HEPATIC DRUG METABOLISM IN PREGNANCY

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ABSTRACT

The results of both isolated tissue and whole animal experimentation, whilst showing some unexplored inconsistencies, suggest that late pregnancy is associated with a reduced ability of the liver to metabolise foreign compounds.

The mechanism of this reduced capacity and the physiological reason for it are unclear but such change does have implication for therapeutic response in pregnancy.

Available results from the limited and often poorly structured studies of drug levels in pregnant women neither prove nor disprove the existence of similar changes in hepatic monooxygenase activity during human pregnancy.

INTRODUCTION

While the type of pharmacological response resulting from the administration of a drug is an intrinsic property of that chemical entity, it is the concentration and contact time of the active drug at its receptors which determines the intensity and duration of any response. This concentration vs. time profile is, in turn, influenced by the various physiological functions that control the rate and extent to which the drug is absorbed into, distributed throughout and eliminated from the body.

Any population of individuals will show genetically determined variations in physiological functions, but non-genetic factors can also influence these functions and hence therapeutic response. The age of a patient is one important determinant of response as the physiology of the neonate, the young adult and the elderly person differ from one another in many respects. As a consequence of these differences drug dosage requirements for a given therapeutic objective may also differ. Similarly diseases that affect any of these physiological functions may also influence therapeutic response; however there is probably no other single event that results in such widespread physiological changes than does pregnancy. This condition is associated with significant and progressive changes in almost all of the parameters that can potentially influence therapeutic response /1/.

Of these, changes in gastrointestinal physiology appear to have minimal effects on the pregnant woman's response to orally administered drugs except perhaps during labour when gastric absorption can be impaired. However, the morphological and fluid changes, which give rise

to a greatly increased, and highly variable environment into which drugs can be distributed, provides scope for significant changes in drug pharmacokinetics in the pregnant woman /1/. Similarly, increases in both renal plasma flow and glomerular filtration have also been shown to influence the clearance of drugs eliminated by this mechanism /2/, but it is the other major pathway of drug elimination, metabolic transformations, about which we have the least information.

The liver is the major site of drug metabolism and it is known that physiological and pathological events in the liver can modify therapeutic response /3/. Physiological indicators of cellular metabolism such as free thyroxine levels and body temperature change during pregnancy /1/ however whether the livers' ability to metabolise drugs also changes during this period is not at all clear.

Obvious legal and ethical reasons explain the lack of systematic examination of hepatic drug metabolism in pregnant women, even by indirect methods. However, the absence of this information means that if pregnancy is associated with significant changes in hepatic metabolic capacity, the drug doses currently being given in pregnancy might not always be the most appropriate.

STUDIES WITH PREGNANT RATS

The inability to study drug metabolism in pregnant women, means that investigation must be made in non-human subjects and while the paucity and generally unsatisfactory nature of drug metabolism studies in human pregnancy is understandable, it is rather more difficult to explain why more comprehensive studies have not been undertaken in pregnant animals, particularly in view of some apparent contradictions that exist between the results obtained by different workers in this field.

The majority of drug metabolism studies have used the rat as an animal model and studies into the influence of pregnancy of drug metabolism are not exceptions. It was studies in 1963 by King and Becker /4/ which showed significantly increased hexobarbital sleeping times in pregnant Osborn-Mendel rats that first indicated possible changes in hepatic drug metabolism during gestation. Thus this review will examine, in some detail, the results obtained and the conclusions reached from rat studies before considering other animals and the results of the information available from investigations of human pregnancy.

Isolated Tissue Studies

Drug transformations have been classified into metabolic and conjugative phases. The former usually involves the formation of a polar group in the parent molecule creating a site with which polar endogenous molecules can subsequently conjugate to increase water solubility and facilitate excretion.

These two phases can be studied separately in isolated liver preparations and this review will first examine the influence of pregnancy on metabolic transformations by liver homogenates, 100,000 x g supernatants or microsomal suspensions.

Direct evidence of changed hepatic metabolism in pregnancy was first demonstrated by Feuer and Liscio /5/ who found a 50 percent reduction in 4-methylcoumarin 3-hydroxylase activity in 14 to 20 day pregnant rats of unnamed strain and by Guarino *et al* /6/ who showed that the V_{max} for aniline parahydroxylase and ethylmorphine demethylase per mg of microsomal protein from 20 day pregnant Sprague Dawley (S.D.) rats were significantly depressed when compared to non-pregnant controls. No depression was found at six days gestation. These rate changes correlated with the reduced concentration of cytochrome P_{450} in microsomes from pregnant rats and were essentially reversed shortly after delivery.

The depression in aniline parahydroxylation in S.D. rats was confirmed by Gabler and Falace /7/, who, while investigating the reduced rate of phenytoin clearance from 19 day pregnant rats, measured the aniline parahydroxylase activity in microsomes isolates from these animals and found only 67 per cent of the enzyme activity present in non-pregnant controls.

Subsequently, more extensive studies, using Wistar rats, were conducted by Neale and Parke /8/ who measured biphenyl hydroxylation, p-nitrobenzoate reduction and cytochrome P_{450} concentrations at 15 to 16 days and also 19 to 20 days of gestation. These workers reported that, whilst both biphenyl-4-hydroxylase and cytochrome P_{450} were significantly reduced at days 19 to 20, the nitroreductase activity and microsomal protein concentration expressed per gram of liver were unchanged. They were unable to show any significant differences in any of the parameters measured at days 15 to 16; however, they did note that the liver weights of 19 to 20 day pregnant rats increased by over 40 percent when compared to non-pregnant females. On this basis they suggested that, if the increased liver size was taken in-

to account, the total cytochrome P_{450} content and biphenylhydroxylase activity in the whole liver would be the same or greater than that in the smaller whole liver of the non-pregnant rat. It would also follow that the whole-liver nitroreductase activity and total microsomal protein were significantly greater in both the 15 to 16 and 19 to 20 day pregnant rat. However, a subsequent report from this same laboratory, using the same rat species and without explanation, recorded no change in cytochrome P_{450} per mg of microsomal protein but a significant reduction in nitroreductase both in terms of specific activity and as activity per whole liver /9/.

Similar investigations were also undertaken by Schlede and Borowski /10/. These workers investigating 14 and 21 day pregnant Wistar rats similarly recorded 50 percent increases in liver weight by day 21 which were associated with significant decreases in benzopyrene hydroxylase activity per gm of liver at days 14 and 21 and also for ethylmorphine at day 14. (Values for day 21 were not recorded.) However, these workers did not find any reduction in hepatic cytochrome P_{450} concentration.

A third major investigation of hepatic drug metabolism by microsomes from Wistar rats was that of Dean and Stock /11/ who measured *in vitro* aniline parahydroxylase, aminopyrine demethylase, p-nitroanisole demythylase and the nitroreduction of p-nitrobenzoic acid at 5, 10, 15 and 20 days gestation then 2 and 5 days post-partum.

The results (Fig. 1) showed a progressive, but not identical, fall in the activity per unit of microsomal protein in the three oxidative pathways which were all significantly different from controls by at least day 15 of gestation. Nitrobenzoic acid reduction, however, was less affected being significantly different only at day 20. In all cases these falls reverted to essentially non-pregnant levels by 2 days post-partum.

This study also showed a similar, progressive fall in hepatic cytochrome P_{450} concentration which was significantly lower than non-pregnant animals at 10, 15 and 20 days of gestation.

In a more restricted study of the period between day 19 and parturition all of the above parameters were measured at daily intervals with the livers in the final study being excised just before delivery. This showed that all of the above metabolic parameters showed maximum depression at 19 to 20 days but from that time this depression was rapidly reversed and by parturition approached non-pregnant values. Over this same period the percentage liver/body weight ratio changed from 3.63 at day 20 to 3.44 at day 21 to 2.9 on day 22. This compared with 3.42 for the non-pregnant female.

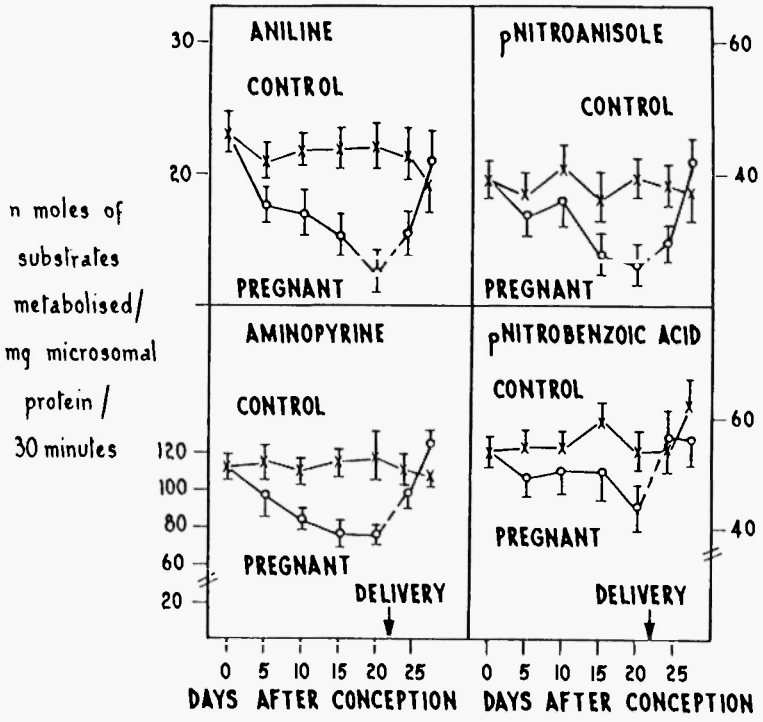

Fig. 1. Levels of *in vitro* hepatic drug metabolism

The levels of *in vitro* aniline parahydroxylation, p-nitroanisole O-demethylation, aminopyrine N-demethylation and p-nitrobenzoic acid reduction at intervals during pregnancy and post partum, are compared with the levels in non-pregnant controls measured at the same time. Each point represents the mean of the product formed (nanomoles per mg of microsomal protein per 30 min) and the S.E. of the data from 12 animals. Significant differences existed between control and pregnant samples at days 5, 10, 15, and 20 (aniline) days 15 and 20 (p-nitroanisole) days 10, 15 and 20 (aminopyrine) and day 20 (p-nitrobenzoic acid). In all cases $p < 0.01$.

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The increased liver size in late pregnancy, which led to the proposal that this can offset, in the whole animal, the decrease in monoxygenase specific activity in microsomal preparations /8,10/, together with this apparent rapid contraction in liver size just before parturition was further investigated. The metabolic capacity of both 20 and 22 day pregnant rats were measured against a range of parameters and compared with equivalent measurements using livers from non-pregnant rats. This showed that both aniline and aminopyrine metabolism were significantly depressed at 20 days gestation against all parameters measured from metabolism per liver cell through to metabolism per whole liver even though the magnitude of the depression was least when expressed in the latter form. However, while aniline metabolism per whole liver was still depressed at day 22 of gestation the aminopyrine metabolism had reverted to non-pregnant levels by parturition. Nevertheless these results would not suggest that increased liver size compensates for the depressed specific activity of late pregnancy.

Another smaller study using Wistar rats, again showed about a 50 percent fall in p-nitroanisole demethylase at day 19 /12/. However, other studies published over this period which used S.D. rather than Wistar rats showed some significant differences in the response of hepatic drug metabolism to pregnancy.

Gut *et al* /13/ could not demonstrate any significant changes in hepatic cytochrome P_{450} concentration, *in vitro* aminopyrine nor hexobarbital metabolism at either day 5, 10, 15 or 20. They did, however, show significant falls in aniline hydroxylation at 10, 15 and 20 days. This change in hydroxylase activity was supported by the results of Blake *et al* /14/ who showed the V_{max} of phenytoin hydroxylase was halved in microsomes from 21 to 22 day pregnant S.D. rats.

In another study with S.D. rats, Tabei and Heinrichs /15/ also found no change in cytochrome P_{450} concentration but they did demonstrate significant depression of dehydroepiandrosterone (DHA)-7-hydroxylase and aminopyrine demethylase at 22 days gestation. Similarly, Mukhtar *et al* /16/ whilst finding significant depressions in hepatic aryl hydrocarbon hydroxylase and epoxide hydrase activities in 19-20 day pregnant S.D. rats found no commensurate depression in microsomal cytochrome P_{450} concentration, while Guenthner and Mannering /17/ could not show any change in cytochrome P_{450}, ethylmorphine demethylation nor benzopyrene hydroxylase during any period of pregnancy.

Studies of phenytoin metabolism by isolated and perfused livers of

pregnant SD rats are ambiguous. Using perfusates containing 20 percent blood and perfusing at 2 ml/minute/gm of liver, there was no significant decrease in phenytoin metabolism from 13 to 21 days of gestation. However, if the perfusate was 10 percent blood and was perfused at 1 ml/minute/gm of liver,the latter part of pregnancy was associated with a large decrease in metabolism. Phenytoin half lives calculated from such experiments showed a change from 38 minutes in non-pregnant livers to 70 and 158 minutes in livers from 17 and 21 day pregnant rats respectively. These workers suggested that reduced oxygen supply might be responsible for this reduced hepatic capacity but why such large differences were found between the non-pregnant and pregnant livers under the same conditions were not really explained /18/.

An indication that pregnancy influenced the conjugative phase of drug metabolism arose with the finding that serum from pregnant women inhibited the glucuronidation of bilirubin by rat liver slices /19/. The fall in *in vitro* bilirubin glucuronidation was confirmed by Halac and Sicignano /20/ who also showed a similar progressive fall in p-nitrophenol glucuronide formation during pregnancy which returned to non-pregnant levels within 3 days of delivery. Further confirmation of reduced glucuronyl transferase activity comes from Feuer and Liscio /5/ who reported a 65 percent reduction in o-aminophenol glucuronide formation in 14-20 day pregnant rats and from Blake *et al* /14/ who found a 35 percent decrease in glucuronic acid conjugation of 5-hydroxyphenyl-5-phenylhydantoin by livers from 21 day pregnant S.D. rats, while a 25 percent reduction in the glucuronyl transferase activity with 4-methylumbelliferone was also recorded in 19-20 day pregnant Wistar rats /8/. It has also been shown in livers from 21 day pregnant Wistar rats the rate of p-nitrophenol sulphation was almost halved /21/.

In contrast to this depressed glucuronide and sulphate conjugation, Bell *et al* /12/ found that the glutathione conjugation of bromosulphophthalein was unchanged at 18 days gestation in the same strain; a finding which was supported by Mukhtar *et al* /16/ who reported no significant change in glutathione-S-transferase activity in the hepatic soluble fraction obtained from 19-20 day pregnant S.D. rats. However, Polidoro *et al* /22/, using soluble fraction preparations from 19-20 day Morini rats, showed significant increases in glutathione-S-transferase activity towards 1,2-epoxy-3-(p-nitrophenoxyl) propane at 10-11 and 19-20 days and towards chloro-2,4-dinitrobenzene at 19-20 days. In the same animals there was, however, a decrease in enzyme activity towards 1,2-dichloro-4-nitrobenzene. These results contrast with measurements

of this same process in liver homogenates from S.D. rats over the last five days of gestation which showed a moderate depression of bromosulphophthalein conjugating activity returning to normal after parturition /23/ and suggests a different response to pregnancy, of the different glutathione conjugating enzymes. The variability in response to pregnancy apparent in the metabolic phase with S.D. rats appears to persist with the conjugative phase as Vaisman *et al* /24/ found that glucuronyl transferase activity towards bilirubin was unchanged during pregnancy with this rat strain, while Pacifici and Rane /25/ reported increased morphine glucuronide formation by livers from 21 day pregnant rats.

Whole Animal Studies

The extent to which changes measured using isolated liver preparations reflects the situation in the pregnant animal has not been established. While Quinn *et al* /26/ demonstrated an inverse correlation between hexobarbital sleeping time and *in vitro* hepatic metabolism in the rat, pregnancy is associated with major changes in other functions that can influence therapeutic response such as distribution and renal excretion of the drug. This makes direct correlation between isolated liver metabolic studies and whole animal response less certain.

During pregnancy changes in hexobarbital sleeping time could be influenced by changes in either or both the distribution and elimination parameters. The results of the original study by King and Becker /4/ with pentobarbital, which showed a markedly increased sleeping time in pregnant rats, is consistent with a reduced metabolic clearance as is a similar study by Neale and Parke /8/. These workers showed that the IP administration of 100 mg hexobarbital/kg to pregnant rats increased their sleeping times from 53 minutes before conception to 113 minutes on day 20 of gestation.

However, it was this latter group who chose to place a somewhat different interpretation on the results of their *in vitro* and *in vivo* studies. They suggested that the significantly increased liver size in late pregnancy would compensate for the lowered enzyme activity per unit of microsomal protein and that the total hepatic monooxygenase capacity in the enlarged pregnant liver was essentially the same as that in the normal non-pregnant rat liver. They supported this proposal by showing that when 20 days pregnant rats were administered a dose of hexobarbital based on their non-pregnant body weight the sleeping time recorded did not differ significantly from that of the non-pregnant rat.

This type of experiment was later repeated by Schlede and Borowski /10/ who also found that the 75% increase in sleeping time in 21 day pregnant rats could be abolished if they receive the same fixed dose of hexobarbital that was given to non-pregnant controls.

The validity of this proposal has however been questioned /11/ on the basis that there was no evidence that the reduced dosage gave serum levels in pregnant rats equivalent to those in their non-pregnant counterparts. In the absence of this, comparisons of the *in vivo* responses cannot legitimately be made. Justification for comparing the results obtained by administering a dose not based on the significantly increased pregnant body weight with one based on the body weight of non-pregnant rats would require either the demonstration that there was no change in hexobarbital's total volume of distribution as a result of the increased foetal-placental mass or that any change was compensated for by corresponding change in the drugs clearance during pregnancy. However, neither group conducted pharmacokinetic experiments to verify either of these possibilities, so that the question was left unresolved.

Apart from the above studies using hexobarbital, whole animal studies with pregnant rats have commonly used the anti-epileptic phenytoin which is essentially cleared by hepatic aromatic hydroxylation followed by conjugation. Thus, on the basis of the consistently recorded depression of aromatic hydroxylation in pregnancy, phenytoin clearance should be significantly reduced.

Gabler and Falace /7/ studied the distribution and metabolism of phenytoin in pregnant S.D. rats. They found that while a single oral dose was essentially cleared from the tissues of non-pregnant rats in 24 hours it took more than 48 hours in the 19 day pregnant rat.

Westmoreland and Bass /27/ also showed that blood levels of phenytoin after both acute and chronic dosage were approximately twice as high in 14 day pregnant Albino Charles River rats as in their non-pregnant controls. The pregnant rats also showed clinical toxicity. However, it was Gutova *et al* /28/ who first attempted to quantify the pharmacokinetics of phenytoin in pregnant rats.

These workers gave S.D. rats, which were between 13 and 19 days pregnant a single dose of 25 mg phenytoin/kg into the tail vein and then took blood samples at selected intervals. The blood concentration vs. time profile allowed calculation of pharmacokinetic parameters. While reduced protein binding resulted in an increase in phenytoin's

volume of distribution in the pregnant rat, this change could not account for large reduction in phenytoin clearance suggesting that reduced metabolism was a significant factor in pregnancy. However, the failure to record any difference between 13 day and 19 day pregnant rats was surprising considering the progressive nature of reduced aromatic hydroxlation in pregnancy /11/. Dean *et al* /29/ also found that pregnancy was associated with a significant reduction in the elimination rate constant for phenytoin in 20 day pregnant Long Evans rats. Similarly, the IP administration of carbamazepine to S.D. rats in late pregnancy showed that these animals had a greatly reduced clearance, with only a very slight increase in the drug's volume of distribution /30/.

Further confirmation of the correlation between hepatic hydroxylation and reduced body clearance in pregnant rats was obtained with the use of antipyrine /31/. This substrate has the advantage of being negligibly plasma protein bound, uniformly distributed throughout body water and extensively metabolised by the liver. It has therefore been widely used as an *in vivo* measurement of hepatic hydroxylation.

A single dose of antipyrine was administered through a cannula into the external jugular vein of both non-pregnant and 20 day pregnant Wistar rats and from which blood samples were withdrawn at intervals for six hours. The pharmacokinetic parameters calculated from this serum concentration vs time profile showed a small increase in antipyrine's volume of distribution but a large reduction in total body clearance which was reflected in a doubling of antipyrine half life in the pregnant animals.

After completion of blood sampling, livers were excised from these animals and *in vitro* incubations showed 32 percent less hydroxylating activity in microsomes from pregnant rats. These results would suggest that at least for antipyrine and phenytoin the diminished hepatic capacity of the 20 day pregnant rat is not compensated for by an increase in liver size as previously implied /8,10/.

Whole animal studies into other metabolic transformations have not been published; however, this laboratory has found that nitrofurantoin, which is subjected to hepatic nitroreduction, shows no significant changes in its pharmacokinetic parameters including elimination rate at day 20 of gestation. This result is consistent with the relatively minor change in *in vitro* metabolism previously shown /8,11/.

Whole animal studies with drugs cleared primarily by conjugative pathways are not available. However, preliminary studies similar to

those described for antipyrine with 20 day pregnant Wistar rats indicate that pharmacokinetic parameters of sulphamethoxazole, a drug primarily acetylated, are unchanged by pregnancy. On the other hand, salicylamide, which is eliminated as sulphate and glucuronide conjugates, does show some changes in pregnancy. While the total body clearance of salicylamide is unchanged, serum metabolite measurements suggest that there is a major reduction in the amount of the sulphate conjugate but that this is offset by a compensatory increase in glucuronide formation. This latter result conflicts with *in vitro* studies which have suggested that glucuronyl transferase activity is reduced in pregnancy /32/.

OTHER ANIMAL SPECIES

Hepatic metabolism in pregnancy has been less well investigated in species other than the rat.

Analysis of blood samples from both the portal and vena cava veins after the oral administration of salicylamide to pregnant rabbits suggested that pregnancy was associated with both reduced absorption and also reduced glucuronide conjugation /33/.

Other studies suggested a 20 percent reduction in *in vitro* glucuronyl transferase activity at full term in New Zealand white rabbits /8/. This same study also recorded a 60 percent reduction in coumarin-7-hydroxylase per gram of liver. However, in contrast to the pregnant rat, there was no apparent change in liver weight, microsomal protein, cytochrome P_{450}, nitroreductase nor biphenylhydroxylase at full term. A similar lack of response to pregnancy was also noted in Dutch Belt Rabbits. No change was found in dimethylaniline demethylase or N-oxidase activity at 10, 20 or 28 days gestation /34/.

In contrast to the results obtained above, Gut and Becker /13/, using 9000 g supernatants from the livers of 29 day pregnant Dutch-belted rabbits showed a dramatic reduction in the metabolism of aminopyrine, benzphetamine and hexobarbital, even though there was no change in the liver/body weight ratio.

The position with pregnancy in rabbits was further complicated by the results of Tabei and Heinrichs /15/. These workers examined both 8 and 14 day pregnant New Zealand rabbits and found significant depressions in cytochrome P_{450} and 7α hydroxylation of DHA at day 8, but no significant changes in 7β or 16α hydroxylation of DHA nor or aminopyrine demethylation. However, studies with livers of 14 day preg-

nant rabbits showed no differences from their non-pregnant controls in any of these parameters including those that were previously depressed at day 8.

Studies of drug metabolism in other animal species appear to have been restricted to whole animal experimentation. McEthatton *et al* /35/ measured primidone and its metabolities, phenylethylmalondiamine and phenobarbital, in plasma obtained 1 and 4 hours after oral administration of primidone to 14 day pregnant mice. Their results suggested delayed absorption and increased hepatic metabolism in these animals.

Studies in sheep do not appear to clarify the effects of pregnancy on hepatic drug metabolism either. The IV administration of hexoprenaline, a β_2 sympathomimetic agent, to pregnant ewes near term provided evidence of a lowered plasma clearance when expressed on a body weight basis and compared with non-pregnant sheep. This change was not associated with a significant change in elimination rate constant but perhaps surprisngly with a reduction in the drug's volume of distribution during pregnancy /36/. However, pharmacokinetic parameters calculated from the results of the IV administration of lidocaine to pregnant crossbred Suffolk ewes within 10 days of parturition (147 to 150 days gestation) showed that the total body clearance was unaltered but that lidocaine's volume of distribution increased during pregnancy resulting in an increase in half life /37/. Thus, although significant pharmacokinetic changes appear to occur in pregnant sheep, it is not possible at this time to attribute these specifically to changes in intrinsic metabolic clearance.

Finally, studies in the Rhesus monkey have suggested that pregnancy in this species is responsible for changes in the clearance of phenytoin. Sampling of both blood and urine for periods after the IV administration of phenytoin to monkeys on the estimated 50th, 100th or 150th day of gestation suggested that pregnancy, particularly late pregnancy, was associated with reduced plasma clearance of this drug. Although not all pharmacokinetic parameters were calculated from this data, the results presented did not indicate marked changes in phenytoin's volume of distribution during pregnancy, suggesting that reduced metabolic transformation was involved. It was also shown that a shift occurred in the metabolic products formed as both plasma and urine samples from the pregnant monkey contained a higher percentage of the 5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-5-phenylhydantoin at the expense of the other major metabolite 5-hydroxyphenyl-5-phenylhydantoin than was present in non-pregnant monkeys /38/.

ENZYME INDUCTION DURING PREGNANCY

Investigations of the influence of pregnancy on the ability of known hepatic monooxygenase inducers to elevate the levels of *in vitro* drug metabolism have again been largely conducted with rats. These studies have used different pretreatment regimens which make direct interstudy comparisons more difficult; however, the majority of them have used phenobarbital as their hepatic monooxygenase inducing agent.

The first report, using 14 to 20 day pregnant rats, showed that, while two IP injections of 46 mg phenobarbital/kg increased by 100 percent the 4-methylcoumarin-3-hydroxylase activity in non-pregnant rat microsomes, it increased the activity by 196 percent in the pregnant animals. However, because of the depressed hepatic activity in the pregnant rat, the induced levels reached only 73 percent of that obtained in the phenobarbital treated non-pregnant rat /5/. The transfer of glucuronic acid to o-aminophenol in these same non-pregnant microsomal preparations showed a 45 percent increase compared with a 65 percent increase in the pregnant animals after phenobarbital treatment.

Another study with microsomes from 19-20 day pregnant Wistar rats which had been previously treated for 3 days with 50 mg phenobarbital/kg daily, showed a 25 percent increase in microsomal protein content. This was similar to that present in the liver of treated non-pregnant rats; however, while the concentration of cytochrome P_{450} rose by 32 percent in the non-pregnant rat, it increased by only 16 percent in the pregnant rat /8/. The nature and magnitude of these changes were difficult to correlate with biphenylhydroxylase activity for while this increased by 50 percent in the phenobarbital treated non-pregnant rats, it doubled in the treated pregnant group.

Another study with hepatic microsomal suspensions from pregnant Wistar rats treated with 75 mg phenobarbital/kg for four days showed a 100 percent increase in benzopyrene hydroxylase and a 190 percent increase in ethylmorphine demethylase at day 14. This compares with 208 and 550 percent increases respectively in these two pathways after similar pretreatment of non-pregnant Wistar rats. By day 21 the benzopyrene hydroxylase activity was increased 200 percent (Ethylmorphine demethylase changes were not recorded). There was, however, again a lack of correlation between these increases in hepatic monooxygenase activity and total cytochrome P_{450} concentrations, for while the phenobarbital pretreatment doubled cytochrome P_{450} concentration in non-pregnant rat livers, it increased this cytochrome by only 17 and 8

percent in the microsomes of 14 and 21 day pregnant rat livers respectively /10/.

Further studies with Wistar rats measured *in vitro* p-nitroanisole demethylation at 4, 8, 15 and 19 days of gestation following oral administration of 75 mg phenobarbital/kg for three days. The increased activity resulting from this exposure varied from increases of 118 percent in the non-pregnant rat to 45 percent at day 4; 100 percent at day 8; 60 percent at day 15 and 140 percent at day 19 of gestation /12/.

Studies with 22 day pregnant S.D. rats after phenobarbital pretreatment showed the same apparent lack of correlation between the concentration of microsomal cytochrome P_{450} and metabolism that was found in control studies of pregnancy with this strain. These animals were injected intraperitoneally for four days at a dose of 40 mg/kg for two days then 80 mg/kg on the third and 120 mg/kg on the fourth day. This treatment of non-pregnant rats resulted in an 84 percent increase in microsomal cytochrome P_{450} and a 128 percent increase in aminopyrine demethylation while microsomes from the 22 day pregnant rats showed only a 41 percent increase in cytochrome P_{450} but a 142 percent in aminopyrine demethylation. The hydroxylation of dehydroepiandrosterone in either the 7α, 7β or 16γ positions showed even less explainable correlations /15/.

Further studies with S.D. rats suggested that the ability of three days pretreatment with subcutaneous injections of 100 mg of phenobarbital/kg to stimulate glucuronyl transferase activity decreased as pregnancy progressed. This was in spite of the fact that these studies showed no change in glucuronyl transferase activity with pregnancy in the non-treated rat. The level of induction fell progressively from increases, equivalent to that in non-pregnant rats at 5, 7 and 9 days gestation of about 62 percent to 31 percent by day 13, 15 percent at 17 days and 19 percent at 21 days of gestation /24/.

Guenthner and Mannering on the other hand, whilst unable to show any reduction in metabolism or cytochrome P_{450} concentration during pregnancy in S.D. rats, found that 40 mg phenobarbital/kg for four days elevated cytochrome P_{450} concentration and ethylmorphine demethylase activity by only 50 percent of that found by pretreating non-pregnant rats. Also there appeared to be very little fluctuation in these parameters over the course of pregnancy. These workers, did, however, establish that this differential in phenobarbital pretreatment between pregnant and non-pregnant rats could be eliminated by doubling the dose to 80 mg of phenobarbital/kg/day /17/.

The above results from phenobarbital treated rats suggests little correlation exists between cytochrome P_{450} and metabolism during pregnancy. However, results from this laboratory in which *in vitro* measurements of cytochrome P_{450} concentrations and aniline parahydroxylation were made at various stages of pregnancy 24 hours after three days pretreatment with 75 mg phenobarbital/kg suggest that such a correlation does exist, at least in Wistar rats. This can be seen by expressing the levels of activity in the pregnant rat as a percentage of the non-pregnant control at each measurement period. This shows that previously recorded /11/ progressive fall during pregnancy with a maximum depression at day 20. If in turn the levels of activity in phenobarbital pretreated pregnant rats are expressed as a percentage of the values obtained with microsomes from phenobarbital pretreated non-pregnant rats, an almost identical pattern is found for both cyrochrome P_{450} concentration and aniline parahydroxylase activity (Figure 2). This apparent correlation would also suggest that the relative increase in cytochrome P_{450} concentration and aniline metabolism as a result of phenobarbital induction are similar both in the pregnant and non-pregnant rat liver.

The only information on animals other than rats comes from two reports of phenobarbital administration to pregnant New Zealand rabbits. In the first, microsomal preparations isolated from rabbits at term after three days treatment with 50 mg phenobarbital/kg showed increases comparable to those obtained by phenobarbital pretreatment of non-pregnant rabbits in hepatic microsomal protein content, biphenyl-4-hydroxylase, coumarin-7-hydroxylase and paranitroreductase activities. However, the increases in 4-methylumbelliferone glucuronyl transferase of 15 percent and cytochrome P_{450} of 61 percent were much less than the phenobarbital mediated increases in the non-pregnant rabbit of 63 and 130 percent respectively /8/.

The second study used 14 day pregnant rabbits after five days treatment with 40 mg phenobarbital/kg and showed similar increases in cytochrome P_{450} (65 percent) together with increases of 116 percent in aminopyrine demethylase. This compared with a 45 percent increase in cytochrome P_{450} and only 19 percent in aminopyrine demethylase in the non-pregnant rabbit. However, even more confusing in this report was the significant decrease in 7α, 7β and 16α dehydroepiandrosterone hydroxylation in microsomes from pretreated pregnant rabbits. Phenobarbital failed to alter these enzymes in any way in the non-pregnant rabbit /15/.

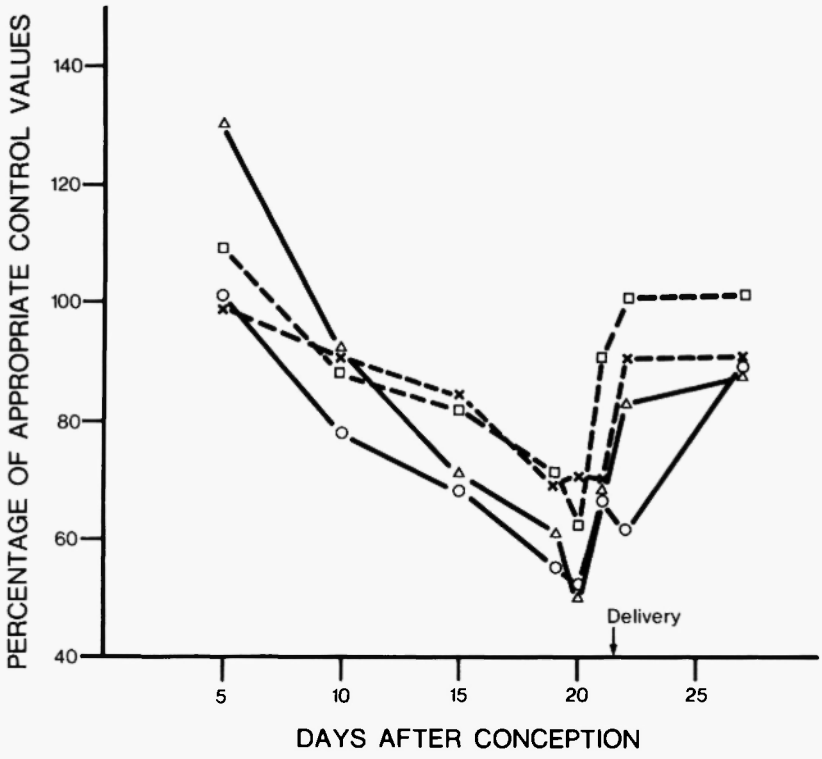

Fig. 2. Cytochrome P_{450} concentration and aniline hydroxylase activity in microsomal preparations from phenobarbital treated and non-treated pregnant and non-pregnant Wistar rats.

The concentration of microsomal cytochrome P_{450} per mg microsomal protein at various stages of pregnancy expressed as a percentage of cytochrome P_{450} concentrations in non-pregnant control rats measured on the same day (x---x). Aniline parahydroxylase activity in the same microsomal preparations were similarly expressed as a percentage of the activities obtained with microsomes from the control rats measured at the same times (○ —— ○). Similar measurements are also shown for both cytochrome P_{450} (□ – – – – – □) and aniline parahydroxylase activity (△ —— △) after both pregnant and non-pregnant control rats were pre-treated with 75 mg phenobarbital/kg for 3 days prior to isolation of the microsomal suspensions. Each point is the mean of data for 6 animals.

Polycyclic aromatic hydrocarbons constitute the other major class of hepatic monooxygenase inducers. Microsomes obtained from 14-20 day pregnant rats which had been given a single dose of approximately 20 mg 4-methylcholanthrene/kg 18 hours previously showed a 400 percent increase in 4-methylcoumarin-3-hydroxylase and a 270 percent increase in o-aminophenol glucuronyl transferase activities. This compared with 210 and 60 percent increases in these enzymes after similar treatment of non-pregnant rats. However, because of the lowered levels of activity in the pregnant rats, methylcholanthrene did not elevate levels to those of the induced non-pregnant rat /5/. In contrast, IP administration of 20 mg/kg daily for three days to Wistar rats prior to sacrifice on day 19-20 of gestation showed no effect on microsomal protein, minimal but identical increase in cytochrome P_{450} concentration and significant and identical increases in biphenyl-4- and biphenyl-2-hydroxylases to those found after treatment of non-pregnant rats /8/.

Sprague Dawley rats, however, showed a 100 percent increase in cytochrome P_{450} concentration and a 10 fold increase in benzopyrene hydroxylation over the whole period of gestation which were essentially the same as comparable non-pregnant rats after treatment with 16 mg 3-methylcholanthrene/kg for four days /17/. This result is consistent with the pattern of stimulation previously recorded by Welch *et al* /19/ after a single oral dose of 60 mg methylcholanthrene/kg to pregnant S.D. rats 24 hours before sacrifice. These workers showed that the benzopyrene hydrxylase activity of maternal liver increased 17 fold at day 14 and 13½ fold at day 19 of gestation. In the same microsomal suspensions the N-demethylation of 3-methyl-4-monomethylaminoazobenzene was increased by 80 and 132 percent at 14 and 19 days respectively.

MECHANISM OF ALTERED HEPATIC DRUG METABOLISM

Whilst there are unexplained inconsistencies in the recorded response of S.D. rats to pregnancy it would appear that in other strains, the latter part of gestation is generally associated with decreases in the specific activity of hepatic monooxygenase enzymes. However what is far from clear is how and why this reduction occurs and what physiological mechanisms mediate these changes.

The earliest explanation of this reduced hepatic capacity followed from the observation that pregnant female serum added to liver preparations inhibited their ability to form bilirubin glucuronide /19/. The similarities between the metabolism of both steroids and xenobiotics to-

gether with the elevated levels of progestogens during the latter half of pregnancy led to the suggestion that the depressed metabolism of xenobiotics results from competition between them and progestogens for the available hepatic cytochrome P_{450} system.

This view was supported by various *in vitro* studies which showed that progesterone and some other steroid derivatives inhibited the glucoronidation of o-aminophenol by guinea pig liver preparations /32/, the metabolism of hexobarbital, zoxazolamine and aniline by 9000 g supernatants from the livers from male Holtzman rats /40/, and also the metabolism of ethylmorphine and hexobarbital by male Holtzman rats /41/. Finally an extensive study involving a large number of progesterone metabolites showed a considerable number competitively inhibited the demethylation of p-nitroanisole by 9000 g supernatants from the livers of male S.D. rats /44/. However, these results do not establish that this competitive inhibition reflects a physiological action of progesterone in pregnancy or that it is even the mechanism of depressed monooxygenase activity. There are, in fact, some compelling arguments against the direct competitive involvement of progesterone as the mechanism for reduced hepatic drug metabolism.

Firstly, kinetic studies /6/ have shown that while the V_{max} for both aniline hydroxylase and ethylmorphine demethylase were significantly reduced in microsomal preparations from 20 day pregnant rats the Km values were unchanged. An increase in Km is a fundamental requirement for competitive inhibition.

Secondly, measurements of the progesterone content in microsomal preparations from 20 day pregnant rats showed the presence of only 2.5×10^{-9}M progesterone which was slightly less than that present in microsomes from non-pregnant female Wistar rats /11/.

Thirdly, the pattern of plasma progesterone concentrations over pregnancy showed maximal levels at around day 14 /43/ while maximum depression of hepatic metabolism did not occur till days 19-20 of gestation /11/.

Whether progesterone exerts an indirect effect on hepatic drug metabolism is not clear. Guenthner and Mannering /17/, who studied the effect of various monooxygenase inducing agents on pregnant, foetal, and neonatal rats postulated that a regulatory mechanism existed in these situations which suppressed the synthesis of new cytochrome P_{450}. These workers, who used S.D. rats, reported no change in microsomal cytochrome P_{450} concentration during pregnancy but found that the

level of phenobarbital induction of this cytochrome during pregnancy was very much less than in the non-pregnant rat. While they were unable to establish any direct involvement of endogenous progesterone in this suppression of cytochrome P_{450} synthesis they did not exclude it. Whether such a suppression mechanism could explain the reduced concentration of cytochrome P_{450} during pregnancy in other rat strains has not been canvassed. However, the administration of exogenous progesterone to non-pregnant Wistar rats gave rise to elevated levels of both aniline and aminopyrine metabolism /11/ rather than depressed enzyme activity.

One interesting suggestion for progesterone involvement in the control of hepatic drug metabolism was advanced by Feuer and Kardish /44/.

Using 18-20 day pregnant Wistar rats, these workers found a significant fall in the activity of enzymes involved in phospholipid synthesis. This reduced phosphatidylcholine and phosphatidylethanolamine synthesis resulted in changes to microsomal phospholipid composition. The microsomal preparations from these rats showed significant reductions in cytochrome P_{450} and monooxygenase activity suggesting that this changed membrane composition was associated with reduced hepatic enzyme activity.

These same workers had previously proposed that high levels of reduced progesterone metabolites in the maternal system were responsible for the lack of significant monooxygenase activity in the foetal and neonatal rat /45/. Extending this hypothesis they suggested that depressed maternal monooxygenase activity of pregnancy resulted from a rise in the reduced/oxidized progesterone metabolities ratio associated with the high levels of progesterone during gestation. They proposed that this change influenced the level of hepatic metabolism via changes to microsomal phospholipid composition.

This postulate was supported by the results obtained with microsomal preparations from female rats administered subcutaneous injections of reduced progesterone metabolities which all paralleled the metabolic changes occurring in the livers of pregnant rats. In contrast, administration of the oxidative metabolite, 16 α hydroxyprogesterone, stimulated the same functions depressed by the reduced metabolites /46/. Unfortunately, however, these workers did not record the influence, if any, that these progesterone derivatives had on the concentration of hepatic microsomal cytochrome P_{450}.

This proposal that oxidative metabolites stimulate hepatic metabolism would explain the rapid reversal of the depressed metabolic activity between day 20 and parturition in the Wistar rat /11/ as it has been shown that this period is associated with a rapid rise in the oxidative metabolite, 20α hydroxyprogesterone /43/.

Another suggestion to explain the reduced monooxygenase activity was also based on altered phospholipid composition of microsomal preparations. This was that these membrane changes were responsible for the reduction in the percentage of cytochrome P_{450} in the high spin state found in the 20 day pregnant rat and that this in turn resulted in lowered NADPH-cytochrome P_{450} reductase activity, which is normally the rate limiting step in drug metabolism /9/. This proposal, however, ignores the significantly reduced cytochrome P_{450} concentrations in 20 day pregnant Wistar rats found by other workers including their own laboratory /8/ so that the relevance, of changes in spin state, if any, to drug metabolism in the pregnant rat needs to be further investigated.

Finally, it is yet to be established if the considerable changes in liver size associated with late pregnancy are related to the changes in monooxygenase activity. The rapid contraction in liver size between day 20 and parturition in the Wistar rat occurred concurrently with a rapid reversal of depressed metabolic capacity /11/. High levels of growth hormone have been shown to correlate with low levels of monooxygenase activity in neonatal rats /47/. However, while pregnant animal livers do contain growth hormone (somatotrophin) binding sites the physiological significance of these, if any, is unclear and previous workers have failed to shown any elevation of growth hormone during pregnancy in the rat /48/.

STUDIES IN HUMAN PREGNANCY

In the final analysis the reason for studying drug metabolism is to establish the rate and extend to which hepatic metabolism contributes to the total body clearance of drugs in humans either directly or via the formation of active metabolites.

The major problem in obtaining definitive information on hepatic drug metabolism is that studies with isolated human liver enzymes are not feasible because the risk associated with liver biopsies can rarely be justified. Thus, human studies are restricted to measurements in blood and urine samples which can be difficult to interpret because physiolo-

gical functions, other than hepatic metabolism, influence the measurements obtained in these biological fluids. Nevertheless, well controlled blood and urine measurements obtained according to appropriate protocols do provide an indirect guide to hepatic metabolism in healthy human subjects. However, while a risk of foetal damage, albeit slight, exists similar deliberate studies in pregnant women are not acceptable. Thus, the limited human studies that have been reported are, of necessity, opportunistic and often poorly structured to provide meaningful measurements of hepatic metabolism's contribution to the intensity and duration of therapeutic response. Also, drug and metabolite measurements in biological fluids will not always detect nor quantify any pregnancy mediated changes in hepatic monooxygenase activity. Thus, drugs whose elimination is limited by hepatic blood flow rather than hepatic enzyme capacity will not reflect the magnitude of change that might be found with those drugs whose clearance relates more directly to intrinsic hepatic metabolism.

There are two situations in pregnant women where studies with drugs have been possible. The first is at delivery, where therapeutic agents are given to assist during parturition. It was in this situation that Crawford and Rudofsky /4/ recorded that the percentage of a 50 mg IV dose of pethidine or promazine appearing as unchanged drug in the urine was more than double that obtained in non-pregnant females and in males. They suggested from this that hepatic metabolism was depressed during pregnancy.

This view was supported by subsequent pharmacokinetic studies with pethidine administered during labour which showed that the total systemic blood clearance of pethidine was substantially less in pregnant than in healthy non-pregnant females /50/. However, while this suggests that pethidine elimination was less in pregnancy, the study could not distinguish if this was due to reduced metabolism or reduced hepatic blood flow during parturition or both. It is this factor, the possibility of significant haemodynamic changes, which makes direct correlation between hepatic metabolic capacity and drug clearances difficult to establish at parturition.

Similar studies by Moore and McBride with IV diazepam at the time of parturition showed a different response /51/. These workers reported that, while the half-life of diazepam in these women was significantly increased, this was the result of a large increase in this drug's volume of distribution during pregnancy and the total body clearance was found

to be equivalent to those previously recorded for non-pregnant females and male controls. However, Greenblatt *et al* /52/ have recently shown that, if corrections are made for the individual differences in plasma protein binding, diazepam clearance is significantly higher in the female than in the male. Thus, as it has been shown that diazepam serum protein binding is significantly reduced in late pregnancy /53/, the equivalence in clearance recorded by Moore and McBride between pregnant females and non--pregnant controls may, in effect, represent a significant fall in hepatic clearance during pregnancy.

In contrast, studies with another benzodiazepine, chlorazepate, administered (by IMI) to women at 37 and 42 weeks gestation suggested that total body clearance was significantly greater in pregnant women. However, the same study also found that elimination of the metabolite nordiazepam was significantly reduced /53/. As Thomson *et al* /55/ have independently shown that the glucuronide conjugation of oxazepam is increased during pregnancy the reduced elimination appears to be associated with a reduced hepatic capacity to hydroxylate nordiazepam, the primary metabolite of chlorazepate, to oxazepam. These apparently conflicting results make it difficult to establish what influence pregnancy does have on chlorazepate clearance.

The second situation which provides a means of studying drug response in pregnancy involves studies in pregnant women who suffer from chronic conditions such as epilepsy and where it is generally believed that the benefit of seizure control during gestation outweighs the potential dysmorphogenic risks to the embryo/foetus of some anticonvulsant drugs.

It has been found clinically that phenytoin dosage needs to be increased during pregnancy to maintain plasma levels which are adequate to control epileptic seizures in many patients /56, 57, 58/.

Increased metabolic clearance was suggested in all these studies as a possible mechanism for these falls in plasma phenytoin concentration; however, direct evidence of increased metabolite formation was not presented. Thus, the more recent finding that a very large percentage of the total oral dose of phenytoin could be isolated unchanged from the stools of pregnant epileptics /59/ and the reduced amount of urinary 4-hydroxyphenytoin excreted during pregnancy suggest that decreased absorption and perhaps changes in serum protein binding /60, 53/ rather than increased metabolic capacity, might explain the reduced plasma levels found in pregnancy. The changes in absorption could also account for the large inter-subject variability in many studies which

makes meaningful interpretation difficult.

In a more detailed study, Dam *et al.* /61/ measured the plasma clearance of phenytoin, phenobarbital and carbamazepine at various times during gestation and post partum. These workers reported that in each case clearance was increased in the last weeks of pregnancy and/or immediately post partum and in the case of carbamazepine the carbamazepine epoxide/carbamazepine ratio increased during late pregnancy, all suggesting an increase in hepatic metabolism. However, the changes in clearance recorded do not correlate with the patterns of change in plasma concentration generally found in pregnancy. Also inter-subject variations and lack of precise experimental information make the significance of these reported changes in clearance difficult to correlate with definite changes in hepatic metabolism.

A more structured pharmacokinetic study with phenobarbital /62/ again showed considerable inter-subject variations but mean values for the total body clearance, elimination rate constant and volume of distribution of phenobarbital in pregnant women were within the range reported for non-pregnant females.

Similarly a study in 8-14 week pregnant women with oral metronidazole, which is largely oxidised by the liver, failed to show any differences in elimination from that recorded in non-pregnant women /63/.

Two other studies, which examined xanthine derivatives over a significant portion of gestation, gave differing results. Aldridge *et al.* /64/ reported changes in the basic pharmacokinetic parameters calculated from oral doses of caffeine taken by women 11 to 38 weeks pregnant. Whilst there were no significant changes in the first trimester thereafter the total body clearance of caffeine decreased. As caffeine's volume of distribution was not significantly altered by pregnancy it would appear that this change was the result of depressed metabolism. However, this contrasts with an earlier study of oral theophylline administration between the 5th and 8th month of pregnancy /65/ which, when compared to literature values of non-pregnant females, showed no change in clearance but a significant increase in volume of distribution for theophylline. This resulted in an increased half life for theophylline during pregnancy.

Finally the case for depressed hepatic monooxygenase activity in pregnancy received some support from a recent pharmacokinetic study with antipyrine in 22 patients with acute viral hepatitis /65/. Included in these were five non-pregnant and five females between 20 to 32 weeks of gestation. All patients were administered 15 mg antipyrine/kg

by IVI and pharmacokinetic parameters were calculated from the blood concentration vs. time data obtained. Liver biopsies were also taken and the tissue obtained used to measure *in vitro* drug metabolism. The results showed that, while metabolic clearance was significantly reduced by acute hepatitis, the elimination rate constants in the pregnant females were less than those in the non-pregnant group. Liver tissue experiments also showed good correlation between aminopyrine-N-demethylase activity and antipyrine half lives in the respective females.

CONCLUSIONS

The inability to study hepatic drug metabolism directly in human pregnancy means that available information derives from indirect studies under less than ideal experimental conditions. However, even with animal experimentation, where such legal and moral constrains are not impediments to more rigorous studies, there is no consistent pattern of response to pregnancy. This is equally true of hepatic monooxygenase activity both in the untreated and inducer pretreated pregnant animal. Species and strain differences to not satisfactorily explain the wide variations and lack of correlation that exist both within and between many studies. However, the most consistent theme that emerges from studies in the rat is that late pregnancy is associated with a reduction in the specific activity of hepatic monooxygenase enzymes and that this depression is reversed just prior to, or at parturition. To date, speculation into the mechanism of this depression has not produced a hypothesis which can satisfactorily explain all the changes reported during pregnancy. However, the importance of hepatic monooxygenase as a major determinant of therapeutic response requires that more definitive studies in both animals and humans be pursued to establish the nature and extent of changed metabolism occurring with those drugs likely to be administered to pregnant women.

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