Biliary Excretion of Foreign Compounds

BIPHENYL, STILBOESTROL AND PHENOLPHTHALEIN IN THE RAT: MOLECULAR WEIGHT, POLARITY AND METABOLISM AS FACTORS IN BILIARY EXCRETION

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1. The extent of biliary excretion of biphenyl, tetralin, stilboestrol and phenolphthalein was studied in the rat. 2. Biphenyl and its 4-hydroxy and 4,4'-dihydroxy derivatives are extensively excreted in the bile as glucuronides in amounts increasing in order of molecular weight. 3. Stilboestrol and its glucuronide are excreted almost quantitatively in the bile mainly as the monoglucuronide, as are also phenolphthalein and its glucuronide. 4. Tetralin is excreted to the extent of about 13% of the dose, mainly as *ac*-tetralyl glucuronides. 5. The results and those of Abou-El-Makarem, Millburn, Smith & Williams (1967) are discussed and it is concluded that the extent of biliary excretion of foreign compounds in rats depends on their molecular weight and their possessing a strongly polar anionic group. There appears to be a minimum value of this molecular weight below which little biliary excretion (i.e. not more than 5–10% of the dose) occurs. There is some latitude in the choice of this molecular weight, which is about 325 ± 50 . The necessary molecular weight and polar group can be acquired by metabolism. Above this minimum value biliary excretion increases with molecular weight. It is suggested that the mechanism of the biliary excretion of foreign compounds may be similar to that of conjugated bile acids, which are highly polar and whose molecular weights exceed 400.

In the preceding paper (Abou-El-Makarem, Millburn, Smith & Williams, 1967) the biliary excretion in the rat of some simple monocyclic benzene derivatives was studied and the results suggested that compounds of molecular weight less than about 300 are poorly excreted in rat bile. In the present paper, the biliary excretion of some compounds containing two or more rings was studied and these include biphenyl and its derivatives, stilboestrol, tetralin (1,2,3,4-tetrahydronaphthalene) and the triphenylmethane derivative, phenolphthalein. Whereas most of the compounds studied in the preceding paper (Abou-El-Makarem *et al.* 1967) were poorly excreted in the bile, those studied in this paper are extensively excreted in the bile. The results obtained have enabled us to define tentatively the properties of compounds that are excreted in relatively large amounts in the bile in the rat.

MATERIALS AND METHODS

Chemicals. Melting points are uncorrected. Biphenyl, m.p. 70°, 4-hydroxybiphenyl, m.p. 164–165°, 4,4'-dihydroxybiphenyl, m.p. 270–272°, and phenolphthalein,

m.p. 258–260° (all from British Drug Houses Ltd., Poole, Dorset), were purified by recrystallization. Phenolphthalein glucuronide (sodium salt) and stilboestrol, m.p. 168°, were purchased (Koch-Light Laboratories Ltd., Colnbrook, Bucks.) and used as such. 4- β -D-Glucuronosido-biphenyl (biphenyl 4-glucuronide), m.p. 182–183°, was prepared by the method of Kamil, Smith & Williams (1951).

4,4'-Dihydroxybiphenyl (2g.) was given by stomach tube as a suspension in water to each of three Chinchilla doe rabbits (3.5kg. wt. each). The bulked 2-day urine (400ml.) was brought to pH 2 with 10N-HCl and kept at 0° overnight. The brown precipitate (1g.) that formed was filtered, washed with water and dried. It gave a strong naphtharesorcinol reaction for glucuronic acid, and on thin-layer chromatography it appeared to be a glucuronide containing a little free 4,4'-dihydroxybiphenyl. It was dissolved in hot aq. 10% (w/v) NaHCO₃ (20ml.), the solution filtered and the filtrate acidified with 2N-HCl. The white precipitate that formed was collected and recrystallized from hot water. The 4- β -D-glucuronosido-4'-hydroxybiphenyl formed a white micro-crystalline powder that charred on heating to 360° (Found: C, 52.9; H, 5.3; loss at 110°, 11.9%; C₁₈H₁₈O₈.2.5H₂O requires C, 53.1; H, 5.7; H₂O, 11.1%). It showed $[\alpha]_D^{25} - 68^\circ$ (c 0.25 in 0.1N-NaOH), and gave a strong naphtharesorcinol test for glucuronic acid and positive tests for a free phenolic group with diazotized sulphanilic acid and Brentamine

Fast Black salt K (see Table 2). On acid hydrolysis it yielded 4,4'-dihydroxybiphenyl, which was identified chromatographically.

[*monoethyl-1-¹⁴C*]Stilboestrol (104 μ C/mg.) was purchased (The Radiochemical Centre, Amersham, Bucks.) and suitably diluted with carrier before use. [*monoethyl-1-¹⁴C*]Stilboestrol monoglucuronide (0.2 μ C/mg.) was prepared biosynthetically by Dr L. J. Fischer (see Fischer, Millburn, Smith & Williams, 1966). [*1-¹⁴C*]Tetralin (0.39 μ C/mg.) was a sample previously synthesized in this Laboratory (Elliott, Hanam, Parke & Williams, 1964).

Animals. Female Wistar albino rats (weighing 200 $\pm$ 10g.) were used and biliary fistulae were established as described by Abou-El-Makarem *et al.* (1967). Compounds were administered intraperitoneally dissolved in 0.75–1.0 ml. of solution. Biphenyl, 4-hydroxybiphenyl, 4,4'-dihydroxybiphenyl and stilboestrol were dissolved in propane-1,2-diol, phenolphthalein and the glucuronides of 4-hydroxybiphenyl, 4,4'-dihydroxybiphenyl and phenolphthalein as sodium salts in water, stilboestrol glucuronide as its sodium salt in aq. 10% (v/v) propane-1,2-diol, and tetralin in arachis oil.

Fluorimetric determination of 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl in bile. An Aminco-Bowman spectrophotofluorimeter was used. At pH 6 in 0.01M-succinate buffer (see Gomori, 1955), 4-hydroxybiphenyl (0.02–0.2 μ g./ml.) fluoresces with maximum intensity at 338 m μ (λ_{exc} , 279 m μ), and at the same pH and wavelengths an equimolecular concentration of 4,4'-dihydroxybiphenyl fluoresces with about (87–98%) the same intensity, although the maximum fluorescence intensity of the dihydroxy compound at pH 6 occurs at 354 m μ (λ_{exc} , 284 m μ) (see Table 1). At pH 13, 4-hydroxybiphenyl fluoresces at 403 m μ (λ_{exc} , 306 m μ), but at this pH and wavelengths 4,4'-dihydroxybiphenyl fluoresces with only 4% of the intensity of the monohydroxy compound. At pH 6 with a mixture of these two phenols the fluorescence intensity is a measure of both, whereas at pH 13 it is a measure of 4-hydroxybiphenyl alone. It was found that 4-hydroxybiphenyl did not quench the fluorescence of 4,4'-dihydroxybiphenyl and vice versa at the concentrations used. For these phenols, fluorescence intensity was found to be proportional to concentration over the range 0–0.2 μ g./ml., which was used in this work.

Bile (2 ml.) from rats dosed with 4-hydroxybiphenyl was refluxed for 3 hr. with an equal volume of 10N-HCl to hydrolyse glucuronides. The solution was neutralized with 5N-NaOH and then filtered to remove pigments. 4-Hydroxybiphenyl plus 4,4'-dihydroxybiphenyl was determined in the filtrate, after diluting it 5000–10000-fold with 0.01M-succinate buffer, pH 6, and measuring the fluorescence intensity at 338 m μ (λ_{exc} , 279 m μ). For the determination of 4-hydroxybiphenyl alone, the filtrate was similarly diluted with 0.1N-NaOH and the fluorescence intensity measured at 403 m μ (λ_{exc} , 306 m μ). 4,4'-Dihydroxybiphenyl was determined by difference. The intensities were compared with those given by standard solutions of 4-hydroxybiphenyl. Blank solutions were prepared from bile collected from rats that had not received 4-hydroxybiphenyl. The recoveries of 4-hydroxybiphenyl and of 4,4'-dihydroxybiphenyl added to bile as their glucuronides (1–3 mg./2 ml. of bile) were 81 and 83% respectively.

4,4'-Dihydroxybiphenyl is excreted in the bile mainly as its monoglucuronide with traces of the free compound (see Table 3). In bile it was determined fluorimetrically at pH 6, after acid hydrolysis, filtration and dilution as above, at 354 m μ (λ_{exc} , 284 m μ). The fluorescences were compared with those of standard solutions of 4,4'-dihydroxybiphenyl.

4-Glucuronosidobiphenyl and 4-glucuronosido-4'-hydroxybiphenyl when injected into rats are excreted in the bile unchanged. They both fluoresce strongly at pH 6 (see Table 1). A suitable volume of the bile was diluted 1000–10000-fold with 0.01M-succinate buffer, pH 6. 4-Glucuronosidobiphenyl was estimated directly in the diluted bile at 328 m μ (λ_{exc} , 274 m μ) and 4-glucuronosido-4'-hydroxybiphenyl at 353 m μ (λ_{exc} , 283 m μ), with standard solutions of the glucuronides for comparison. For both glucuronides, fluorescence intensity was found to be proportional to concentration over the range 0–0.4 μ g./ml., which was used in this work. The recovery by the fluorimetric method of either glucuronide added to normal rat bile (0.1–2 mg./ml. of bile) was 102%.

Determination of phenolphthalein and its glucuronide. Bile samples (1 ml.) containing phenolphthalein were diluted to 10 ml. with 0.4M-glycine buffer, pH 10.4 (Talalay, Fishman & Huggins, 1946), and the red colour was measured at 550 m μ in a Unicam SP 600 spectrophotometer.

Table 1. *Fluorescence of the hydroxybiphenyls and their glucuronides*

Concentration was 1.2 μ M in 0.01M-succinate buffer, pH 6, or in 0.1N-NaOH, pH 13. Wavelengths are instrumental and intensities are uncorrected.

Compound	pH	Excitation λ_{max} , (m μ)	Fluorescence λ_{max} , (m μ)	Relative fluorescence intensity*
4-Hydroxybiphenyl	6	279	338	(100)
	13	306	403	138
4,4'-Dihydroxybiphenyl	6	284	354	141
	6	279	338	98†
	13	316	400	5
4-Glucuronosidobiphenyl	6	274	328	214
4-Glucuronosido-4'-hydroxybiphenyl	6	283	353	171

* Relative to that of 4-hydroxybiphenyl = 100 at pH 6.

† These are not peak values, but are included for comparison with those of 4-hydroxybiphenyl at the same excitation wavelength.

Table 2. *R_F* values and colour reactions

Descending chromatography on Whatman no. 1 paper or ascending chromatography by the thin-layer technique was used (see the text). The solvent systems employed were: *A*, butan-1-ol-acetic acid-water (4:1:2, by vol.); *B*, propan-1-ol-aq. NH₃ (sp. gr. 0.88) (7:3, v/v); *C*, pyridine-pentan-1-ol-water (7:7:6, by vol.); *D*, butan-1-ol-ethanol-acetic acid-water (32:8:1:12, by vol.); *E*, ethanol-butan-1-ol-1.5*N*-ammonium carbonate buffer, pH 7.4 (1:4:2, by vol.) (Fewster & Hall, 1951); *F*, acetone-chloroform (1:1, v/v). The paper chromatograms were run for 10-14 hr. Thin-layer chromatograms in solvents *A* and *C* were run for 2-3 hr., and in solvent *F* for 0.5 hr.

Compounds	<i>R_F</i> values in solvents						Colour reactions			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	Fluorescence in u.v. light	Naphtharesorcinol test†	Pauly reagent†	Brentamine Fast Black salt K†
Thin-layer chromatography										
4-Hydroxybiphenyl	0.91	—	0.87	—	—	0.40	Blue	None	Pink	Purple
4,4'-Dihydroxybiphenyl	0.89	—	0.82	—	—	0.20	Blue	None	Pink	Yellow
4-Glucuronosidobiphenyl	0.65	—	0.32	—	0.26	—	Purple	Blue	None	None
4-Glucuronosido-4'-hydroxybiphenyl	0.61	—	0.30	—	0.24	—	Blue	Blue	Pink	Yellow
Paper chromatography										
Phenolphthalein*	0.80	0.70	—	0.91	—	—	Dark spot	None	—	—
Phenolphthalein glucuronide*	0.40	0.35	—	0.61	—	—	Dark spot	Blue	—	—
Stilboestrol	—	0.94	0.94	0.88	0.86	—	Blue	None	—	—
Stilboestrol glucuronide	—	0.78	0.81	0.75	0.30	—	Blue	Blue	—	—
					0.50					
α- and β-Tetralyl glucuronides‡	0.74	0.50	0.53	—	—	—	Dark spot	Blue	—	—

* Phenolphthalein gives a pink colour on spraying with 2*N*-NH₃ solution; its glucuronide, however, only gives the colour with NH₃ 16 hr. after spraying the paper with β-glucuronidase.
† See the text.
‡ We are grateful to Mr J. Hanam for these values (see Elliott *et al.* 1964).

To estimate phenolphthalein glucuronide, bile samples (1 ml.) were heated with an equal volume of 8*N*-HCl in a boiling-water bath for 1 hr. The solution was neutralized with solid Na₂CO₃, made up to 10 ml. with the glycine buffer and its red colour measured at 550 mμ. In some cases bile pigments were precipitated after hydrolysis and these were removed by centrifugation. The recovery of either phenolphthalein or phenolphthalein glucuronide (0.25-0.5 mg./ml.) added to the bile was 97 ± 5%.

Measurement of ¹⁴C radioactivity and chromatography. ¹⁴C was estimated by end-window or scintillation counting (Abou-El-Makarem *et al.* 1967). Bile samples (0.01-0.02 ml.) from rats treated with [¹⁴C]stilboestrol were chromatographed on strips (1.5 in. wide) of Whatman no. 1 paper with solvent *E* (see Table 2). The dried chromatogram was scanned for radioactivity with a Packard radiochromatogram scanner (model 7200). The compounds responsible for the radioactive peaks on the chromatogram were identified by comparing their *R_F* values with those of authentic substances.

Compounds in bile were identified by comparison of their *R_F* values and colour reactions with those given by authentic compounds. Bile samples (0.01-0.2 ml.) were chromatographed by the descending technique on Whatman no. 1 paper. For thin-layer chromatography, alumina supported on 8 in. × 8 in. glass sheets was used and the chromatograms were developed by the ascending technique in

glass tanks (8.5 in. × 8.5 in. × 4 in.). Satisfactory separation was achieved when the solvent had run 10 cm. from the origin. The composition of the solvents and the colour reactions used are given in Table 2. Glucuronides were detected by the naphtharesorcinol test (see Bridges, Kibby & Williams, 1965). Phenols were detected with diazotized sulphanilic acid and an aq. 0.1% solution of Brentamine Fast Black salt K (G. T. Gurr Ltd., London, S.W. 6) followed by saturated NaHCO₃ solution.

Determination of distribution ratios. This was carried out as described in the preceding paper (Abou-El-Makarem *et al.* 1967).

RESULTS AND DISCUSSION

The qualitative and quantitative aspects of the biliary excretion by the rat of the ten compounds studied are shown in Tables 3 and 4. All these compounds are excreted in the bile in amounts greater than 10% of the dose and in some cases the excretion is nearly 100% of the dose.

The first five compounds are derivatives of biphenyl and, as Table 4 shows, biliary excretion increases with molecular weight. Further, the forms in which these compounds are excreted in the bile are glucuronides of molecular weight about

Table 3. *Compounds found in rat bile after administration of various cyclic compounds*

The method of administration of the compounds and the collection of bile are described in the text.

Compound administered	Metabolites found in bile	R_F value (solvent†) of metabolite
4-Hydroxybiphenyl	Unknown glucuronide*	0.16 (A)
	4-Glucuronosido-4'-hydroxybiphenyl	0.59 (A), 0.28 (C), 0.21 (E)
	4-Glucuronosidobiphenyl	0.63 (A), 0.32 (C), 0.27 (E)
4,4'-Dihydroxybiphenyl	Unknown glucuronide*	0.16 (A)
	4-Glucuronosido-4'-hydroxybiphenyl	0.60 (A), 0.29 (C), 0.23 (E)
4-Glucuronosidobiphenyl	Compound administered only	0.64 (A), 0.31 (C), 0.26 (E)
4-Glucuronosido-4'-hydroxybiphenyl	Compound administered only	0.61 (A), 0.30 (C), 0.23 (E)
Phenolphthalein	Phenolphthalein glucuronide	0.41 (A), 0.34 (B), 0.57 (D)
	Phenolphthalein*	0.78 (A), 0.68 (B), 0.90 (D)
Phenolphthalein glucuronide	Compound administered only	0.40 (A), 0.33 (B), 0.60 (D)
Stilboestrol	Unidentified	0 (D), 0 (E)
	Unidentified (glucuronide?)	0.44 (D), 0.07 (E), 0.11 (E)
	Stilboestrol glucuronide	0.75 (D), 0.33 (E)
	Stilboestrol*	0.90 (D), 0.89 (E)
Stilboestrol glucuronide	Unidentified (glucuronide?)	0.10 (E)
	Stilboestrol glucuronide	0.38 (E)
Tetralin	<i>cis</i> - and/or <i>trans</i> -Tetralin-1,2-diol glucuronides	0.64 (A), 0.47 (B), 0.47 (C)
	α - and/or β -Tetralyl glucuronides	0.74 (A), 0.51 (B), 0.52 (C)

* Minor metabolites, i.e. less than 5% of dose (see also Table 4).

† The letters in this column refer to solvents given in Table 2.

350. Biphenyl (mol.wt. 154) is excreted (12% of dose) as two glucuronides, i.e. the monoglucuronides of 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl, which occur in roughly equal amounts. This means that biphenyl has to be hydroxylated, first to the monohydroxy and then to the dihydroxy compound, and then conjugated before it appears in the bile. 4-Hydroxybiphenyl (mol.wt. 170) appears in the bile as the same two glucuronides but now in larger amounts (37%), and it appears that it undergoes two separate reactions, one being direct conjugation and the other hydroxylation to the dihydroxy compound, which is then conjugated. 4,4'-Dihydroxybiphenyl (mol.wt. 186) is extensively excreted (65%) in the bile as one glucuronide, which is formed by direct conjugation at one of the hydroxyl groups. When the glucuronides themselves are administered they are excreted to a very large extent in the bile unchanged, and the excretion (92%) of 4-glucuronosido-4'-hydroxybiphenyl (mol.wt. 362) is greater than that (59%) of 4-glucuronosidobiphenyl (mol.wt. 346). It is to be noted that when 4-hydroxybiphenyl is administered it is excreted as two glucuronides, but that when the glucuronide of 4-hydroxybiphenyl is administered only one glucuronide, i.e. the one administered, is excreted. The fact that biphenyl, 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl, which have molecular weights between 150 and 200, are excreted in the bile in appreciable amounts,

whereas phenylthiourea, 4-nitrobenzoic acid and sulphanilic acid, which also have molecular weights between 150 and 200, are not excreted in the bile (see Abou-El-Makarem *et al.* 1967), suggests that, though molecular weight is an important factor in biliary excretion, there are other factors involved for compounds of this magnitude of molecular weight.

Since these biphenyl derivatives are excreted in the bile as glucuronides, it is pertinent to examine the nature of these conjugates, since this may give us a clue as to what properties a compound needs to be excreted in the bile in appreciable amounts. The glucuronides derived from biphenyl are highly polar compounds of molecular weight of about 350. One could suggest therefore that, for appreciable biliary excretion in the rat, a compound should have a polar anionic group and molecular weight about 350 or can be converted metabolically into such a compound. 4-Glucuronosidobiphenyl and 4-glucuronosido-4'-hydroxybiphenyl possess these properties and are excreted in large amounts as such in the bile. Biphenyl, 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl are metabolized to such compounds and are therefore excreted in the bile in appreciable amounts, which probably depend on the extent and rate at which they are metabolized. 4-Nitrobenzoic acid and sulphanilic acid are already highly polar compounds, but their molecular weights are about 170 and they are not

Table 4. *Quantitative aspects of the biliary excretion of cyclic compounds in the rat*

The method of administration of compounds and collection of bile are described in the text. Average values are given, with ranges in parentheses; numbers of animals are indicated by superscripts.

Compound	Mol.wt.	Dose		% of dose in bile in 24 hr.	Metabolites found in bile (% of dose)
		(mg./kg.)	(m-mole/kg.)		
Biphenyl	154	31	0.20	12 (5-17) ³	4-Glucuronosidobiphenyl (5), 4-glucuronosido-4'-hydroxybiphenyl (7)
4-Hydroxybiphenyl	170	35	0.20	37 (35-39) ⁴	4-Glucuronosidobiphenyl (21)*, 4-glucuronosido-4'-hydroxybiphenyl (nearly 16)
4,4'-Dihydroxybiphenyl	186	38	0.20	65 (48-73) ⁴	4-Glucuronosido-4'-hydroxybiphenyl (nearly 65)*
4-Glucuronosidobiphenyl	346	12	0.035	59 (38-68) ³	4-Glucuronosidobiphenyl (59)
4-Glucuronosido-4'-hydroxybiphenyl	362	9-45	0.025-0.124	92 (78-106) ⁵	4-Glucuronosido-4'-hydroxybiphenyl (92)
Stilboestrol	268	10	0.037	94 (89-99) ³	Stilboestrol glucuronide (67), stilboestrol (3), three unidentified metabolites (24)
		0.05	0.00018	98 (95-99) ⁴	Not determined
Stilboestrol glucuronide	445	5	0.011	100 (98-103) ³	Stilboestrol glucuronide (89), unidentified glucuronide (11)
Phenolphthalein	318	50	0.157	100 (94-104) ³	Phenolphthalein glucuronide (98), phenolphthalein (2)
Phenolphthalein glucuronide	495	10	0.02	75 (67-87) ³	Phenolphthalein glucuronide (75)
Tetralin	132	45	0.34	13 (7-17) ³	ac-Tetralyl glucuronide mainly, tetralin-1,2-diol glucuronide

* These biles contained small amounts of unidentified glucuronide (possibly the diglucuronide of 4,4'-dihydroxybiphenyl) and traces of the free aglycones.

converted into suitable conjugates, and therefore they are not excreted in the bile in appreciable amounts. Phenylthiourea (mol.wt. 152) is metabolized *in vivo* (Scheline, Smith & Williams, 1961), but it is not converted into a polar metabolite of suitable molecular weight and therefore its biliary excretion is relatively small (Abou-El-Makarem *et al.* 1967). 4-Aminophenyl glucuronide is an example of a polar conjugate that is not excreted in the bile to any appreciable extent (its excretion is 0.1%; see Abou-El-Makarem *et al.* 1967). In this case it appears that, although it is a polar conjugate, its molecular weight (285) may not be high enough for extensive biliary excretion.

The next four compounds in Table 4 are stilboestrol (mol.wt. 268), phenolphthalein (mol.wt. 318) and their glucuronides (mol.wt. 445 and 495 respectively). Neither stilboestrol nor phenolphthalein are highly polar, but they are both converted *in vivo* into glucuronides whose molecular weights are much higher than 350. The biliary excretion of stilboestrol is over 90% of the dose and its main metabolite in the bile is stilboestrol monoglucuronide (67% of the dose). A small

amount of free stilboestrol (3%) and three other metabolites (24%) are also excreted in the bile. When stilboestrol glucuronide is administered it is entirely excreted in the bile, mainly (89%) as such but to a smaller extent (11%) as a second glucuronide, possibly the diglucuronide for on hydrolysis it yields stilboestrol.

Phenolphthalein (mol.wt. 318) is virtually completely excreted (100%) in the bile, almost entirely (98%) as its glucuronide. Its glucuronide (mol.wt. 495) is also excreted in the bile to a very high degree (75% of the dose) in the unchanged state but to a somewhat smaller extent than phenolphthalein itself. Whether the difference (about 25%) between the values for the biliary excretion of phenolphthalein and its glucuronide has any significance is difficult to determine at present. The distribution ratios between organic solvents and phosphate buffer, pH 7.4, of stilboestrol, phenolphthalein and their glucuronides are shown in Table 5, and it is clear that stilboestrol and phenolphthalein are many hundred times more soluble in organic solvents than are their glucuronides, which occur as anions at pH 7.4. Although

Table 5. *Distribution ratios of stilboestrol, phenolphthalein and their glucuronides between organic solvents and phosphate buffer, pH 7.4*

0.1 M-Phosphate buffer, pH 7.4, was used with chloroform, ethyl acetate or hexane. *C* means concentration in the solvent indicated by the subscript (EtAc refers to ethyl acetate). The values are taken from Abou-El-Makarem (1967).

Compound	$C_{\text{CHCl}_3}/C_{\text{buffer}}$	$C_{\text{EtAc}}/C_{\text{buffer}}$	$C_{\text{hexane}}/C_{\text{buffer}}$
Stilboestrol	7.12	25.8	2.0
Stilboestrol glucuronide	0.018	0.096	0.004
Phenolphthalein	9.0	4.0	—
Phenolphthalein glucuronide	<0.0001	0.01	—

stilboestrol and its glucuronide have obviously very different lipid-solubilities at pH 7.4, both compounds are equally well excreted in the bile. It is to be noted, however (see Table 5), that the distribution ratios of phenolphthalein glucuronide are much smaller than those of stilboestrol glucuronide. This may suggest that phenolphthalein glucuronide is much less lipid-soluble at pH 7.4 than is stilboestrol glucuronide. It is possible that the smaller biliary excretion of phenolphthalein glucuronide mentioned above compared with phenolphthalein (see Table 4) may be due to the glucuronide having a lower rate of penetration into the liver in view of its relatively low lipid-solubility. This would allow it to be partially lost in the urine.

Tables 3 and 4 also contain results for tetralin (mol.wt. 132). The metabolites of this compound in the urine in rabbits have been examined in this Laboratory and reported on by Elliott *et al.* (1964). In rat bile, two glucuronide spots were found by paper chromatography (Table 3). The major spot corresponded to *ac*- α - or - β -tetralyl glucuronide or both and the other is probably a glucuronide of tetralin-1,2-diol. These glucuronides have molecular weights 324 and 340 respectively, and are of sufficient size to be excreted in the bile to an appreciable but not high extent. The extent of biliary excretion is 13% (Table 4) and this value is probably influenced, as with biphenyl, by the rate and extent to which tetralin is oxidized to tetralol and tetralindiol and subsequently conjugated with glucuronic acid.

It is concluded from the results given in this and in the preceding paper (Abou-El-Makarem *et al.* 1967) that the extent of biliary excretion of a compound in the rat depends on its molecular weight, polarity and metabolism. To be excreted in the bile in appreciable amounts (i.e. more than 5–10% of the dose), a compound must possess a strong polar anionic group and a molecular weight not less than about 300. This molecular weight can be attained, if needs be, by a metabolic change, usually by conjugation. There is some latitude in the definition of this minimum molecular weight but it appears to be about 325 ± 50 , for the rat.

The rate at which the metabolic change occurs may also affect the amount of a metabolite in the bile. This is illustrated by biphenyl, 4-hydroxybiphenyl and 4-glucuronosidobiphenyl. These three compounds are excreted in the bile, as practically the same metabolites, but their biliary excretions are respectively 12, 37 and 59% of the dose. These differing values may be a reflection of their metabolism, for biphenyl has to be hydroxylated and conjugated to form the metabolite in the bile, whereas 4-hydroxybiphenyl has only to be conjugated and 4-glucuronosidobiphenyl requires no metabolism.

It might be interesting to speculate as to why a foreign compound needs a minimum molecular weight of about 325 ± 50 and a strongly polar anionic group before it can be excreted in the bile in appreciable quantities. It is possible that a transport mechanism that is normally used for the biliary excretion of endogenous compounds is being utilized. Such endogenous compounds are glycocholic acid (mol.wt. 466; pK_a 4.54), taurocholic acid (mol.wt. 516; pK_a 1.56) (Sobotka, 1938) and bilirubin mono- and di-glucuronides (mol.wt. 761 and 937; most glucuronides have pK_a 3–4) (Williams, 1959). It is to be noted that each of these compounds possesses a highly polar anionic group and a relatively high molecular weight. It is therefore possible that, if a foreign compound is highly polar and has a molecular weight approaching the smallest of these endogenous compounds or can acquire these properties by metabolism, then it will be excreted in the bile in relatively large amounts. The extent to which biliary excretion occurs will depend on how close the molecular weight approaches some value such as 466 or 516. There is obviously considerable latitude in the approach to this value, for stilboestrol glucuronide (445) is entirely excreted in the bile, 4-glucuronosido-4'-hydroxybiphenyl (362) is about 90% excreted and 4-glucuronosidobiphenyl (346) is about 60% excreted in the bile.

Sperber (1963) has pointed out that the renal tubules secrete best those compounds of molecular weight 200–400, whereas compounds of molecular

weight greater than 400 are efficiently excreted in the bile.

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