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The adverse influence of pregnancy upon sulphation: a clue to the pathogenesis of intrahepatic cholestasis of pregnancy?

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Sulphation of oestrogens and monohydroxy bile acids is important in attenuating their cholestatic potential. Thus, impairment of sulphation could lead to retention of cholestatic compounds and precipitate intrahepatic cholestasis in susceptible individuals. We tested the hypothesis that such a mechanism may be involved in the pathogenesis of intrahepatic cholestasis of pregnancy. *In vivo* and *in vitro* assessment of sulphation capacity was performed in patients with cholestasis of pregnancy, compared with control females on and off the oestrogen-containing oral contraceptive pill and control individuals during normal pregnancy and post partum, to assess the influence of high oestrogen states upon this metabolic pathway. During *in vivo* studies utilising paracetamol as a metabolic probe, the proportion of paracetamol sulphate and sulphate:glucuronide ratio were decreased in those with elevated oestrogens, whether the rise in oestrogens was endogenous, in pregnancy (paracetamol sulphate $p<0.05$; paracetamol sulphate:glucuronide ratio $p<0.01$), or exogenous, with the contraceptive pill (paracetamol sulphate $p=0.2$; paracetamol sulphate:glucuronide ratio $p<0.001$). *In vitro*, platelet sulphotransferase activity was measured, utilising phenol as substrate. Sulphotransferase activity decreased during pregnancy compared with repeat measurements post partum ($p<0.005$) and compared with non-pregnant individuals ($p<0.05$). In conclusion, we have shown that elevated oestrogens are associated with significant impairment in sulphation capacity. An imbalance of sulphation with glucuronidation provoked by high circulating oestrogen levels may be contributory in the pathogenesis of cholestasis of pregnancy. © Journal of Hepatology.

Key words: Cholestasis of pregnancy; Estrogen; Pregnancy; Sulphation

Intrahepatic cholestasis of pregnancy (COP) is a disease of unknown aetiology (1). A high prevalence of the disorder in countries such as Chile and Bolivia (2), plus the aggregation of cases amongst blood relatives, indicates a genetic predisposition (3); however, seasonal variation and marked differences in the intra-regional prevalence of the disease, suggest an additional role of environmental factors (4). The importance of oestrogens in the aetiology of this condition is suggested by its occurrence and recurrence during pregnancy (3,5), a higher prevalence in twin-, compared with single pregnancies (6) and the tendency for susceptible females to develop intrahepatic cholestasis when challenged with the oestrogen-containing oral contraceptive pill.

Oestrogens and monohydroxy bile acids may be conjugated by sulphate or glucuronide in the liver (7,8). Although sulphation renders these substances nontoxic and polar, facilitating excretion, the glucuronide conjugates may be hepatotoxic and cholestatic (8–10). Oestrogen 17-beta glucuronide, for example, is the most cholestatic of all oestrogen metabolites. Cholestasis of pregnancy may be the consequence of production of an abnormal oestrogen metabolite or undue susceptibility to the cholestatic effect of normal oestrogens. Diminished ability to sulphate during pregnancy, either because of substrate competition or downregulation of sulphotransferase activity would result in the accumulation of more cholestatic glucuronide conjugates.

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Benign recurrent intrahepatic cholestasis has some features which parallel COP and the two conditions have been described in the same patients (11–13). It is postulated that both are due to similar mechanisms (13). In benign recurrent intrahepatic cholestasis, bile acid pool studies indicate increased faecal spillover of primary bile acids, resulting in a contracted bile acid pool during the non-cholestatic phase, with a relative rise of cholestatic secondary monohydroxy bile acids (14). Serum bile acids rise rapidly as an episode of cholestasis develops. Serum bile acid levels are also markedly elevated in COP (15). In the presence of a low sulphate state, conjugation with the more cholestatic glucuronide is likely to predominate. It is possible that those who develop COP have an increased secondary monohydroxy bile acid pool. An increased load of monohydroxy bile acids in an environment in which sulphation is already compromised will result in an increase of glucuronide conjugates and would perpetuate the cholestatic environment until demand for sulphation decreased with the fall in oestrogen levels.

We have studied *in vivo* and *in vitro* sulphotransferase activity in groups with a history of COP and control groups during pregnancy and after delivery, as well as control groups on and off the oestrogen-containing oral contraceptive pill.

Patients and Methods

1) In vivo *study*

Assessment of paracetamol sulphation capacity:

Group 1: COP – Pregnant (n=15) – third trimester.
Group 2: COP – Post partum (n=10) – 4–8 weeks post delivery.
Group 3: Control females – Pregnant (n=11) – third trimester.
Group 4: Control females – Post partum (n=10) – 4–8 weeks post delivery.
Group 5: Control females – on oral contraceptive pill (n=11).
Group 6: Control females – not on oral contraceptive pill (n=17).

Group 1 consisted of 15 females who were previously fit and well, but had developed cholestasis of pregnancy. They were otherwise healthy. Pregnancies were progressing otherwise normally in all cases.

Group 2 consisted of 10 patients from group 1. Studies upon this group were carried out within 2 months of delivery. Five patients from group 1 were unable to carry out the test post partum.

Group 3 consisted of 11 females studied during the third trimester of an entirely normal pregnancy. None had a history of previous COP or cholestasis in relationship to the oral contraceptive pill.

Group 4 consisted of 10 subjects from group 3. Studies were performed within 2 months of delivery. One woman from group 3 was unable to carry out a repeat study post partum.

Group 5 consisted of healthy volunteers who worked at the Queen Elizabeth Medical Centre. They were taking the oral contraceptive pill for contraception, not for any other gynaecological indication. None had a past history of COP or of developing cholestasis on the oral contraceptive pill. Individuals were studied during the 3-week phase of oral contraceptive pill ingestion.

Group 6 consisted of a group of healthy volunteers who worked at the Queen Elizabeth Medical Centre. None of them was taking the oral contraceptive pill or any other drugs and none was pregnant. There was no past history of COP. This group was unrelated to group 5.

Patients fulfilling the following criteria were diagnosed as suffering from cholestasis of pregnancy (4).

1) Commencement of pruritus during pregnancy.
2) Continuation of pruritus until parturition.
3) Disappearance of pruritus after delivery.
4) Absence of liver or dermatological disease.
5) Absence of recurrent pruritus except when pregnant or on oral contraceptive pill.

Standard liver function tests were also tested. If these were abnormal during COP, normalisation post partum was required, to exclude patients with intrinsic liver disease from this group. Individuals with allergy to paracetamol were excluded.

The sulphation status was assessed in these groups using paracetamol as a metabolic probe (16–19). Individuals fasted from midnight. Paracetamol (one 500-mg Panadol, Winthrop Pharmaceuticals, UK) was dispensed at 7 h with 100 ml water and 30 min later they were permitted to eat. Urine was collected for 8 h, and then an aliquot was frozen immediately at −20°C until analysed.

All pregnant females were tested during the third trimester of pregnancy. Each individual was given a detailed explanation of the project, and informed consent was obtained. Permission to perform this study was obtained from the local ethical committee.

Analysis of paracetamol metabolites in urine. The urine samples were initially filtered through a Sep-pak C-18 cartridge (Millipore Waters). The analysis was performed according to the method of Howie et al. (20) using high performance liquid chromatography (HPLC).

The mobile phase consisted of 1% (v/v) acetic acid-methanol-ethyl acetate (90:15:0.1 by vol); the flow rate was 1.6 ml/min, and the C18 column was Techopak 10[1], 5.0 μm, 250 mm and 3.9 mm internal diameter, the press-

ure being 150 bar. Detection was by u/v spectrophotometry at 250 nm (0.32 aufs). Appropriate amounts of paracetamol were added to drug-free urine to give final concentrations of 25–250 μg/ml. These standards and urine samples were filtered and injected onto the column. The HPLC assay had an intra-assay variation of 8.7%. Conjugate formation was detected by incubation with either glucuronidase (bovine beta-glucuronidase type B1 (Sigma) or sulphatase (Helix promatia sulphatase type H1, Sigma) incubation. For the former, 1.0 ml urine was diluted to 2.2 ml with 0.05 m acetate buffer (pH 5.0) and incubated with 1000 units of bovine liver beta-glucuronidase. Sulphate conjugation was estimated as above, except that 200 units per incubation of sulphatase type H1 and 10 mg D-saccharic acid-1,4,lactone (21) were added to inhibit beta-glucuronidase activity. All enzyme hydrolyses were carried out at 37°C for 18 h and 10 μl aliquots were used for HPLC analysis. The quantity of the total dose excreted as glucuronide and sulphate conjugates was expressed as mg equivalent of paracetamol (22). There was no increase in the quantity of paracetamol released following enzyme hydrolysis after 16, 18, 24, 48 or 72 h, showing that the reaction had proceeded to the point of completion. All solvents used were of HPLC grade.

2) In vitro *study*

Assessment of platelet phenol sulphotransferase activity: 5 groups.

Group 1: Pregnant females ($n=16$) - on day of delivery.

Group 2: Post-partum females ($n=16$). Females from group 1 each reassessed 3–4 weeks post partum.

Group 3: Non-pregnant females - past COP ($n=18$).

Group 4: Females on oral contraceptive pill ($n=20$) - between day 10 and 21 of cycle.

Group 5: Females not on oral contraceptive pill ($n=21$).

Group 1 consisted of volunteers who were recruited at the time of their routine ante-natal clinic appointments at the Birmingham Maternity Hospital. These same volunteers provided post-partum samples (group 2).

Pregnant females in group 1 were notified of the study when they attended routine antenatal clinics. A venous sample of blood was taken on the day of delivery, where possible at the same time as other blood sampling.

Group 3 consisted of patients who had a previous history of COP, but none of them was pregnant at the time of this assay.

Groups 4 and 5 consisted of healthy volunteers who all worked in the Queen Elizabeth Medical Centre.

A total of 13.5 ml blood was drawn from each subject for the platelet phenol sulphotransferase assay, in addition to a simultaneous sample obtained for platelet count. Following phlebotomy, the sample was immediately put on ice, and then rapidly processed.

Each individual was given a detailed explanation of the project, and informed consent was obtained. Permission to perform this study was obtained from the local ethical committee.

Analysis of platelet phenol sulphotransferase (PST) activity. Platelets were prepared according to the method of Anderson et al. (23). Blood samples were spun at 200 g for 10 min at 20°C before platelet-rich plasma was aspirated. This was transferred and re-spun at 2500 g for 20 min at 4°C. Plasma was aspirated and discarded. The platelet pellet was rinsed twice with phosphate buffer (10 mM, pH 7.4), then resuspended in 1 ml of buffer and homogenised for 15 s. The platelet homogenate thus formed was used for platelet phenol sulphotransferase assay. Platelet phenol sulphotransferase was assayed by a modification of the method of Littlewood et al. (24).

Aliquots (20 μl) of platelet homogenate were placed in 1.5 ml plastic Ependorf tubes. To these was added 20 μl of the substrate – phenol (BDH, Poole, UK) at a concentration of 0.1 mM. The reaction was initiated by adding non-radioactive and radioactive (35-S) 3′-phosphoadenosine 5′-phosphosulphate (PAPS), with a final concentration of 6.7 μM. The mixture was made up to a final volume of 150 μl with 10 mM phosphate buffer (pH 7.4).

Samples were incubated in a water bath at 37°C for 30 min. The reaction was terminated by adding 200 μl of 0.1 mol/l barium acetate. Unreacted 3′-phosphoadenosine 5′-phosphosulphate was removed by precipitating twice each with 200 μl of 0.1 mol/l barium hydroxide and 0.1 mol/l zinc sulphate. Samples were centrifuged for 5 min. The supernatant (700 μl) was placed in scintillation vials and counted with 3.5 ml scintillant (Optiphase "Hi Safe 3" scintillation fluid – LKB, Loughborough, UK).

Platelet counts for each individual were measured on an automated Syfmex NE1500 coulter counter. Results were expressed as activity per 10^9 platelets per 30 min incubation.

Statistical analysis

Wilcoxon's rank sum (25) test was applied for comparison of quantitative biochemical data from the analysis of paracetamol metabolites and platelet sulphotransferase activity, for non-paired data. Wilcoxon's signed rank tested the paired hypothesis between subjects who were tested both pre and post partum. (In the *in vivo* study, 10 subjects (Groups 1 and 2); and 10 subjects (groups 3 and 4) had paired analyses performed pre and post partum. In the *in vitro* study, 16 subjects were tested both pre and post partum; groups 1 and 2.)

TABLE 1

Results of paracetamol metabolism in the six groups

Groups	% Free paracetamol	% Paracetamol sulphate	% Paracetamol glucuronide	* Ratio SO_4/GLU
Group 1. Pregnant, cholestasis of pregnancy $n=15$				
Median	3.8	8.2	17.9	0.4
Conf interval	3–4.3	7.1–9.2	14.5–20.8	0.32–0.56
Group 2. Post partum, cholestasis of pregnancy $n=10$				
Median	3.1	14.8	15.4	0.98
Conf interval	1.3–4.6	8.0–19.6	1.0–33.3	0.64–1.57
Group 3. Pregnant control $n=11$				
Median	3.2	6.2	22.3	0.39
Conf interval	2.0–4.4	1.2–13.2	12.2–30.2	0.1–0.49
Group 4. Post partum, control pregnant $n=10$				
Median	2.6	20.3	25.1	0.69
Conf interval	1.4–4.5	7.8–24.6	11.5–33.6	0.32–0.97
Group 5. Non pregnant on oral contraceptive pill $n=11$				
Median	1.4	7.7	17.9	0.38
Conf interval	0.6–3.4	2.2–11.9	7.8–24.3	0.26–0.53
Group 6. Control female, not on oral contraceptive pill $n=17$				
Median	1.6	9.1	10.8	0.72
Conf interval	0.9–2.8	5.8–12.1	9.2–19.9	0.6–0.85

*Conf interval: confidence intervals expressed at 95% levels.

Results presented of paracetamol analysis by HPLC in patients with COP, pregnant and post partum, healthy controls, pregnant and post partum and healthy controls either on or off the oral contraceptive pill. Median values, and 95% confidence intervals for percentage recovery of parent compound, paracetamol sulphate, paracetamol glucuronide and the paracetamol sulphate to paracetamol glucuronide ratio. Statistical analysis:

1) Paracetamol sulphate			
Wilcoxon signed rank, $n=10$:			
COP	pregnant v post partum	group 1 v group 2	$p<0.05$
Control females	pregnant v post partum	group 2 v group 4	$p<0.05$
Wilcoxon rank sum:			
Control females	OC pill v no OC pill	group 5 v group 6	$p=0.2$
2) Paracetamol Glucuronide			
Wilcoxon signed rank, $n=10$:			
COP	pregnant v post partum	group 1 v group 2	$p=$NS
Control females	pregnant v post partum	group 3 v group 4	$p=$NS
Wilcoxon rank sum:			
Control females	OC pill vs no OC pill	group 5 v group 6	$p=$NS
3) Paracetamol sulphate/glucuronide ratios			
Wilcoxon signed rank, $n=10$:			
COP	pregnant v post partum	group 1 v group 2	$p<0.001$
Control females	pregnant v post partum	group 3 v group 4	$p<0.01$
Wilcoxon rank sum:			
Control females	OC pill vs no OC pill	group 5 v group 6	$p<0.001$

Results

A) Paracetamol metabolism (Table 1)

i) *Total recovery.* There was no significant difference in total recovery of paracetamol and its conjugates between the pairings; Wilcoxon signed rank, $n=10$, group 1 versus group 2, $p=0.58$; Wilcoxon signed rank, $n=10$, group 3 versus group 4, $p=0.24$ and Wilcoxon rank sum, group 5 versus group 6, $p=0.85$.

ii) *Paracetamol sulphate.* All three groups with elevated oestrogens, whether pregnant with COP, control pregnant females or control females on the oral contraceptive pill, produced less sulphate conjugate than their equivalent groups post partum or not on the oral contraceptive pill. The difference was significant in both groups during pregnancy compared with post partum, $p<0.05$ (Wilcoxon signed rank), although it did not achieve significance in the group on the oral contraceptive pill, compared with the group not on the oral contraceptive pill, $p=0.2$ (Wilcoxon rank sum).

iii) *Paracetamol glucuronide.* There was no significant difference in the quantity of paracetamol glucuronide produced between the various pairs or groups.

iv) *Paracetamol sulphate/glucuronide ratio.* The sulphate/glucuronide ratios were similar ($p>0.1$) when females with elevated oestrogens, groups 1, 3 and 5 were compared with each other. Similarly there were no significant differences when the sulphate/glucuronide in the lower oestrogen level groups 2, 4 and 6 were compared ($p>0.1$). The sulphate/glucuronide ratios of post partum females and females not on the oral contraceptive pill in groups 2, 4 and 6, however, were significantly higher than pregnant females or females on the oral contraceptive pill, groups 1, 3 and 5 respectively (COP, pregnant versus post partum, Wilcoxon signed rank, $p<0.01$, control females, pregnant versus post partum, Wilcoxon signed rank, $p<0.01$ and control females, on versus off the oral contraceptive pill, Wilcoxon rank sum, $p<0.001$) (Table 1). Thus females with an increased oestrogen load (groups 1, 3 and 5) had significantly lower sulphate/glucuronide ratios than matched female groups with normal oestrogen levels, i.e. females in groups 2, 4 and 6. The sulphate/glucuronide ratio of the pregnant groups was also significantly lower than the group 6 non-pregnant control females off the oral contraceptive pill (Wilcoxon rank sum); COP, pregnant vs group 6, $p<0.01$, control pregnant vs group 6, $p<0.001$).

B) Platelet phenol sulphotransferase (PST) activity

Table 2 shows the median values and range for platelet phenol sulphotransferase activity, as indicated by scintillation activity per 30 min incubation per 10^9 platelets.

i) *The effect of pregnancy.* Pregnant females, Group 1, have significantly lower platelet phenol sulphotransferase activity than the same individuals post partum, Wilcoxon signed rank $n=16$, ($p<0.005$). Pregnant females also have significantly lower activity of platelet phenol sulphotransferase compared with healthy non-pregnant control groups (Wilcoxon rank sum) with previous COP, on the oral contraceptive pill and off the oral contraceptive pill ($p<0.05$).

ii) *The effect of previous COP.* There is no statistically significant difference in the platelet phenol sulphotransferase activity of those with a past history of COP (group 3) and control individuals on or off the oral contraceptive pill; groups 4 and 5.

iii) *The effect of the oral contraceptive pill.* Platelet phenol sulphotransferase activity is slightly higher in those on the oral contraceptive pill in group 4, than those not on the oral contraceptive pill group 5, but the difference is not significant ($p=0.4$).

Discussion

Sulphation is an important metabolic pathway in the detoxification of many endogenous and exogenous compounds (16,26,27). The reaction is catalysed by a family of enzymes, the sulphotransferases, which are widely distributed throughout the body, including liver, lung, cerebral cortex, intestine, kidney and platelets. All sulphotransferases described to date utilise 3′-phosphoadenosine 5′-phosphosulphate as the sulphate donor. 3′-phosphoadenosine 5′-phosphosulphate is formed *in vivo* from inorganic sulphate and under certain circumstances, the availability of inorganic sulphate may limit the rate of sulphate conjugation (9).

Sulphate conjugation is readily saturated in human liver, due to the relatively poor supply of inorganic sul-

TABLE 2

Results of platelet phenol sulphotransferase activity in the six groups

Group	1 Pregnant	2 Post partum	3 Previous COP	4 OC pill	5 No OC pill
Number (N)	16	16	18	20	21
Age	29	29	32	27	30
Plt* count	233	348	241	277	258
PST activity	12.3	42	20.1	26.6	16.8
Act/Plt	0.066	0.134	0.094	0.110	0.085
Range	0.017–0.24	0.003–0.33	0.003–0.37	0.003–0.28	0.026–0.36
Conf int†	0.03–0.1	0.10–0.25	0.05–0.13	0.10–0.12	0.05–0.135

*OC pill=oral contraceptive pill. †Confidence intervals expressed at the 95% level. *Plt count=platelet count$\times 10^9$/l. Conf int=confidence interval. Plt=platelet.

Results are presented of median age, platelet count $\times 10^9$ and PST activity, plus PST activity/ platelet count $\times 10^9$, with range and 95% confidence intervals for the latter, measured in healthy females pregnant and post partum and healthy females either on or off the oral contraceptive pill or females with previous COP. Pregnant females, Group 1, have significantly lower PST/platelet count activity than the same individuals post partum, Wilcoxon signed rank ($p<0.005$). Pregnant females, have significantly lower activity of PST/platelet count compared with non-pregnant control groups combined (groups 3,4 and 5) previous COP, on oral contraceptive pill and off oral contraceptive pill, Wilcoxon rank sum ($p<0.05$) and compared with individual groups using Wilcoxon rank sum, group 1 compared with group 4, females on oral contraceptive pill, $p=0.01$; and a non-significant trend to reduced activity in group 3, previous COP, $p=0.18$; and group 5, not on oral contraceptive pill, $p=0.18$.

phate and the high energy sulphate donor 3′-phosphoadenosine 5′-phosphosulphate (16,26–28). Increased competition for the limited supply of sulphate results in a decrease in the efficiency with which substrates are sulphated. For example, increasing the oral dose of paracetamol from 5 mg/kg to 20 mg/kg reduces the proportion of drug that is sulphated (29). Similarly, for those on the oestrogen-containing oral contraceptive pill, concomitant medication with vitamin C or paracetamol (both of which are sulphated) reduces the metabolism of the ethinyloestradiol component (30–32). Whenever demand for sulphation increases, alternative conjugation reactions such as glucuronidation or methylation are increasingly utilised (28).

In our *in vivo* study assessing paracetamol sulphation, pregnant females with or without COP had significantly lower sulphate/glucuronide ratios than the same females after delivery or a group of age-matched female controls not taking the oral contraceptive pill. This reduced ratio resulted from a decrease in the quantity of sulphate conjugation in absolute terms. This might have resulted from a reduced supply of inorganic sulphate, down-regulation of sulphotransferase by oestrogens or competitive inhibition of the sulphotransferase enzymes by pregnancy-related increases in substrate hormones. A reduced sulphate/glucuronide ratio was also seen in the group on the oral contraceptive pill. In this case, however, the reduced ratio was not simply due to a decline in sulphate conjugate, which was reduced non-significantly, but also an increase in glucuronide conjugates. A similar pattern has been noted by others assessing the influence of the oral contraceptive pill upon paracetamol metabolism (33). The difference in proportion of paracetamol sulphate between females on and off the oral contraceptive pill may have failed to reach statistical significance because of a type 2 error, since sample size was small.

In vitro assessment of platelet phenol sulphotransferase reveals a detrimental influence of pregnancy on the activity of this enzyme. Total activity and activity per 10^9 platelets was lower in the pregnant group than the other control groups. During the post-partum period, this activity appears to rebound to supranormal levels.

A similar reduction in platelet phenol sulphotransferase activity was not seen in the group receiving exogenous oestrogens. Activity was actually greater in these individuals than in those not on the oral contraceptive pill, although the difference did not achieve significance. This may be because the dose of ethinyloestradiol, 30 μg in all subjects, was too low to influence the activity of platelet phenol sulphotransferase. Alternatively, it may reflect the pattern of oestrogen ingestion whilst on the pill. All subjects take the oestrogen-containing oral contraceptive pill for the first 21 days of their cycle and for the subsequent 7 days take none. During this time it is possible that platelet phenol sulphotransferase activity rebounds to higher than normal levels, as it does following pregnancy.

Oestrogen has been incriminated as the causative agent in COP (1,4–6,8); yet the mechanism, and in particular the reason why some females should be affected and not others, is unexplained. Cholestasis may develop in susceptible individuals who produce a specific, atypical oestrogen metabolite. Alternatively, it may result from undue susceptibility to normal oestrogens in those with an excessive imbalance between the capacity for sulphate generation, sulphate conjugation and a prediliction to form the most cholestatic glucuronide conjugates.

Although it is known that several different sulphotransferase enzymes are present in man, to date only a single steroid/bile acid sulphotransferase has been isolated and purified from human liver cytosol, named dehydroepiandrosterone sulphotransferase. This enzyme appears responsible for sulphating oestrogens and bile acids such as lithocholic acid (16,34). During this study, *in vivo* assessment of sulphotransferase activity utilised paracetamol as the probe. This is a substrate for phenol sulphotransferase. Similarly, the *in vitro* studies assessed phenol sulphotransferase activity, but with phenol as the substrate. Paracetamol was chosen for the *in vivo* studies, because of its known safety in the third trimester of pregnancy.

During cholestasis serum bile acids rise greatly, and urinary excretion of bile acids becomes a major route of excretion (35–39). A large proportion of urinary bile acids, including cholestatic lithocholic acid, are excreted in their sulphated form. This may be because the addition of sulphate increases polarity, facilitating urinary excretion.

Sulphation of oestrogens and bile acids may be of benefit in one of three ways: firstly sulphation of lithocholic acid reduces its enterohepatic circulation enhancing its faecal excretion; secondly sulphation may reduce the cholestatic potential of the particular oestrogen/bile acid; and finally sulphation may enhance urinary excretion if bile acids begin to rise. Elevation of oestrogen levels, by interfering with sulphation, may therefore predispose to cholestasis in any of these ways.

Since the results do not show a deficiency which is specific to COP, we therefore believe they demonstrate a milieu interieur with great susceptibility to cholestasis in pregnancy. We have not been able to examine dehydroepiandrosterone sulphotransferase activity directly, and acknowledge that a factor, additional to our findings, such as a genetically determined relative deficiency of dehydroepiandrosterone sulphotransferase activity is necessary to account for individual susceptibility in intrahepatic cholestasis of pregnancy.

Whether dehydroepiandrosterone sulphotransferase activity is regulated in parallel with platelet phenol sulphotransferase activity is unknown. In rats, bile acid sulphotransferase activity is highly expressed in the female liver and almost undetectable in male liver. Hydroxysteroid sulphotransferase activity in the rat is increased following administration of oestrogen and diminished by androgens (35). Although it is unlikely that platelet phenol sulphotransferase is capable of sulphating bile acids, it is possible that dehydroepiandrosterone sulphotransferase also has the capacity to sulphate simpler phenolic compounds including paracetamol. Our *in vivo* observations therefore suggest that pregnancy not only produces a critical deficiency and downregulation of platelet phenol sulphotransferase and paracetamol sulphating capacity, but would infer, by implication that hepatic dehydroepiandrosterone sulphotransferase capacity is similarly limited.

In conclusion, we have shown that elevated oestrogen levels are associated with a significant impairment in sulphation capacity. An imbalance of sulphation with glucuronidation provoked by high circulating oestrogen levels may be of pathogenetic importance in this disease.

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