Glutathione *S*-Aryltransferase: The Effect of Treating Male and Female Rats with Phenobarbitone on the Apparent Kinetic Parameters for the Conjugation of 1,2-Dichloro-4-nitrobenzene and 1-Chloro-2,4-dinitrobenzene with Glutathione

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There are several reports that the treatment of rats with barbiturates increases the rate of conjugation of GSH with 1,2-dichloro-4-nitrobenzene and sulphobromophthalein by hepatic subcellular fractions *in vitro* (Grover & Sims, 1964; Klaasen & Plaa, 1968; Suga & Akagi, 1970). The elimination of sulphobromophthalein and γ -hexachlorocyclohexane from rats *in vivo* is also increased by barbiturate treatment of the animals (Koransky *et al.*, 1964; Schellhaus *et al.*, 1965; Klinger *et al.*, 1968; Klaasen & Plaa, 1968). The present paper describes the effect of phenobarbitone treatment of rats on the kinetic parameters K_m and V_{max} for the conjugation of 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene with GSH by hepatic supernatant preparations, and on the rate of conjugation of GSH with sulphobromophthalein *in vitro*.

Experimental

Materials. All chemicals were of AnalaR grade except for chlorodinitrobenzene (OAS grade), dichloronitrobenzene (both purchased from British Drug Houses Ltd., Poole, Dorset, U.K., and used without further purification), sulphobromophthalein and GSH (both purchased from Koch-Light Laboratories, Colnbrook, Bucks., U.K.).

Animals. Male (260–280 g) and female (230–250 g) rats of the Wistar strain (Scientific Products Farm, Ash, Canterbury, Kent, U.K.) were injected intraperitoneally (50 mg/kg) with phenobarbitone sodium solution (Macarthy's Ltd., Romford, Essex, U.K.) once daily for 2 consecutive days and killed 2 days later. Control animals were injected with 0.9% NaCl and treated similarly. One control animal and one phenobarbitone-treated animal were killed by cervical dislocation at approximately the same time and the whole livers were homogenized (1:5, w/v) in ice-cold 1.15% (w/v) KCl containing 0.005 M-potassium phosphate, pH 7.4 (Darby, 1971). The homogenates were centrifuged at 10000 g_{av} for 20 min and the resulting supernatants were centrifuged at 105000 g_{av} for 60 min. The '100000 g ' supernatants were decanted carefully and used as such in the assays for enzyme activity.

Enzyme assays. The measurement of the conjugation of dichloronitrobenzene and chlorodinitrobenzene with GSH was modified from the spectro-

photometric method described by Grover & Sims (1964) and Clark *et al.* (1967). GSH was present at a final concentration of 4.0 mM and the final volume was 3.0 ml, including 2.75 ml of 0.1 M-potassium phosphate buffer, pH 7.4. Chlorodinitrobenzene (0.125–0.4 mM) or dichloronitrobenzene (0.067–0.4 mM) was added in acetone (20 μ l). A portion of 100000 g supernatant was added to one cuvette to start the reaction, either 100 μ l (male animals) or 200 μ l (female animals) for assay of dichloronitrobenzene or 25 μ l of supernatant diluted 1:10 (v/v) with the buffered 1.15% KCl for assay of chlorodinitrobenzene. Reference cuvettes contained GSH, 0.1 M-potassium phosphate buffer, dichloronitrobenzene or chlorodinitrobenzene, and the appropriate volume of buffered 1.15% KCl instead of supernatant, and the reactions were followed at 344 nm in a Perkin-Elmer 402 double-beam recording spectrophotometer. The conjugation of sulphobromophthalein with GSH was measured similarly but at 330 nm (Goldstein & Combes, 1966), sulphobromophthalein being present at a final concentration of 0.1 mM (added in water). Reference cuvettes contained 0.1 M-potassium phosphate buffer, pH 7.4, sulphobromophthalein, GSH and 100 μ l of buffered 1.15% KCl instead of 100000 g supernatant.

The protein concentrations in the 100000 g supernatants were measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and discussion

The reported increase in the activity of hepatic GSH *S*-aryltransferase caused by treatment of rats with barbiturates is relatively small (Grover & Sims, 1964; Klaasen & Plaa, 1968; Suga & Akagi, 1970). Grover & Sims (1964) found that, after the initial increase after phenobarbitone treatment, the activity (with dichloronitrobenzene) declined in a few days to a value 40% below that found in untreated rats. Table 1 of the present paper is in broad agreement with the three studies quoted, phenobarbitone treatment (50 mg/kg) of the rat daily for 2 days, 2 days before the animal was killed, being associated with only small changes in V_{max} , but for chlorodinitrobenzene and not for dichloronitrobenzene. The apparent K_m values are unchanged. The

Table 1. *Effect of phenobarbitone treatment of male and female rats on the K_m and V_{max} for 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene, and on the rate of conjugation in vitro of sulphobromophthalein and glutathione*

For experimental details see the text. All results are means $\pm$ S.E.M., for the numbers of animals given in parentheses. Ratios are K_m (dichloronitrobenzene)/ K_m (chlorodinitrobenzene) and V_{max} (chlorodinitrobenzene)/ V_{max} (dichloronitrobenzene). P values were calculated by using Student's t test: N.S., not significantly different; * $0.10 > P > 0.05$; **, $\dagger P < 0.02$ or better for the appropriate pairs of values in each horizontal group of four values. With dichloronitrobenzene, V_{max} for the female was highly significantly different ($P < 0.001$) from V_{max} for the male. With chlorodinitrobenzene, V_{max} for the female was significantly different from V_{max} for the male only for control animals. With sulphobromophthalein, v for the female was highly significantly different from v for the male. The V_{max} ratio for the female was highly significantly different from that for the male, whereas the K_m ratio for the female only tends to be different from that for the male in phenobarbitone treatment.

	K_m (mM)				V_{max} (ΔE_{344} /min per mg of supernatant protein)			
	Male		Female		Male		Female	
	Control	+Pheno-barbitone	Control	+Pheno-barbitone	Control	+Pheno-barbitone	Control	+Pheno-barbitone
1,2-Dichloro-4-nitrobenzene	0.62 ± 0.03 (6)	0.56 ± 0.06 (6)	0.67 ± 0.17 (7)	0.63 ± 0.08 (7)	0.46 ± 0.03 (6)	0.50 ± 0.05 (6)	0.15 ± 0.04 (7)	0.17 ± 0.02 (7)
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
1-Chloro-2,4-dinitrobenzene	$0.13 \pm 0.01^{**}$ (6)	$0.12 \pm 0.02^*$ (6)	$0.19 \pm 0.02^{**}$ (7)	$0.21 \pm 0.04^*$ (7)	$12.8 \pm 0.6^\dagger$ (6)	16.1 ± 0.7 (6)	$9.8 \pm 0.5^\dagger$ (7)	13.6 ± 1.8 (7)
	N.S.	N.S.	N.S.	N.S.	$P < 0.01$		$0.10 > P > 0.05$	
Ratio	5.2 ± 0.7 (6)	$5.1 \pm 0.7^*$ (6)	3.6 ± 0.7 (7)	$3.3 \pm 0.5^*$ (7)	28.1 ± 1.6 (6)	33.4 ± 2.8 (6)	81.6 ± 12.3 (7)	83.5 ± 8.4 (7)
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Sulphobromophthalein					v (ΔE_{330} /min per mg of supernatant protein)			
	—	—	—	—	0.0265 ± 0.001 (5)	0.0299 ± 0.001 (5)	0.0083 ± 0.0006 (7)	0.0098 ± 0.0007 (7)
					$0.10 > P > 0.05$		$0.10 > P > 0.05$	

increase in $V_{\max}$ for the conjugation of chlorodinitrobenzene with GSH is nonetheless statistically highly significant for male animals, whereas the increase for female rats shows only a trend to significance. There is a trend to an increase (12%) in the rate of conjugation of sulphobromophthalein with GSH for both male and female animals. Klaasen & Plaa (1968) found a significant increase of 23% with larger numbers of animals and a larger dose of phenobarbitone (75 mg/kg daily for 3 days). The dosage of phenobarbitone used in the present experiments is sufficient to induce the microsomal drug-metabolizing enzyme system by twofold or more (Darby, 1972), and it is reasonable to conclude that the GSH *S*-aryltransferase for dichloronitrobenzene, chlorodinitrobenzene and sulphobromophthalein is not dependent on the microsomal mixed-function oxidase system, unlike the conjugations with bromobenzene (Suga & Akagi, 1970) and γ -hexachlorocyclohexane (Koransky *et al.*, 1964).

Table 1 indicates that the hepatic GSH *S*-aryltransferase in female rats has different properties from that in male rats. The rate of conjugation of sulphobromophthalein with GSH for male rats is three times that for female rats, as is $V_{\max}$ (dichloronitrobenzene) but not $V_{\max}$ (chlorodinitrobenzene), which is only marginally higher. K_m (dichloronitrobenzene) is similar for males and females, but K_m (chlorodinitrobenzene) is significantly higher for females. That phenobarbitone does not alter the intrinsic properties

of GSH *S*-aryltransferase is readily apparent from comparison of the ratios K_m (dichloronitrobenzene)/ K_m (chlorodinitrobenzene) and $V_{\max}$ (chlorodinitrobenzene)/ $V_{\max}$ (dichloronitrobenzene). The sex difference is emphasized, and it will be interesting to see whether other species show similar sex differences.

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