Br. J. clin. Pharmac. (1983), **16**, 503–509

INFLUENCE OF SEX AND ORAL CONTRACEPTIVE STEROIDS ON PARACETAMOL METABOLISM

J.O. MINERS, J. ATTWOOD & D.J. BIRKETT

Department of Clinical Pharmacology, Flinders University of South Australia, Flinders Medical Centre, Bedford Park, Adelaide, South Australia, 5042

1 Paracetamol metabolism was investigated in eight healthy males, eight healthy females and eight healthy females receiving oral contraceptive steroids (OCS).

2 Paracetamol clearance was 22% greater in males compared to the control female group. This difference was entirely due to increased activity of the glucuronidation pathway in males, there being no sex-related differences in the sulphation or oxidative metabolism of paracetamol.

3 Paracetamol clearance in females using OCS was 49% greater than in the control females. Glucuronidation and oxidative metabolism were both induced in OCS users (by 78% and 36% respectively) but sulphation was not altered.

4 Although sex-related differences in paracetamol metabolism are unlikely to be of clinical importance, induction of paracetamol metabolism by OCS may have clinical and toxicological consequences.

Keywords paracetamol contraceptive steroids sex difference drug conjugation

Introduction

Although it has been known for some time that sex hormones influence the activity of certain forms of cytochrome P-450 in the rat (Kato, 1974), only recently have systematic studies been performed in man which demonstrate an effect of sex on oxidative drug metabolism. In particular, differences between males and females in the metabolism of antipyrine (Teunissen *et al.*, 1982), chlordiazepoxide (Roberts *et al.*, 1979), desmethyldiazepam (Allen *et al.*, 1980) and diazepam (Greenblatt *et al.*, 1980a) have been reported. There is also considerable evidence showing that the elimination of a number of drugs metabolised by the hepatic mixed function oxidase system is impaired in women receiving oral contraceptive steroids (OCS). Thus, OCS reduce the clearance of aminopyrine (Sonnenberg *et al.*, 1980), antipyrine (Chambers *et al.*, 1982; Teunissen *et al.*, 1982), caffeine (Patwardhan *et al.*, 1980), chlordiazepoxide (Roberts *et al.*, 1979) and metoprolol (Kendall *et al.*, 1982). The clearance of nitrazepam, a drug which undergoes reductive metabolism, is also diminished in women taking OCS (Jochemsen *et al.*, 1982).

Whereas the effect of sex and OCS on oxidative drug metabolism is now recognised, both in rats and humans, little data are available on the influence of these factors on drug conjugation reactions. The clearance of oxazepam and temazepam, drugs biotransformed by glucuronide conjugation, have been shown to be significantly higher in males compared to females (Greenblatt *et al.*, 1980b; Divoll *et al.*, 1981) while gender had no apparent effect on the clearance of another glucuronidated benzodiazepine, lorazepam (Greenblatt *et al.*, 1979). Recent reports (Wojcicki *et al.*, 1979; Mucklow *et al.*, 1980; Divoll *et al.*, 1982) have also indicated that paracetamol clearance is greater in males than females. Paracetamol is biotransformed in the liver primarily by conjugation with glucuronic acid and sulphate, with a small proportion of the dose being transformed by cytochrome P-450 to a reactive intermediate which is detoxified by conjugation with glutathione (Hinson, 1980). Thus, paracetamol serves as a useful model drug for simultaneously determining effects on both glucuronide and sulphate conjugation, as well as a (minor) oxidative pathway. The present study was therefore undertaken to investigate the effect of gender and OCS on the individual metabolic pathways for paracetamol.

Methods

Subjects

The subjects were 24 volunteers; eight males (mean age 24.8 ± 2.1 years; mean weight 73.8 ± 4.8 kg), eight females not taking OCS (mean age 22.5 ± 2.6 years; mean weight 64.9 ± 4.2 kg), and eight females receiving OCS (mean age 22.2 ± 1.3 years; mean weight 61.7 ± 4.0 kg). All subjects were non-smokers

and were healthy as determined by medical history, physical examination, and biochemical and haematological parameters. No medications, other than those required for the study, were taken for 1 week before or during the study. The OCS were all combination pills and included Microgynon and Nordette (both ethinyloestradiol 0.03 mg, levonorgestrol 0.15 mg), and Orthonovum (mestranol 0.08 mg, norethisterone 1.0 mg). With the female subjects, study days were not standardised to any particular phase of the menstrual cycle. Of the control group females four were studied in the follicular phase and four in the luteal phase, while all OCS users were studied between days 5 and 22 of the pill cycle. Written informed consent was obtained from each subject and the study was approved by the Clinical Investigation Committee of Flinders Medical Centre.

Protocol

After an overnight fast the subjects received 2 x 500 mg paracetamol tablets (Panadol) with 150 ml tap water. Three mouth washes with warm water were performed in the 10 min following the paracetamol dose. Saliva samples were collected prior to and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6 and 8 h after drug administration. In addition, urine was collected for 24 h following the paracetamol dose. Saliva samples were stored at −20°C until analysis but urine samples were assayed within 12 h of collection.

Analytical procedures

Salivary paracetamol concentrations were measured by a specific high performance liquid chromatographic method. A 0.05 ml aliquot of saliva was mixed with an equal volume of 10% trichloroacetic acid and after centrifugation 0.01 ml of the clear supernatant was injected onto a Waters Associates μ-Bondapak C-18 reversed phase column (30 cm x 3.9 mm i.d.). The mobile phase was 5% acetonitrile—95% phosphate buffer (20 mM, pH 3.0) at a flow rate of 2 ml min and an ultraviolet absorbance detector operating at 250 nm was used for quantitation of paracetamol. Calibration curves for paracetamol peak height were linear in the range 0.5–100 mg l^{-1} and passed through the origin. The method was shown to be reproducible, with the mean (± s.d.) coefficient of variation of the normalised peak heights from 40 standard curves prepared over 1 year being 3.1 ± 1.4%. Concentrations of unchanged paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates in urine were determined by high performance liquid chromatography (Miners *et al.*, 1984).

Analysis of results

The following parameters were estimated from the individual saliva paracetamol concentration-time curves. Apparent first-order elimination rate constant (k) was calculated by linear least squares regression analysis using experimental data points from 2 to 8 h after dosing, and half-life ($t_{½}$) determined as $0.693/k$. Clearance (CL_p) and apparent volume of distribution (V) were calculated from the area under the saliva concentration-time curve (AUC) as determined by the trapezoidal rule with extrapolation to infinite-time. Thus,

$$CL_p = D \text{ (AUC x B.W.) and } V = CL_p\ k$$

where B.W. is the body weight in kg.

The clearance to each metabolite (CL_m) was calculated as:

$$CL_m = f_m \cdot CL_p$$

where f_m is the fractional excretion of the metabolite compared to total paracetamol-derived products. This expression follows from the assumption that CL_p is the sum of the individual clearances involved in paracetamol elimination, i.e.

$$CL_p = CL_R + CL_G + CL_S + CL_{GSH}$$

where Cl_R is the renal clearance of unchanged paracetamol and CL_G, CL_S and CL_{GSH} are metabolic clearances to the glucuronide, sulphate and glutathione-derived conjugates respectively. The glutathione derived conjugate was measured as the sum of the cysteine and mercapturic acid derivatives.

Results are expressed as mean ± s.e.mean. The significance of the differences between study groups was determined using the Mann-Whitney U test. Values of $P < 0.05$ were regarded as significant.

Results

Table 1 summarises the paracetamol kinetic parameters in each of the three study groups. Paracetamol clearance (CL_p) was higher in the male (5.62 ± 0.18 ml min^{-1} kg^{-1} ($P < 0.001$) than in the control (non-OCS) female group (4.61 ± 0.18 ml min^{-1} kg^{-1}) but $t_{½}$ was not significantly different. Paracetamol clearance was markedly increased (by 49%) in OCS users compared to control females ($P < 0.01$). The magnitude of this effect was such that CL_p was higher in OCS using females than in males although this was of marginal statistical significance ($P < 0.065$). Paracetamol half-life ($t_{½}$) was shorter in the OCS users (1.49 ± 0.12 h) compared to both the males (2.09 ± 0.09 h) ($P < 0.001$) and control females (2.16 ± 0.17 h) ($P < 0.01$). The volumes of distribution for paracetamol were similar in the two female groups (control females, 0.83 ± 0.05 1 kg^{-1}; OCS users, 0.85 ± 0.05 1 kg^{-1}) and in both cases were significantly lower than for males (1.00 ± 0.06 1 kg^{-1}) ($P < 0.05$ for both the control female and OCS user groups).

Table 1 Paracetamol kinetics in males, females and OCS-using females

Group	CL_P (ml min^{-1} kg^{-1})	$t_{½}$ (h)	V (l kg^{-1})
Males	5.62 ± 0.18	2.09 ± 0.09	1.00 ± 0.06
Females	4.61** ± 0.18	2.16 ± 0.17	0.83* ± 0.05
OCS-using females	6.88^{+} ± 0.65	1.49**$^{+}$ ± 0.12	0.85* ± 0.05

Compared to males; * $P < 0.05$, ** $P < 0.001$
Compared to females; $^{+}P < 0.01$

The individual metabolic and renal clearances of paracetamol are shown in Table 2. Glucuronidation was enhanced in males (3.42 ± 0.16 ml min^{-1} kg^{-1}, $P < 0.01$) and in OCS using females (4.74 ± 0.51 ml min^{-1} kg^{-1}, $P < 0.01$) compared to control females (2.67 ± 0.20 ml min^{-1} kg^{-1}). Cl_G was higher in the OCS users than in the male subjects ($P < 0.05$). Oxidative metabolism of paracetamol (clearance to glutathione derived metabolites, CL_{GSH}) was also induced by OCS, being 36% greater in OCS users than in control females ($P < 0.05$). Although CL_{GSH} was higher in males (0.51 ± 0.05 ml min^{-1} kg^{-1}) than in control females (0.44 ± 0.05 ml min^{-1} kg^{-1}) the difference was not statistically significant. There were no significant differences between any of the groups in clearance by sulphation or in the renal clearance of unchanged paracetamol. No differences were observed in any parameter when females studied in the luteal phase of their cycle were compared to those studied during the follicular phase.

The urinary excretion profile of paracetamol and its metabolites in each group is shown in Table 3. In all groups the recovery of paracetamol was essentially quantitative. There was no significant difference between males and females in the percentage excretion of any of the metabolites. In the OCS users, the marked induction of the glucuronidation pathway caused an increase in the proportion of the dose excreted as the glucuronide ($P < 0.05$) and a decrease in sulphate excretion ($P < 0.05$) compared to both males and control females.

Discussion

Throughout this study salivary paracetamol concentrations have been used to determine paracetamol clearance. In a preliminary experiment the relationship between plasma and salivary paracetamol concentrations was investigated in six subjects. The sub-

Table 2 Metabolic and renal clearances of paracetamol in males, females and OCS-using females

Group	CL (ml min^{-1} kg^{-1}) to:			
	G[1]	S	GSH	P
Males	3.42 ± 0.16	1.43 ± 0.13	0.51 ± 0.05	0.27 ± 0.04
Females	2.67** ± 0.20	1.24 ± 0.12	0.44 ± 0.05	0.27 ± 0.03
OCS-using females	4.74*$^{++}$ ± 0.51	1.21 ± 0.16	0.60+ ± 0.09	0.32 ± 0.04

[1] G = glucuronide, S = sulphate, GSH = glutathione-derived conjugates (cysteine + mercapturic acid), P = unchanged paracetamol.

Compared to males; * $P < 0.05$, ** $P < 0.01$
Compared to females; $^{+}P < 0.05$, $^{++}P < 0.01$

Table 3 Urinary excretion of paracetamol and its metabolites in males, females and OCS-using females

Group	*Mean fractional excretion (% recovered dose)*[1]				*Mean % dose recovered*[2]
	G[3]	*S*	*GSH*	*P*	
Males	60.5 ± 1.6	25.4 ± 1.3	8.1 ± 1.3	4.9 ± 0.6	101.5 ± 2.3
Females	57.6 ± 1.9	26.9 ± 1.9	8.7 ± 1.5	4.9 ± 0.8	98.3 ± 3.5
OCS-using females	68.8*+ ± 2.3	17.6*+ ± 1.6	9.1 ± 0.9	4.4 ± 0.7	100.0 ± 4.2

[1] Expressed as percentage of total paracetamol-derived products recovered.
[2] Total paracetamol-derived products recovered expressed as a percentage of the dose administered.
[3] See Table 2 for explanation of symbols.
Compared to males, * $P < 0.05$
Compared to females, + $P < 0.05$

jects, all healthy male volunteers, were administered paracetamol (1 g) and saliva and blood samples were taken at 30 min intervals to 8 h. Using the h.p.l.c. procedure described earlier for the determination of paracetamol, there was a highly significant correlation ($r = 0.975$, $P < 0.001$) between AUCs calculated from salivary and plasma paracetamol concentrations. Although this result is in concurrence with the data of Glynn & Bastain (1973) and Mucklow *et al.* (1980), a more recent report (Adithan & Thangam, 1982) failed to show a significant correlation between AUCs calculated from salivary and plasma paracetamol concentrations. The reason for the variability observed in the latter study is not clear but may be due to the spectrophotometric procedure used for the determination of paracetamol concentration.

In this study paracetamol clearance has been determined following oral doses of the drug. While there is evidence that paracetamol undergoes presystemic hepatic metabolism, this first-pass effect is only approximately 10% for a 1 g dose of paracetamol (Rawlins *et al.*, 1977). Thus, paracetamol clearance calculated in this manner will slightly overestimate systemic clearance and volume of distribution. However, clearance determined after oral administration is a measure of intrinsic hepatic clearance (Wilkinson & Shand, 1975) and is therefore a better indicator of drug metabolising enzyme capacity than systemic clearance. Similarly, it should be noted that throughout this work CL_{GSH} has been assumed to reflect the cytochrome P-450 mediated metabolite formation. Animal studies have shown that covalent binding of reactive metabolite to hepatocyte macromolecules does not occur after low doses of paracetamol (Hinson, 1980) suggesting that glutathione conjugate formation is a valid measure of the oxidative pathway under these conditions.

The data presented here show that paracetamol clearance is 22% greater in males compared to females. This result is consistent with previous reports (Wojcicki *et al.*, 1979; Mucklow *et al.*, 1980; Divoll *et al.*, 1982) which have suggested that paracetamol clearance is faster in males than in females. Paracetamol volume of distribution was lower in females (including the OCS users), probably due to the greater proportion of total body weight as fat in women together with incomplete distribution of paracetamol into fat (Divoll *et al.*, 1982). There was no difference in paracetamol half-life between males and the control females, half-life being a hybrid kinetic variable related directly to volume of distribution and inversely to clearance.

Although there was no significant difference between males and females in the fraction of each metabolite excreted in the urine, the metabolic clearance to the glucuronide conjugate was 28% higher in males. There were no sex-related differences in the renal clearance of paracetamol or in the clearances to the sulphate and glutathione-derived conjugates. Thus, it would appear that the difference in paracetamol clearance observed between the sexes is entirely due to an increased activity of the glucuronidation pathway in males. To date, the only other report of sex-related differences in the clearance of drugs dependent on glucuronide conjugation for their elimination are for the benzodiazepines, temazepam and oxazepam (Greenblatt *et al.*, 1980b; Divoll *et al.*, 1981). The clearances of oxazepam and temazepam were 40% and 32% greater respectively in males compared to females. By contrast, sex was not a significant factor in accounting for the variability observed in the disposition of the similarly metabolised benzodiazepine lorazepam, a discrepancy which has been suggested as being due to incomplete matching of the

respective study populations (Greenblatt *et al.*, 1979). Variable effects of sex on oxidative metabolism have been reported in humans. The demethylation of chlordiazepoxide was found to be faster in males than in females (Roberts *et al.*, 1979), but the intrinsic clearances of desmethyldiazepam and diazepam and the metabolic clearance of antipyrine to norantipyrine, 4-hydroxyantipyrine and 3-hydroxymethylantipyrine were greater in females (Allen *et al.*, 1980; Greenblatt *et al.*, 1980a; Teunissen *et al.*, 1982).

There is some evidence in animals showing a direct effect of sex hormones on UDP-glucuronyltransferase activity. The glucuronidation of 2-aminophenol was shown to be approximately four times greater in liver microsomes from male rats compared to microsomes from female rats (Inscoe & Axelrod, 1960). Chronic administration of oestradiol to male rats decreased glucuronyltransferase activity while the chronic administration of testosterone to female rats increased glucuronidation. Specific UDP-glucuronyltransferase activities have been shown to be decreased in pregnant rats where oestrogen and progesterone levels are high (Feuer & Liscio, 1969; Neale & Parke, 1973). However, the relative importance of the individual sex hormones on glucuronidation in humans remains unclear. In the present study there was no difference in any of the parameters measured between female subjects studied in the luteal and follicular phases of their cycles. Similarly, Wojcicki *et al.* (1979) reported that, although there was a trend to higher AUC for paracetamol during the luteal phase compared to the follicular phase, this difference was not significant.

In females taking OCS paracetamol clearance was 49% greater compared to the control females and was 22% higher than in males. Paracetamol half-life was correspondingly decreased in the OCS users. In a study of the variability of paracetamol disposition in a group of London factory and office workers Mucklow *et al.* (1980) also found a trend to higher paracetamol clearance in OCS users, although effects on specific metabolic pathways were not determined. The results of the present study show that the increased paracetamol clearance in OCS users was largely due to induction of UDP-glucuronyl transferase in this group. Thus, for the OCS users CL_G was 78% greater than in the control females and 39% greater than in males. Furthermore, the clearance to the glutathione-derived products was significantly greater in OCS users compared to non-pill using females, the extent of induction (36%) being approximately half that observed for glucuronidation. While the fractional excretion of the sulphate conjugate was lower in OCS users compared to the other groups, clearance to this metabolite was not altered. The latter result is not surprising since sulphotransferase appears to be a non-inducible enzyme and, even though ethinyloestradiol is extensively sulphated (Orme, 1982), the low dose of oestrogen in the OCS would not be expected to result in significant competitive inhibition of paracetamol sulphation. It might be speculated, however, that co-administration of paracetamol and OCS would reduce the first-pass metabolism of ethinyloestradiol thereby increasing its bioavailability.

Little other data is available on the effect of OCS on xenobiotic glucuronidation. Addition of ethinyloestradiol to rat liver slices decreased the rate of glucuronidation of 2-aminophenol and bilirubin, while addition of progestogens did not inhibit the conjugation of these substrates (Hargreaves *et al.*, 1971). Furthermore, only the oestrogenic component appears to be responsible for the hyperbilirubinaemia observed in some women receiving OCS (Aldercreutz & Ikonen, 1964). These observations suggest that induction of glucuronidation is more likely to be due to the progestogen since oestrogens appear to inhibit this pathway. Animal studies have been initiated to test this hypothesis.

The oxidative metabolism of paracetamol, determined as clearance to the glutathione-derived conjugates, was induced in the OCS users compared to the control female group. While some pathways of antipyrine biotransformation may be induced by OCS (Teunissen *et al.*, 1982), these results contrast to the inhibition of clearance by OCS for the oxidised drugs aminopyrine (Sonnenberg *et al.*, 1980), antipyrine (Chambers *et al.*, 1982; Teunissen *et al.*, 1982), caffeine (Patwardhan *et al.*, 1980), chlordiazepoxide (Roberts *et al.*, 1979) and metoprolol (Kendall *et al.*, 1982). Again, the oestrogenic component of OCS appears to be responsible for the decreased elimination of these drugs (Chambers *et al.*, 1982). Progestogens, in particular lynestrenol, increase the activity of a range of liver microsomal enzymes in rats and mice (Briatico *et al.*, 1976), consistent with a selective effect of OCS on the various forms of cytochrome P-450. The oxidative metabolism of paracetamol is of major toxicological significance since it results in the formation of the reactive intermediate responsible for paracetamol-mediated hepatotoxicity. Induction of this pathway enhances paracetamol toxicity in laboratory animals (Hinson, 1980) and microsomal enzyme induction has also been reported to increase the susceptibility of humans to liver damage following paracetamol overdose (Wilson *et al.*, 1978; Wright & Prescott, 1973) and chronic low-dose treatment with paracetamol (Goldfinger *et al.*, 1978; Johnson & Tolman, 1977; McClain *et al.*, 1980). The results presented here show that OCS may be a relative risk factor in the development of paracetamol-induced hepatotoxicity in females.

In summary, this study has demonstrated that the faster elimination of paracetamol in males compared to females is due to increased activity of the glucuronidation pathway in males. A previous study has shown that liver microsomes isolated from male and female rats show differences in the activity of UDP-glucu-

ronyltransferase for a number of substrates (Winsnes, 1971). Hence it is likely that sex-related differences occur for a range of drugs metabolised by glucuronide conjugation in man, although data for paracetamol and the glucuronidated benzodiazepines show this effect is generally small and unlikely to be of clinical importance. By contrast, OCS markedly induced the glucuronidation and oxidative metabolism of paracetamol and these effects may have clinical consequences in terms of duration of action and the development of hepatotoxicity. Further studies are under way to investigate the specificity of the effects of sex and OCS on xenobiotic glucuronidation in humans.

This study was supported by grants from the National Health and Medical Research Council of Australia and Winthrop Laboratories (Sterling Pharmaceuticals (Aust.) Ltd). The expert clinical assistance of Mrs Belinda Harrington, R.N., is gratefully acknowledged.

References

ADITHAN, C. & THANGAM, J. (1982). A comparative study of saliva and serum paracetamol levels using a simple spectrophotometric method. *Br. J. clin. Pharmac.*, **14**, 107–109.

ALDERCREUTZ, H. & IKONEN, E. (1964). Oral contraceptives and liver damage. *Br. med. J.*, **2**, 1133.

ALLEN, M.D., GREENBLATT, D.J., HARMATZ, J.S. & SHADER, R.I. (1980). Desmethyldiazepam kinetics in the elderly after oral prazepam. *Clin. Pharmac. Ther.*, **28**, 196–202.

BRIATICO, G., GUISO, G., JORI, A. & RAVAZZANI, C. (1976). Effects of contraceptive agents on drug metabolism in various animal species. *Br. J. Pharmac.*, **58**, 173–181.

CHAMBERS, D.M., JEFFERSON, G.C., CHAMBERS, M. & LOUDON, N.B. (1982). Antipyrine elimination in saliva after low-dose combined or progestogen-only oral contraceptive steroids. *Br. J. clin. Pharmac.*, **13**, 229–232.

DIVOLL, M., ABERNATHY, D.R., AMEER, B. & GREENBLATT, D.J., (1982). Acetaminophen kinetics in the elderly. *Clin. Pharmac. Ther.*, **31**, 151–156.

DIVOLL, M., GREENBLATT, D.J., HARMATZ, J.S. & SHADER, R.I. (1981). Effect of age and gender on disposition of temazepam. *J. pharm. Sci.*, **70**, 1104–1107.

FEUER, G. & LISCIO, A. (1969). Origin of delayed development of drug metabolism in the newborn rat. *Nature*, **223**, 68–69.

GLYNN, J.P. & BASTAIN, W. (1973). Salivary excretion of paracetamol. *J. Pharm. Pharmac.*, **25**, 420–421.

GOLDFINGER, R., AHMED, K.S., PITCHUMON, C.S. & WESELEY, S.A. (1978). Concomitant alcohol and drug abuse enhancing acetaminophen toxicity. *Am. J. Gastroenterology*, **70**, 385–388.

GREENBLATT, D.J., ALLEN, M.D., LOCNISKAR, A., HARMATZ, J.S. & SHADER, R.I. (1979). Lorazepam kinetics in the elderly. *Clin. Pharmac. Ther.*, **26**, 103–113.

GREENBLATT, D.J., ALLEN, M.D., HARMATZ, J.S. & SHADER, R.I (1980a). Diazepam disposition determinants. *Clin. Pharmac. Ther.*, **27**, 301–312.

GREENBLATT, D.J., DIVOLL, M., HARMATZ, J.S. & SHADER, R.I. (1980b). Oxazepam kinetics: effects of age and sex. *J. Pharmac. exp. Ther.*, **215**, 86–91.

HARGREAVES, T., PIPER, R.F. & CAM, J. (1971). Effect of oral contraceptives on glucuronyl transferase. *Nature (New Biol.)*, **234**, 110–111.

HINSON, J.A. (1980). In *Reviews in Biochemical Toxicology*, eds. Hodgson, E., Bend, J.R. & Philpot, R.M., **2**, 103–129. North Holland (New York): Elsevier.

INSCOE, J.K. & AXELROD, J. (1960). Some factors affecting glucuronide formation *in vitro*. *J. Pharmac. exp. Ther.*, **129**, 128–131.

JOCHEMSEN, R., VAN DER GRAAFF, M., BOEIJINGA, J.K. & BREIMER, D.D. (1982). Influence of sex, menstrual cycle and oral contraception on the disposition of nitrazepam. *Br. J. clin. Pharmac.*, **13**, 319–324.

JOHNSON, G.K. & TOLMAN, K.G. (1977). Chronic liver disease and acetaminophen. *Ann. Int. Med.*, **87**, 302–304.

KATO, R. (1974). Sex-related differences in drug metabolism. *Drug Metab. Rev.*, **3**, 1–32.

KENDALL, M.J., QUARTERMAN, C.P., JACK, D.B. & BEELEY, L. (1982). Metoprolol pharmacokinetics and the oral contraceptive pill. *Br. J. clin. Pharmac.*, **14**, 120–122.

McCLAIN, C.J., KROMHOUT, J.P., PETERSON, F.J. & HOLTZMAN, J.L. (1980). Potentiation of acetaminophen toxicity by alcohol. *J. Am. med. Ass.*, **244**, 251–253.

MINERS, J.O., ADAMS, J.F. & BIRKETT, D.J. (1984). A simple hplc assay for urinary paracetamol metabolites and its use to characterise the C3H mouse as a model for paracetamol metabolism studies. *Clin. exp. Physiol. Pharmac.* (in press).

MUCKLOW, J.C., FRASER, H.S., BULPITT, C.J., KAHN, C., MOULD, G. & DOLLERY, C.T. (1980). Environmental factors affecting paracetamol metabolism in London factory and office workers. *Br. J. clin. Pharmac.*, **10**, 67–74.

NEALE, M.G. & PARKE, D.V. (1973). Effects of pregnancy on the metabolism of drugs in the rat and rabbit. *Biochem. Pharmac.*, **22**, 1451–1461.

ORME, M. L'E. (1982). The clinical pharmacology of oral contraceptive steroids. *Br. J. clin. Pharmac.*, **14**, 31–41.

PATWARDHAN, R.V., DESMOND, P.V., JOHNSON, R.F. & SCHENKER, S. (1980). Impaired elimination of caffeine by oral contraceptive steroids. *J. lab. clin. Med.*, **95**, 603–608.

RAWLINS, M.D., HENDERSON, D.B. & HIJAB, A.R. (1977). Pharmacokinetics of paracetamol after intravenous and oral administration. *Eur. J. clin. Pharmac.*, **11**, 283–286.

ROBERTS, R.K., DESMOND, P.V., WILKINSON, G.R. & SCHENKER, S. (1979). Disposition of chlordiazepoxide: sex differences and effects of oral contraceptives. *Clin. Pharmac. Ther.*, **25**, 826–831.

SONNENBERG, A., KOELZ, H.R., HERZ, R., BENES, I. & BLUM, A.L. (1980). Limited usefulness of the breath test in evaluation of drug metabolism: a study in human oral

contraceptive users treated with dimethylamino-antipyrine and diazepam. *Hepatogastroenterology*, **27**, 104–108.

TEUNISSEN, M.W.E., SRIVASTAVA, A.K. & BREIMER, D.D. (1982). Influence of sex and oral contraceptives on antipyrine metabolite formation. *Clin. Pharmac. Ther.*, **32**, 240–246.

WILKINSON, G.R. & SHAND, D.G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmac. Ther.*, **18**, 377–390.

WILSON, J.T., KASANTIKUL, V., HARBISON, R. & MARTIN, D. (1978). Death in an adolescent following an overdose of acetaminophen and phenobarbital. *Am. J. Dis. Child.*, **132**, 466–473.

WINSNES, A. (1971). Age and sex-dependent variability of the activation characteristics of UDP-glucuronyltransferase *in vitro*. *Biochem. Pharmac.*, **20**, 1249–1258.

WOJCICKI, J., GAWRONSKA-SZKLARZ, B., KAZIMIERCZYK, J., BASKIEWICZ, Z. & RACZYNSKI, A. (1979). Comparative pharmacokinetics of paracetamol in men and women considering follicular and luteal phases. *Arzneim-Forsch.*, **29**, 350–354.

WRIGHT, N. & PRESCOTT, L.F. (1973). Potentiation by previous drug therapy of hepatotoxicity following paracetamol overdose. *Scot. med. J.*, **18**, 56–59.

(Received April 18, 1983, accepted July 4, 1983)