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The biliary excretion of tartrazine. Sex differences in the rat and species differences in the rat, guinea-pig and rabbit

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The excretion of tartrazine in the bile and urine has been studied in biliary cannulated rats, rabbits and guinea-pigs. This dye is excreted unchanged by these species. In the rat a sex difference in the relative amounts of tartrazine excreted in bile and urine has been found. Male rats excrete in 3 h about 17% of an intravenous dose (50 μmol/kg) in the bile and about 70% in the urine, whereas females excrete about 40 and 45% respectively. The biliary excretion of tartrazine in the rat appears to be influenced by dose level, for at the lower level of 4·5 μmol/kg male rats excrete about 9% of an intravenous dose in the bile and 64% in the urine in 3 h, the corresponding values for female rats being 30% and 50%. There is also a species difference in the extent of biliary excretion of tartrazine (50 μmol/kg intravenously). The female rat and female guinea-pig excrete in 3 h about 40% of the dose in the bile and a similar amount in the urine whereas the female rabbit excretes only 6% in the bile and nearly 70% in the urine. Previous work in this laboratory has shown that molecular weight is an important factor in the biliary excretion of foreign compounds and the present results fit in with this view.

Ryan & Wright (1961; 1962) reported that the yellow food colour, tartrazine, was poorly excreted in the bile (1% of dose in 6 h) when injected intravenously into rats. This azo dye is the sodium salt of a tribasic acid whose trianion has a molecular weight of 465. Millburn, Smith & Williams (1967) suggested that a relation exists between the extent of biliary excretion and the polarity and molecular weight of a compound, and that in the rat biliary excretion begins to become appreciable, i.e. 5–10% or more of the dose, when the molecular weight of the compound reaches the value of 325 ± 50 and provided that the compound is polar. There are now many data available to support this suggestion (see reviews by Millburn, 1970; Smith, 1971). The findings of Ryan & Wright would appear to be contrary to this view. The biliary excretion of tartrazine was therefore reinvestigated using rats of both sexes since the results of Millburn & others (1967) were obtained with female rats whereas the sex of Ryan & Wright's rats was not defined. It will be shown that in the rat there is a sex difference in the biliary excretion of tartrazine and that this excretion is more extensive in both sexes than that found by Ryan & Wright.

MATERIALS AND METHODS

Tartrazine was obtained from Williams Ltd., Hounslow, Middlesex. When chromatographed on thin-layer plates of alumina G it ran as a single yellow spot in the

Table 1. *R_F values of tartrazine.* Thin-layer chromatography was carried out (ascending technique) on alumina G on glass plates.

Solvent system	R_F in*		
	Water	Rat urine, guinea-pig or rabbit bile or urine	Rat bile
A	0·96	0·96	0·70
B	0·60	0·60	0·50
C	0·30	0·30	0·25

The solvent systems used were A: 2% sodium citrate in ammonia solution (sp. gr. 0·88)—water (1:19 vol); B: ethanol–butan-1-ol–ammonia solution (sp. gr. 0·88)—water (42:28:1:28 by vol); C: propan-2-ol–ammonia solution (sp. gr. 0·88) (7:3 by vol).

* Tartrazine appeared as a yellow spot on the plate and as a dark spot when viewed with ultraviolet light (254 nm; Hanovia Chromatolite lamp).

three solvent systems used (see Table 1). The R_F values of tartrazine in water, bile and urine are shown in Table 1.

Animals. Male and female Wistar albino rats, 190–210 g, female Duncan Hartley albino guinea-pigs, 0·5–0·6 kg, and female Dutch rabbits, 2–3 kg, were used. Biliary fistulae were established and bile collected as previously described (Abou-El-Makarem, Millburn & others, 1967). Tartrazine was dissolved in water and 1 ml of the solution/kg was injected intravenously. For intraperitoneal injection in rats, the dose of tartrazine was dissolved in 1 ml of water.

Estimation of tartrazine. Tartrazine in bile and urine was estimated by the method of Ryan & Wright (1961) and by two other methods, one (*a*) involving thin-layer chromatography and the other (*b*) direct spectrophotometry.

(*a*) The urine or bile (0·1 ml) was chromatographed on thin-layer alumina G using solvent B (Table 1). The yellow band corresponding to tartrazine was scraped from the glass plate and eluted with water (2 × 2·5 ml). The absorbance of the eluate was determined at 427 nm in a Unicam SP 600 spectrophotometer. The recovery of tartrazine added to rat bile in the range 20–1500 μg/ml was 99 $\pm$ 6%. This method was particularly useful for concentrations of tartrazine in bile of the order 20–200 μg/ml.

(*b*) For concentrations of tartrazine greater than 200 μg/ml direct spectrophotometry could be used. The urine or bile was diluted 50–100 fold with water and the absorbance at 427 nm measured against blanks of appropriately diluted bile or urine. The recovery of tartrazine by this method was 95 $\pm$ 3% over the range 200–1600 μg/ml.

(*c*) The recovery of tartrazine from bile over the range 30–80 μg/ml by the method of Ryan & Wright (1961) was 93 $\pm$ 10%.

RESULTS

Rats

The quantitative aspects of the biliary and urinary excretion of tartrazine injected intravenously (2·4 and 26·7 mg/kg) in male and female rats are shown in Table 2. Both male and female rats excreted 80–90% of the dose (26·7 mg/kg; 50 μmol/kg) of tartrazine in the bile and urine in 3 h. Chromatography of the urine in solvents A, B and C (Table 1) showed the presence of a single yellow spot with the same R_F value

Table 2. *The excretion of tartrazine in the bile and urine of rats.* Tartrazine dissolved in water was injected intravenously into biliary cannulated rats as described in the text. Bile and urine were collected for 3 h and analysed for tartrazine. The results are given as the mean for 3 or more animals with ranges in parentheses.

Sex	Dose mg/kg	Dose μmol/kg	Assay method*	% dose excreted in 3 h in Bile	Urine	Total
Male			*a*	8·6 (6·1–12)	64 (31–78)	73 (37–90)
	2·4	4·5	*c*	5·3 (2·6–7·2)	48 (22–69)	53 (24–75)
			a	17 (14–18)	72 (67–82)	89 (84–96)
	26·7	50	*b*	18 (16–19)	73 (63–88)	91 (82–104)
			c	16 (14–20)	71 (62–76)	87 (82–91)
Female			*a*	29 (22–33)	49 (44–52)	78 (71–85)
	2·4	4·5	*c*	26 (23–28)	33 (26–40)	59 (54–67)
			a	44 (39–48)	46 (43–50)	90 (87–92)
	26·7	50	*b*	33 (29–37)	47 (45–51)	80 (78–82)
			c	39 (36–43)	44 (40–47)	83 (79–84)

* See text.

as tartrazine. Chromatography of the bile in the same solvents showed the presence of one yellow spot of R_F value less than that of tartrazine dissolved in water. When tartrazine was added to normal rat bile and then chromatographed, its R_F values were found to be less than those in water and the same as the yellow spot present in the bile of rats injected with the dye, which is thus excreted unchanged.

Further evidence that tartrazine was excreted in the urine and bile in the unchanged state was obtained by comparing the absorption spectrum in water of the compound isolated from bile or urine by preparative t.l.c. with that of authentic tartrazine. They gave identical spectra with peaks at 258 nm and 427 nm.

Table 2 shows that there is a sex difference in respect of the relative amounts of tartrazine excreted in the bile and urine. Male rats excreted about 17% of the dose (50 μmol/kg) in the bile and about 70% in the urine in 3 h whereas the females excreted about 40 and 45%, respectively. This sex difference is also seen at the lower dose level (2·4 mg/kg; 4·5 μmol/kg) although the total recovery (50–60%) was less when determined by method *c* than by method *a* (70–80%). Male rats were found to excrete about 9% of the dose in the bile and 64% in the urine in 3 h whereas the corresponding values for female rats were about 30 and 50%. Chromatography of bile and urine from rats given the lower dose of tartrazine showed that only the unchanged dye was being excreted.

The sex difference in the biliary and urinary excretion of tartrazine in biliary cannulated rats is also evident in the urinary excretion of tartrazine in the intact rat. When the intact rat is injected intraperitoneally with tartrazine (100 μmol/kg), 85 (78–96)% of the dose is excreted in the urine unchanged in 24 h in male rats and 51 (40–63)% in female rats, these values being the averages and the ranges for 3 or more animals.

The biliary and urinary excretion of tartrazine given by intraperitoneal injection to female biliary cannulated rats is shown in Table 3. The dye is rapidly absorbed, and then excreted in almost equal proportions in bile and urine. At the lower dose level of

Table 3. *Biliary excretion of tartrazine injected intraperitoneally into biliary cannulated female rats.* Results are mean values for 3 or more animals; ranges are shown in parentheses.

Dose mg/kg	Dose μmol/kg	Assay method	Period of collection of excreta (h)	% dose in Bile	% dose in Urine	% dose in Total
2·4	4·5	*c*	3	30 (26–31)	37 (30–42)	67 (61–74)
53·5	100	*b*	3	24 (22–26)	—	—
53·5	100	*b*	24	44 (42–46)	47 (43–49)	91 (90–95)

2·4 mg/kg (4·5 μmol/kg) about 30% of the dose was excreted in 3 h in the bile and 37% in the urine. At a dose of 53·5 mg/kg (100 μmol/kg) about 24% of the dose appeared in the bile in 3 h and about 44% in 24 h.

Guinea-pigs and rabbits

Table 4 shows that there is a marked species difference in the extent of biliary excretion of tartrazine injected intravenously at a dose of 26·7 mg/kg (50 μmol/kg). Thus, the female guinea-pig and the female rat excreted about 40% of the dose in the bile and a similar amount in the urine in 3 h, whereas the doe rabbit eliminated only 6% of the dose in the bile and nearly 70% in the urine in 3 h. Chromatography in three solvent systems (Table 1) of the bile and urine from the three species injected with tartrazine showed the presence of only unchanged dye.

Table 4. *Biliary excretion of tartrazine in different species.* Tartrazine (26·7 mg/kg; 50 μmol/kg) was injected intravenously. All the animals used were females. Results are mean values with ranges in parentheses. Tartrazine was assayed by method *b*.

Species (No.)	% dose in 3 h in Bile	Urine	Total
Guinea-pig (3)	39 (30–49)	38 (24–51)	77 (74–81)
Rat (7)	42 (29–63)	46 (27–56)	88 (78–105)
Rabbit (4)	5·8 (2·4–7·7)	67 (51–87)	73 (58–94)

DISCUSSION

Jones, Ryan & Wright (1964) found that when a small dose of tartrazine (2·4 mg/kg) was injected intraperitoneally into rats, the dye was excreted almost entirely unchanged in the urine and Ryan & Wright (1961; 1962) showed that only 1% of the dye was excreted in the bile. Jones & others (1964) also make the statement "with sufficiently large doses, some biliary excretion did occur". The present work, however, shows that tartrazine on injection into rats is excreted in the bile in significant amounts and that the extent of this biliary excretion depends upon sex and upon dose. We found that at a dose of 26·7 mg/kg some 40% of the dose was excreted in the bile and 46% in the urine by female rats and some 17 and 70% respectively by male rats in 3 h. At the lower dose of 2·4 mg/kg used by Wright and his coworkers, the biliary excretion was less, being nearly 30% in the female and 9% of the dose in the male (Table 2). In the studies of Ryan & Wright (1961; 1962) neither the sex nor the strain of albino

rats used was stated, but the body weights of the rats were given as 300–400 g which is higher than the weights of the rats used in this study, i.e. 190–210 g. Sex differences in the extent of the biliary excretion of certain compounds in the rat have been observed. Thus Hart, Guarino & Adamson (1969) have reported that the biliary excretion of indocyanine green is greater in female than in male rats, but the reverse is true for chlorothiazide. Daniel & Gage (1965) also reported that in experiments with single animals, there was a much greater biliary excretion of ^{14}C after the oral administration of [^{14}C] butylated hydroxytoluene to a male than to a female rat. It would appear that with some compounds biliary excretion is greater in the female than in the male rat whereas the reverse may be true for other compounds.

Millburn & others (1967) have pointed out that molecular size and polarity are important physico-chemical factors in the biliary excretion of foreign compounds. For significant biliary excretion, that is 5–10% or more of the dose, to occur in the rat, it was suggested that the minimum molecular weight of 325 $\pm$ 50 was required and that the compound should possess a polar anionic group or acquire one by metabolism. Since tartrazine is a strong acid with an anion of molecular weight 465, it could be expected to be extensively excreted in the bile in the rat. The results given in this paper show that the biliary excretion of tartrazine conforms with this view, although the sex of the rat and the dose also play a role.

Species differences in the extent of biliary excretion have been reported by Abou-El-Makarem & others (1967). The biliary excretion of tartrazine also shows a species difference, for in the female rat and female guinea-pig the extent of biliary excretion is about 40% of the dose but in the female rabbit only 6% (Table 4). Other work in progress in this laboratory suggests that the minimum molecular weight for significant biliary excretion is about 325 $\pm$ 50 in the female rat, 400 $\pm$ 25 in the guinea-pig and 500 $\pm$ 50 in the rabbit (Abdel Aziz, Hirom & others, 1971). Since the tartrazine anion has a molecular weight of 465, extensive biliary excretion could be expected in the rat and guinea-pig, but not in the rabbit.

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