0·2	13 ± 3	24 ± 3†	2·0 ± 0·1	2·3 ± 0·2	17 ± 1	27 ± 4†
4-Methylumbelliferone-glucuronyl transferase (μmole/hr)					106 ± 6	78 ± 8†	910 ± 70	910 ± 110
Cytochrome P-450 (nmole)	21 ± 3	17 ± 1	146 ± 16	169 ± 9	21 ± 1	16 ± 1†	161 ± 9	168 ± 7
Microsomal protein (mg)	29 ± 1	31 ± 1	206 ± 13	314 ± 22*	29 ± 1	32 ± 1	202 ± 13	340 ± 19*

Rats were 19–20 days pregnant or non-pregnant (control). The mean values of 6–12 animals are given together with ± S.E.M. Significant differences from control animals are shown by * $P < 0.01$, † $P < 0.05$.

‡ Liver weight, expressed as per cent total body weight are as follows: 15–16 days pregnant, 3·3 ± 0·2; non-pregnant controls 2·9 ± 0·2; 19–20 days pregnant, 3·4 ± 0·1; non-pregnant controls, 3·3 ± 0·2.

TABLE 2. HEPATIC MICROSOMAL ENZYME ACTIVITIES AND PROTEIN CONTENT FROM PREGNANT RATS PRETREATED WITH ENZYME INDUCERS

Enzyme	Pretreatment	Non-pregnant	Pregnant
Biphenyl-4-hydroxylase (μmole/g liver/hr)	None	3·2 ± 0·2	2·6 ± 0·3
	Phenobarbital	4·8 ± 0·5	5·3 ± 0·6
	None	3·4 (3·2, 3·6)	2·6 ± 0·3
	Methylcholanthrene	6·2 (6·4, 6·0)	5·9 (6·7, 5·2)
Biphenyl-2-hydroxylase (μmole/g liver/hr)	None	0·2	0·2
	Phenobarbital	0·2	0·2
	None	0·2 (0·2, 0·2)	0·2
	Methylcholanthrene	2·3 (2·1, 2·6)	2·5 (2·8, 2·3)
p-Nitrobenzoic acid reductase (μmole/g liver/hr)	None	1·2 (1·3, 1·1)	1·5 (1·4, 1·6)
	Phenobarbital	1·7 (1·9, 1·5)	2·2 (2·3, 2·1)
Cytochrome P-450 (nmole/g liver)	None	25 ± 1	19 ± 1
	Phenobarbital	33 ± 3	22 ± 1
	None	19 (18, 19)	19 ± 1
	Methylcholanthrene	25 (24, 25)	24 (24, 24)
Microsomal protein (mg/g liver)	None	31 ± 1	32 ± 1
	Phenobarbital	39 ± 2	40 ± 2
	None	30 (30, 30)	32 ± 1
	Methylcholanthrene	30 (29, 31)	31 (32, 30)

Rats, 19–20 days pregnant or non-pregnant, were pretreated by intraperitoneal injection of an aqueous solution of sodium phenobarbitone (50 mg/kg body wt) a solution of methylcholanthrene (20 mg/kg body wt) in arachis oil, or with vehicle alone (none), daily for 3 days. Mean value of three animals are given ± S.E.M., or when only two animals were used the individual values are given in parentheses.

was the enzyme-inducing effect of phenobarbitone pretreatment diminished by pregnancy, and the effects of methylocholanthrene appeared to be unaffected by pregnancy. The effect of phenobarbitone on biphenyl-4-hydroxylase activity was to produce an increase such that the inhibitory effect of pregnancy was completely annulled.

Pregnancy in the rabbit was found to have somewhat different effects on the hepatic microsomal drug-metabolizing enzymes to those found in the rat (see Table 3). The only major difference between the pregnant and non-pregnant rabbits was in the 7-hydroxylation of coumarin, a reaction that does not occur in rats. In the pregnant rabbits the specific activity of this enzyme was decreased by 70 per cent. The specific activities of biphenyl-4-hydroxylase, *p*-nitrobenzoic acid reductase, 4-methylumbelliferase glucuronyl transferase, microsomal protein and cytochrome P-450, showed no significant differences between pregnant and non-pregnant animals, and as there was no significant increase in liver weight during the pregnancy of these rabbits

TABLE 3. HEPATIC MICROSOMAL ENZYME ACTIVITIES, AND MICROSOMAL PROTEIN CONTENT OF PREGNANT RABBITS PRETREATED WITH PHENOBARBITAL

Enzyme	Pretreatment	Non-pregnant	Pregnant
Biphenyl-4-hydroxylase (μmole/g liver/hr)	None	4·0 (0·3)[10]	4·6 (0·2)[5]
	Phenobarbital	5·9 (0·1)[3]	7·6 (6·6, 8·6)
Coumarin-7-hydroxylase (μmole/g liver/hr)	None	1·1 (0·2)[8]	0·4 (0·1)[4]
	Phenobarbital	1·5 (0·2)[3]	1·0 (0·9, 1·0)
p-Nitrobenzoic acid reductase (μmole/g liver/hr)	None	1·1 (0·2)[4]	1·3 (0·2)[3]
	Phenobarbital	1·9 (0·3)[3]	2·4 (2·6, 2·3)
4-Methylumbelliferone-glucuronyl transferase (μmole/g liver/hr)	None	160 (10)[3]	135 (15)[3]
	Phenobarbital	260 (20)[3]	155 (150, 160)
Cytochrome P-450 (nmole/g liver)	None	37 (6)[4]	36 (2)[4]
	Phenobarbital	85 (4)[3]	68 (58, 77)
Microsomal protein (mg/g liver)	None	22 (1)[4]	21 (2)[4]
	Phenobarbital	25 (1)[3]	26 (26, 26)
Liver weight (g)	None	115 (10)[10]	110 (10)[5]
	Phenobarbital	105 (10)[3]	100 (100, 103)
Liver wt/body wt (g/kg)	None	30 (3)[10]	25 (1)[5]
	Phenobarbital	33 (4)[3]	23 (22, 24)

Rabbits, full-term pregnant, or non-pregnant, were pretreated by intraperitoneal injection of an aqueous solution of sodium phenobarbitone (50 mg/kg), or vehicle alone (none) daily for the previous three days. Mean values are given with S.E.M. in parentheses and number of animals as superscripts, or individual results in parentheses.

there are obviously no differences in the total hepatic content of any of these enzymes or proteins. Furthermore, pretreatment with phenobarbital, gave rise to an increase of all measured parameters except liver weight. The coumarin 7-hydroxylase activity which was increased by about 40 per cent by phenobarbital pretreatment of non-pregnant animals, was more than doubled by phenobarbital treatment of the pregnant rabbits.

The results obtained *in vitro* using preparations of the hepatic, microsomal, drug-metabolizing enzymes were substantiated to some extent by the *in vivo* study of the effects of pregnancy on the duration of action of hexobarbital in rats. When the animals were dosed on a basis of pregnant body weight, the hexobarbital sleeping time was found to progressively increase over the 15–20 day period of pregnancy

TABLE 4. DURATION OF HEXOBARBITAL ANAESTHESIA IN RATS AT DIFFERENT STAGES OF PREGNANCY

Duration of pregnancy (days)	Body wt of pregnant rats (g)	Duration of anaesthesia (min)	
		Dosage of 100 mg/kg pregnant wt	Dosage of 100 mg/kg non-pregnant wt
Non-pregnant	271 (7)	53 (3)[22]	47 (4)[5]
3	274 (8)	46 (4)[7]	
7	284 (7)		43 (1)[3]
10	295 (8)	54 (2)[2]	42 (2)[2]
13	305 (9)	64 (2*)[6]	
15	316 (8)	68 (3*)[6]	33 (6)[3]
17	330 (10)	87 (4†)[6]	
20	361 (5)	113 (2†)[6]	44 (5)[3]
A day post-partum	304 (7)	56 (3)[3]	

Animals were given a single intraperitoneal dose of hexobarbital.
Mean values are given with S.E.M. in parentheses and numbers of animals as superscripts.
Significant difference from non-pregnant, *P = < 0·01, †P = < 0·001.

confirming that the rate of metabolism decreased (see Table 4). It is over this same period of 15–20 days gestation that the greatest increase in body weight takes place, due largely to growth of the foetuses. It appears, therefore, that the maternal liver of a pregnant rat is unable to metabolize a dose of hexobarbitone related to its total body weight as rapidly as can the non-pregnant rat. Indeed, a similar increase in sleeping time was obtained by giving these increased doses of hexobarbitone to non-pregnant rats. Thus, a 30 per cent increase in the dose of hexobarbitone from 100 to 130 mg/kg, corresponding to the 30 per cent increase in body weight of the pregnant rat, almost doubled the sleeping time of non-pregnant rats from 69 $\pm$ 4 to 113 $\pm$ 6 min. Furthermore, when pregnant rats were dosed with hexobarbitone (100 mg/kg) on a basis of their weight before mating (non-pregnant weight) no increase in the sleeping time was observed (see Table 4).

DISCUSSION

The results of these studies on rats and rabbits confirm earlier reports that the activities of the hepatic microsomal enzymes concerned in the hydroxylation and glucuronide conjugation of drugs and other xenobiotics may be reduced in late pregnancy.[5,10] The observations of reduced specific activities of the microsomal drug-metabolizing enzymes found with *in vitro* preparations of liver have been confirmed by demonstration of increased anaesthesia when hexobarbital is administered *in vivo*. This is in agreement with the findings of King and Becker[28] using pentobarbital and, similarly, other workers[13,29] have shown that diphenylhydantoin administered on the basis of pregnant body weight, is also more slowly metabolized by pregnant rats. The activities of other, non-microsomal, enzymes, for example, serum lactate dehydrogenase, acetylcholinesterase[30] and the hepatic enzyme respon-

sible for glutathione conjugation[31] have also been shown to be reduced during pregnancy.

The microsomal enzymes of the liver which are responsible for the metabolism of drugs are in many ways similar to those responsible for the metabolism of endogenous steroid hormones and it is believed that drugs and steroids may often be metabolized by the same enzyme system.[32–35]. It has consequently been suggested that the high levels of circulating steroid hormones which occur during pregnancy[36,37] might be responsible for the decreased activity of the drug-metabolizing enzymes by inhibition as competitive substrates.[10,38]

The present findings show that the reduction in the activities of the microsomal drug-metabolizing enzymes in the rat is paralleled by a similar reduction in the content of cytochrome P-450. This has also been observed by Guarino *et al.*,[15] who, furthermore showed, with the substrates aniline and ethylmorphine, that although pregnant animals displayed significantly lower V_{max} values than the controls, the K_m values were unchanged. These points indicate that the decreased activity of the microsomal drug hydroxylating enzymes observed in full-term pregnant rat liver is due rather to a reduction of the enzymic haemoprotein than to competitive inhibition from the high levels of circulating steroids. Factors other than cytochrome P-450, such as cytochrome P-450 reductase, and possible latency of the enzyme activity and the membrane environment of the endoplasmic reticulum, might also be involved in the reduction of the microsomal hydroxylase activity observed in pregnancy. For in the rat, phenobarbital pretreatment completely restores the loss of activity of biphenyl-4-hydroxylase, but produces only a marginal increase in cytochrome P-450, whereas in the rabbit pregnancy greatly diminishes the coumarin-7-hydroxylase activity but has no significant effect on biphenyl-4-hydroxylase and cytochrome P-450.

Any satisfactory explanation for the reduction of drug-hydroxylation and cytochrome P-450 in full-term pregnancy must accommodate the findings that the inhibitory effect is not so apparent in 15–16 day pregnant rats and that it can largely be annulled by pretreatment with the enzyme inducers phenobarbitone or methylcholanthrene. It has been established that cytochrome P-450 and the microsomal drug-metabolizing enzymes are lower than normal in many conditions characterized by the rapid growth of liver cells,[39] for example, in the foetus and neonate,[40–42] after partial hepatectomy[43] and in hepatic tumours.[44,45] In all of these circumstances the presence of increased growth-promoting factors has been reported and this is also found in pregnancy.[45] This increase in growth-promoting factors could, therefore, be responsible for the inhibitory effect of pregnancy on drug metabolism, and indeed Wilson[46,47] has shown that pituitary growth hormone (somatotropin) in rats does cause a similar decrease in the hepatic metabolism of hexobarbital, ethylmorphine and aminopyrine.

This inhibition of the specific activities of the drug-metabolizing enzymes which has been observed during pregnancy is counteracted by the increase that occurs in the maternal liver weight so that the total activity in the whole liver of these enzymes and cytochrome P-450, is similar to the total activity in the non-pregnant female. Consequently, when pregnant and non-pregnant rats were given the same dose weight of hexobarbital, the duration of action was similar in both groups.

The significance of these findings to human therapeutics may only be speculated, for although we have shown that pregnancy may be accompanied by a reduction in the hepatic metabolism of drugs in the rat and rabbit, even these two species show

differences in the pattern of response, so that extrapolation to other species is extremely difficult. Furthermore, even in the same species (rabbit) the extent of inhibition of a particular enzyme (cytochrome P-450) varies according to substrate (hydroxylation of coumarin but not biphenyl is decreased). However, in view of the importance of metabolism in the deactivation of drugs, and the possible effects this could have on the foetus, further studies in human subjects are well merited.

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