J. Pharm. Pharmac, 1972, **24**, 620–624 *Received March* 6, 1972

Sex and species differences in the biliary excretion of tartrazine and lissamine fast yellow in the rat, guinea-pig and rabbit. The influence of sex hormones on tartrazine excretion in the rat

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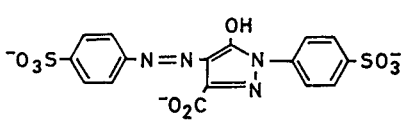
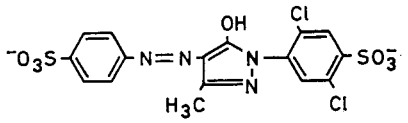
A sex difference in the biliary excretion of tartrazine, which occurs in the rat, has been found not to occur in the guinea-pig and rabbit. Male and female rats excrete 13 and 29% respectively of an intravenous dose of tartrazine (50 μmol/kg) in the bile in 3 h. The corresponding figures for male and female guinea-pigs and rabbits were 33 and 39%, and 5 and 6%, respectively. The sex difference in the rat was unrelated to any differences in renal function, for when the renal pedicles were ligated 22 and 63% of a dose was excreted in the bile of males and females respectively. Treatment of male and female rats with oestradiol and testosterone respectively influenced this sex difference in hepatic function but males were not affected by treatment with progesterone. Thus the extent of biliary excretion of tartrazine in males was increased by oestradiol pretreatment from 14 to 33% of the dose whereas testosterone pretreatment of females decreased excretion from 31 to 16% of the dose. The related dye lissamine fast yellow does not exhibit marked sex or species differences, 75–90% of a dose being excreted in the bile of males and females of all three species. Both dyes are excreted unchanged in the bile and urine of the three species.

In a previous paper (Gregson, Hirom & others, 1972) from this laboratory, a marked sex difference in the biliary excretion in the rat of the food dye, tartrazine (I), was described. The extent of biliary excretion was much greater in the female than in the male. The dye was also found to be more extensively excreted in the bile by the rat and guinea-pig than by the rabbit. The sex difference has now been further investigated and a comparison made with the structurely-related dye lissamine fast yellow (II).



I (mol wt 465)



II (mol wt 505)

MATERIALS AND METHODS

Dyes. Lissamine fast yellow was obtained from Edward Gurr, Ltd., London, S.W.14. When chromatographed by the ascending technique on thin-layer plates

of Alumina G (E. Merck, A.G., Darmstadt; 0·25 mm thick), it ran as a single yellow spot of R_F 0·94 in 2% sodium citrate in ammonia solution (sp.gr. 0·88)–water (1 : 19 by vol), 0·73 in ethanol–butan-1-ol–ammonia solution (sp.gr. 0·88)–water 42 : 28 : 1 : 28, by vol) and 0·59 in propan-2-ol–ammonia solution (sp.gr. 0·88) (7 : 3, by vol). When viewed with ultraviolet light (254 nm) it appeared as a dark spot. Tartrazine and its chromatography have been described by Gregson & others (1972).

Animals. Male and female Wistar albino rats (190–350 g), Duncan Hartley albino guinea-pigs (500–800 g) and Dutch rabbits (2–3 kg) were used. Biliary fistulae were established and bile collected as described by Abou-El-Makarem, Millburn & others (1967). The dyes were administered intravenously in aqueous solution (50 μmol/ml) at a dose of 50 μmol/kg.

Hormone treatment. Male rats were injected subcutaneously with oestradiol benzoate (0·1 mg/kg) or progesterone (2·5 mg/kg) dissolved in ethyl oleate (0·1 ml) daily for 28 days. Control male rats were injected daily with 0·1 ml ethyl oleate only. Female rats were injected subcutaneously with testosterone propionate (2·5 mg/kg) dissolved in 0·1 ml of a solvent consisting of 10% benzyl alcohol in arachis oil daily for 28 days. Control females were injected with the solvent (0·1 ml/rat/day). Biliary fistulae were established 24 h after the final injection and the biliary and urinary excretion of tartrazine was measured.

Estimation of dyes. Tartrazine was estimated by direct spectrophotometry of diluted bile and urine as described previously (Gregson & others, 1972). Lissamine fast yellow was similarly estimated by measuring the absorbance of suitably diluted samples at 400 nm. The recovery of this dye added to bile at concentrations of 200–1800 μg/ml and analysed by this procedure was 95–100%.

RESULTS

Biliary excretion of tartrazine. Table 1 shows that there is a marked species and, in rats, a sex difference in the pattern of excretion of tartrazine. The female rat and guinea-pig excrete 29 and 39%, respectively, of the dye in the bile and 55 and 38%, respectively, in the urine in 3 h. The female rabbit, however, excretes only 6% of the dose in the bile and a correspondingly greater amount, 67%, in the urine in 3 h. There is, however, a sex difference in the extent of the biliary excretion in the rat but not in the guinea-pig and rabbit. The female rat excretes more than twice as

Table 1. *The excretion of tartrazine in rats, guinea-pigs and rabbits.* Tartrazine in water was injected intravenously (50 μmol/kg) into biliary cannulated animals. Bile and urine were collected for 3 h and analysed for tartrazine. Results given are mean values for 3 or more animals with ranges in parentheses.

Species	Sex	% Dose excreted in 3 h		
		Bile	Urine	Total
Rat	Male	13 (7·4–17)	61 (55–73)	74 (67–82)
	Female	29 (21–34)	55 (52–60)	84 (78–90)
Guinea-pig	Male	33 (31–37)	47 (41–51)	80 (72–87)
	Female	39 (30–49)	38 (24–51)	77 (74–81)
Rabbit	Male	4·5 (3·2–6·0)	61 (52–68)	66 (55–72)
	Female	5·8 (2·4–7·7)	67 (51–87)	73 (58–94)

much tartrazine in the bile as does the male but no difference between the sexes was observed in the guinea-pig and rabbit in which the biliary excretion was for the male and female guinea-pig, 33 and 39%, respectively, and for the male and female rabbit, 5 and 6%, respectively.

This sex difference in rats was further investigated. In the first place, it could be due to more rapid renal excretion of the dye in the males, less being then available for biliary excretion. The biliary excretion of the dye was therefore examined in male and female rats in which the renal pedicles were tied to prevent urinary excretion. This procedure did not alter the sex difference although biliary excretion is increased in both sexes from 13 (7·4–17, n = 3) to 22 (15–35, n = 3) % in the male and from 29 (21–34, n = 3) to 63 (58–66, n = 3) % in the female for intact and ligated animals, respectively. These results suggest that the sex difference in the hepatic excretion of tartrazine in the rat is not related to any difference in renal function. However, the sex difference observed is apparently under the influence of sex hormones. Table 2 shows that the extent of biliary excretion of tartrazine can be altered in

Table 2. *The effect of sex hormones on the excretion of tartrazine in the rat.* Rats (5–11 animals per group) were treated for 28 days with sex hormones as described in the text and were biliary cannulated 24 h after the final injection of hormone. Tartrazine in water was injected intravenously (50 μmol/kg) and bile and urine collected for 3 h and analysed for the dye. Other rats (10–20 per group) were also treated with sex hormones and used to measure the hexobarbitone sleeping time. For this purpose hexobarbitone sodium (100 mg/kg) was injected intraperitoneally and the sleeping time was taken as the period of time from loss of until recovery of the righting reflex. Results are mean values ± standard error of the mean.

Sex	Treatment	Hexobarbitone sleeping time (min)	% Dose excreted in 3 h		
			Bile	Urine	Total
Male ..	None	24·9 ± 1·2	13·4 ± 1·3	61·0 ± 2·7	74·4 ± 2·1
	Solvent alone	30·5 ± 1·8	13·3 ± 1·2	63·0 ± 2·1	76·2 ± 2·2
	Progesterone	29·9 ± 0·9	14·3 ± 0·5	63·0 ± 3·4	77·2 ± 3·2
	Oestradiol	68·0 ± 7·9	33·2 ± 2·7	35·8 ± 1·7	68·9 ± 2·9
Female ..	None	110·8 ± 4·1	29·0 ± 1·6	55·2 ± 1·0	84·2 ± 1·6
	Solvent alone	102·8 ± 4·8	31·0 ± 3·2	47·1 ± 1·4	78·1 ± 2·3
	Testosterone	57·0 ± 4·8	16·3 ± 3·0	61·2 ± 2·0	77·5 ± 2·1

both male and female rats by pretreatment with the appropriate sex hormone. The efficacy of the hormone treatment was checked by measuring the hexobarbitone sleeping time before and after the administration of the hormones (Quinn, Axelrod & Brodie, 1958). Table 2 shows that pretreatment of male rats with oestradiol more than doubles the amount of tartrazine excreted in the bile in 3 h, whereas pretreatment of female rats with testosterone reduces their ability to excrete the dye in the bile to the values found in untreated normal males. These treatments also altered the hexobarbitone sleeping times. However, pretreatment of males with the progestational hormone, progesterone, neither altered the biliary excretion nor the hexobarbitone sleeping time.

Biliary excretion of lissamine fast yellow. In contrast with tartrazine, there appear to be no marked species or sex differences in the extent of biliary excretion of lissamine

fast yellow (see Table 3). In both sexes of the three species studied some 75–90% of the intravenously injected dose of lissamine is excreted unchanged in the bile in 3 h. In the rat there would appear to be a slight sex difference since in 1 h after dosing the females excreted 84% of the dye in the bile whereas the males excreted 69% and at 3 h after dosing the values were 90% for females and 80% for males. In guinea-pigs and rabbits, there is practically no difference between the males and females in the extent of biliary excretion of the dye.

Table 3. *The excretion of lissamine fast yellow in rats, guinea-pigs and rabbits.* Lissamine fast yellow dissolved in water was injected intravenously (50 μmol/kg) into biliary cannulated animals. Bile and urine were collected for 3 h and analysed for the dye. Results are mean values for 3 or more animals with ranges in parentheses.

Species	Sex	% Dose excreted in 3 h		
		Bile	Urine	Total
Rat	Male	80 (77–82)	3·1 (2·7–3·5)	83 (80–84)
	Female	90 (86–97)	1·8 (1·7–1·9)	92 (88–99)
Guinea-pig	Male	77 (73–80)	13 (11–14)	90 (87–93)
	Female	75 (72–77)	12 (7·0–19)	87 (81–96)
Rabbit	Male	89 (88–90)	6·1 (5·8–6·5)	95 (94–96)
	Female	85 (84–86)	7·9 (7·4–8·4)	93 (91–94)

DISCUSSION

The results show that there is a marked sex difference in the biliary excretion of tartrazine but not of the related compound lissamine fast yellow. Furthermore, this sex difference is seen only in the rat. Sex differences in certain hepatic functions in the rat are known and one which is well established is that of the drug-metabolizing enzymes of the endoplasmic reticulum of the liver (see Gillette, 1963; Conney, 1967). Sex differences in drug-metabolizing enzymes have not been observed in the rabbit and guinea pig. In the rat, the sex differences in drug metabolism are under hormonal control, for example, the sex difference in hexobarbitone metabolism, as measured by the sleeping time, is affected by pretreatment of males and females with oestradiol and testosterone respectively (Quinn & others, 1958). Prolonged treatment of male rats with progesterone, however, appears not to influence hexobarbitone metabolism *in vitro* (Juchau & Fouts, 1966). Our studies indicate that the sex difference in tartrazine excretion is similar to that found for the drug-metabolizing enzymes in that both are influenced by oestradiol and testosterone but not by progesterone. However, it would appear that, since tartrazine is not metabolized (at least not to stable metabolites), the drug-metabolizing enzymes are not involved in its biliary excretion (see Levine, Millburn & others, 1970). Possibly, the sex hormones influence some unrecognized hepatic factor involved in the biliary excretion of certain foreign compounds.

The marked sex difference in the biliary excretion of tartrazine but not in that of lissamine is difficult to explain. It is possible that sex differences may occur when certain physicochemical characteristics of the compound which include polarity, molecular weight, chemical structure and lipid solubility are appropriate. Tartrazine

and lissamine possess similar structures (see I and II) but the CO_2H group of the former is replaced by CH_3 in the latter which also has two Cl groups in one of the aromatic rings. These structural differences modify the relative positions of water-soluble to lipid-soluble groups in the molecules and this could alter their ability to be excreted in the bile (see Hirom, Millburn & others, 1972). These two dyes differ in molecular weight by only 40 units but the biliary excretion of lissamine (mol wt 505) is at least 2–3 times that of tartrazine (mol. wt. 465) (see Tables 1 and 3) suggesting that other factors apart from molecular weight are involved. Abdel-Aziz, Hirom & others (1971) have suggested that the threshold molecular weights for significant biliary excretion (>5–10% of the dose) of anions varies with species being about 325 ± 50 for the rat, 400 ± 25 for the guinea-pig and 500 ± 50 for the rabbit. Furthermore, it was suggested that anions with molecular weights in excess of 500 would be excreted to a marked extent in all species. Our findings agree with these ideas, although the high biliary excretion of lissamine in all three species is probably also influenced by its molecular structure. The possibility exists that marked sex differences in the biliary excretion of anions in the rat may only occur with those compounds which also exhibit marked species differences.

Acknowledgements

F. O. Osiyemi, supported by the International Atomic Energy Agency, participated in this work during a sabbatical leave from the Department of Biochemistry, University of Ibadan, Nigeria. P. Bertagni participated during a short leave from Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

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