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Biliary Excretion of Foreign Compounds

BENZENE AND ITS DERIVATIVES IN THE RAT

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1. The extent of the excretion in the bile of the rat of benzene and 21 of its simple derivatives was studied. 2. Some 16 compounds of molecular weight less than 200, and including neutral molecules (benzene and toluene), aromatic acids, aromatic amines and phenols, were injected in solution intraperitoneally into biliary-cannulated rats. Metabolites in the bile were identified and estimated. The extent of biliary excretion of these compounds was low, i.e. 0–10% of the dose in 24hr., and most appeared in the bile mainly as conjugates. 3. The biliary excretion of six conjugates of molecular weight less than 300, including three glycine conjugates, one sulphate conjugate, one glucuronic acid conjugate and two acetyl derivatives, was low (less than 3% of the dose). 4. It is concluded that simple benzene derivatives of molecular weight less than about 300 are poorly excreted in rat bile.

During the study of the fate of foreign compounds in animals in this Laboratory over many years, it had been noticed that the faecal excretion of certain substances in the rat was greater than in the rabbit. It was thought that this difference might be due to a more extensive biliary excretion of these compounds in the rat, and that it was indicative of a species difference in the extent of biliary excretion. There were also indications from the literature that conjugation with glucuronic acid, glycine and other agents might play a role in biliary excretion, for bile acids (Haslewood, 1955), bilirubin (Klatskin, 1961), thyroxine (Flock & Bollman, 1962), sulphobromophthalein (Combes & Stakelum, 1960) and morphine (Way & Adler, 1962) are excreted in the bile as conjugates (see also Smith, 1966). Further, the above-mentioned compounds are of relatively high molecular weight (morphine, 285; cholic acid, 409; bilirubin, 585; thyroxine, 777; sulphobromophthalein sodium, 838). These observations suggested to us that species, conjugation and molecular weight may play a role in the biliary excretion of foreign compounds. A systematic examination of the extent of biliary excretion of a variety of foreign compounds in the rat and then in other species was therefore undertaken. Preliminary reports of this work have appeared (Millburn, Smith & Williams, 1964; Williams, Millburn & Smith, 1965; Williams, Smith & Millburn, 1965; Abou-El-Makarem, Millburn, Smith & Williams, 1966).

The present paper deals with the extent of biliary excretion in the rat of some substituted benzenes of low molecular weight.

MATERIALS AND METHODS

Chemicals. 4-Nitrobenzoic acid, 2- and 4-aminobenzoic acid, 4-acetamidobenzoic acid, 4-aminohippuric acid, 4-acetamidohippuric acid, 2-, 3- and 4-aminobenzenesulphonic acid (orthanilic acid, metanilic acid and sulphanilic acid), salicylic acid and 2-, 3- and 4-aminophenol were either commercial samples or prepared as described in the literature and were purified by recrystallization. Their melting points agreed with values given in the literature. 4-Aminophenyl glucuronide was prepared biosynthetically (Williams, 1943). Potassium 3-aminophenyl sulphate was available in this Laboratory.

Radioactive compounds. [U-^{14}C]Benzene, [U-^{14}C]phenol, [U-^{14}C]aniline hydrogen sulphate, [*carboxy*-^{14}C]benzoic acid (all from The Radiochemical Centre, Amersham, Bucks.) and [U-^{14}C]toluene (Packard Ltd., Wembley, Middx.) were purchased. [2-^{14}C]Phenylthiourea was a sample previously prepared in this Laboratory (Scheline, Smith & Williams, 1961).

[^{14}C]Hippuric acid was prepared by refluxing [*carboxy*-^{14}C]benzoic acid (890mg., 90μc) with thionyl chloride (1·5ml.) for 1hr. The product was fractionally distilled and the [^{14}C]benzoyl chloride collected at b.p. 192–198°. The chloride (0·9g.) was added to a solution of glycine (0·55g.) in 20% (w/v) NaOH (5ml.) and vigorously shaken. The solution was cooled, acidified with conc. HCl and kept at 0° overnight. The [^{14}C]hippuric acid was filtered, washed with water and recrystallized from hot water (8ml.). The yield was 0·9g. with specific activity 17μc/g. and m.p. 188–190°.

Treatment of the rats. Female Wistar albino rats (weighing 200$\pm$10g.) were anaesthetized with hexobarbitone sodium (100mg./kg.; 20mg./ml. intraperitoneally). The common bile duct was exposed through a small mid-line incision in the abdominal wall and a polythene cannula (0·4mm. internal diam. and 0·8mm. external diam.) inserted and tied in position, the tip being about 3mm. from the junction

of the right and left bile ducts. The cannula was sewn in position on the abdominal wall and directed, after the cavity had been closed, between the skin and the abdominal wall until it emerged through a small conveniently situated lateral incision in the skin. The rats were kept in Bollman (1948) restraining cages for up to 24hr. with free access to water containing 5% (w/v) glucose and 1% (w/v) NaCl and kept warm with a warming blanket over each cage. The other end of the cannula led into a measuring cylinder (20ml.) for bile collection. Urine was collected in a polythene tray fitted beneath the restraining cage.

In some experiments the excretion of urine was prevented. During this type of experiment the rats were anaesthetized as described above for the whole duration of bile collection (3hr.). The common bile duct was cannulated as described above and both renal pedicles (i.e. renal artery, vein and ureter) were ligated, thus stopping urine formation.

Determination of salicylic acid. Bile (0·1ml.) was chromatographed on Whatman no. 1 paper with solvent *E* (see Table 1). After the paper had been dried the salicylic acid area was located by its fluorescence in u.v. light, cut out and eluted with 0·5M-borate buffer, pH10. The eluate was made up to 10ml. with the buffer and its salicylic acid content determined fluorimetrically (Aminco–Bowman spectrophotofluorimeter) as described by Chirigos & Udenfriend (1959). The recovery of salicylic acid added to bile (0·01–0·1mg./ml.) was 90%.

Determination of free and total aromatic amino compounds. The Bratton & Marshall (1939) method was used. For 2- and 4-aminobenzoic acid, 4-amino- and 4-acetamido-hippuric acid and 2, 3- and 4-aminobenzenesulphonic acid, the bile was diluted either fivefold or tenfold with water. The diluted bile (1ml.) was mixed with 2N-HCl (0·5ml.) followed by 0·1% $NaNO_2$ solution (0·5ml.). After 3min. 0·5% ammonium sulphamate (0·5ml.) was added, the solution was shaken and then 3min. later aq. 0·1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride (0·5ml.) was added. The sample was diluted to 10ml. with water and after 30min. the red–purple colour was determined in a Unicam SP.600 spectrophotometer at 550mμ for 4-aminohippuric acid and at 540mμ for 4-aminobenzoic acid and the aminobenzene-sulphonic acids. For 2-aminobenzoic acid the colour was measured at 550mμ after 24hr. For total primary amine, bile (2–5ml.) mixed with an equal volume of 2N-HCl was heated in a boiling-water bath for 1hr. The mixture was filtered and the filtrate (2ml.) diluted with water to 10ml., and with this solution the procedure for free primary amine was followed as described above. Control bile gave no colour with the Bratton & Marshall (1939) reagents before or after acid hydrolysis.

2- and 4-Aminophenol appear in bile mainly as the corresponding *O*-glucuronides and were estimated as such by the Bratton & Marshall (1939) method, with the modifications that the diazotization time was increased to 15min. and the colour allowed to develop for 24hr. before measurement. For 4-aminophenyl glucuronide it was also necessary to add 1ml. of 5N-HCl to the sample after the addition of the coupling agent to ensure maximum colour production. For the sulphate ester, 3-aminophenyl sulphate, the diazotization time was 15min. and maximum colour development occurred after 3hr. The colours from 2-aminophenyl glucuronide and 3-aminophenyl sulphate were measured at 550mμ and that from 4-aminophenyl glucuronide was measured at 570mμ. The recoveries of the above compounds when they were added to bile (0·005–2mg./ml.) were 86–98%.

Determination of 4-nitrobenzoic acid. The bile (0·5ml.) was treated with 15% (w/v) titanous chloride (2ml.) and 2N-HCl (1ml.). The 4-aminobenzoic acid formed was then determined as above.

Measurement of ^{14}C radioactivity. ^{14}C was measured by either end-window or scintillation counting. For the former, bile samples (1ml.) were counted on stainless-steel planchets with a Panax end-window counter (type D.657), the background of which was 9–11counts/min. For scintillation counting, the bile sample (0·1–1·0ml.) was added to about 20ml. of liquid scintillator [composition: naphthalene (60g.), 2,5-diphenyloxazole (4g.), 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0·2g.), methanol (100ml.) and ethylene glycol (20ml.) added to dioxan and made up to 1l.] contained in a glass vial. The samples were counted at an efficiency of 35–65% (determined by the twin-channel ratio method) in a Packard Tri-Carb scintillation spectrometer (model 3214).

Chromatograms of radioactive bile and urine were scanned in a Packard radiochromatogram scanner (model 7200). Bile (0·01–0·2ml.) or diluted urine (0·02–0·5ml.) was chromatographed on thin strips (1·5–1·75in. wide) of paper (Whatman no. 1) and R_F values of the ^{14}C-labelled peaks were compared with those of known reference compounds.

Chromatography. The R_F values of the compounds relevant to this work are given in Table 1. For the detection of compounds in bile, 0·01–0·2ml. samples of bile were chromatographed with the solvents listed in Table 1. Compounds were identified by their R_F values and colour reactions. Detection of compounds on paper by u.v. light, *p*-dimethylaminocinnamaldehyde and naphtharesorcinol was essentially as described by Bridges, Kibby & Williams (1965). *p*-Dimethylaminobenzaldehyde in acetic anhydride was used to detect hippuric acid and its derivatives (see Gaffney, Schreier, DiFerrante & Altman, 1954).

Determination of distribution ratios. A solution (1ml.) of the compound in water (0·2–0·5mg./ml.) was added to 49ml. of 0·1M-phosphate buffer, pH7·4. This was then shaken mechanically for 1hr. at room temperature with 50ml. of $CHCl_3$ or ethyl acetate. The compound remaining in the aqueous layer was determined by one of the above methods. For the organic-solvent layer, the glucuronides, the sulphate and 4-amino- and 4-acetamido-hippuric acid were extracted from the solvent by saturated aq. Na_2CO_3 and then determined, whereas the aminophenols were extracted with 2N-HCl. For the ^{14}C-labelled compounds, a portion of the organic-solvent layer was evaporated in a scintillation-counting vial, phosphor added and the ^{14}C radioactivity counted. All determinations were done in duplicate.

RESULTS AND DISCUSSION

The extent of biliary excretion of 22 simple benzene derivatives of molecular weight less than 300 is shown in Table 2. The biliary excretion of these compounds does not exceed 10% of the dose. Although bile was collected for 24hr. after the compounds were injected, other experiments have shown that the excretion of these compounds in the bile was probably complete in about 3hr. Table 2

Table 1. *Chromatography and colour reactions of some aromatic acids and phenols and their derivatives*

Descending chromatography on Whatman no. 1 paper was used. The solvent systems employed were: *A*, butan-1-ol–acetic acid–water (4:1:2, by vol.); *B*, butan-1-ol–aq. NH_3 (sp.gr. 0·88)–water (10:1:1, by vol.); *C*, aq. 80% (v/v) ethanol; *D*, butan-1-ol–ethanol–water–acetic acid (3:1:1:0·1, by vol.); *E*, propan-1-ol–aq. NH_3 (sp.gr. 0·88) (7:3, v/v). All chromatograms were run for 12–14hr. except with solvent *C*, which was run for 8–10hr. Abbreviations: DMAC, *p*-dimethylaminocinnamaldehyde; DMAB, *p*-dimethylaminobenzaldehyde (see the text).

Compound	R_F values in solvent					Colour reactions*	
	A	*B*	*C*	*D*	*E*	DMAC	DMAB
Phenol	0·91	0·92	—	—	0·89	—	—
Phenyl glucuronide	0·65	0·09	—	—	0·41	—	—
Quinol	0·85	Unstable	—	—	Unstable	—	—
Benzoic acid	0·96	—	—	0·81	0·65	None	None
Hippuric acid	0·87	—	—	0·56	0·60	None	Orange
Benzoyl glucuronide	0·69	—	—	0·32	0·44	None	None
2-Hydroxybenzoic acid	0·89	0·30	—	—	0·72	None	None
4-Nitrobenzoic acid	0·87	0·48	0·71	—	—	None	None
2-Aminobenzoic acid	0·88	0·22	0·66	—	0·42	Red–purple	Yellow
4-Aminobenzoic acid	0·82	0·03	0·50	—	0·22	Red–purple	Yellow
4-Acetamidobenzoic acid	0·88	0·16	—	—	0·40	None, but red–purple after 6–24hr.	Yellow after 6–24hr.
4-Aminohippuric acid	0·58	0·09	0·28	—	0·25	Red–purple	Orange
4-Acetamidohippuric acid	0·73	0·19	0·35	—	0·37	None, but red–purple after 6–24hr.	Orange
Aniline hydrochloride	0·60	—	—	0·56	Unstable	Red–purple	—
2-Aminophenol	0·71	—	—	0·71	0·78	Red–orange Red–purple in 24hr.	—
3-Aminophenol	0·70	0·68	—	0·69	0·74	Red–purple	—
4-Aminophenol	0·62	—	—	0·52	Unstable	Red–orange Red–purple in 24hr.	—
3-Aminophenyl sulphate	0·23	0·02	—	0·07	0·28	Red–purple after 3hr.	—
2-Aminophenyl glucuronide	0·40	—	—	0·18	0·36	Red–purple	—
4-Aminophenyl glucuronide	0·23	0·02	—	0·06	0·19	Red–orange	—
Sulphanilic acid	0·22	0·12	—	—	0·33	Red–purple	—
Metanilic acid	0·30	0·14	—	—	0·41	Red–purple	—
Orthanilic acid	0·44	0·25	—	—	0·49	Red–purple	—

* The glucuronides give a blue colour with the naphtharesorcinol spray; in u.v. light (254mμ; Hanovia Chromatolite lamp) all the compounds showed up as dark spots except 2-hydroxybenzoic acid, 2- and 4-aminobenzoic acid, 4-aminophenol, 4-aminophenyl glucuronide, 4-aminohippuric acid and the aminobenzenesulphonic acids, which fluoresce blue or purple.

lists compounds of differing physicochemical properties including hydrocarbons, acids, bases, phenols and amphoteric substances such as aminophenols. These compounds also differ widely in lipid-solubility at pH 7·4, e.g. aniline and benzoic acid, as shown in Table 3. However, what the compounds in Table 2 have in common is a relatively low molecular weight, i.e. less than 300, and it appears that this may be associated with a relatively low extent of biliary excretion in the rat. Although the actual numerical values of the biliary excretion of these compounds vary within the limits 0–10%, to draw conclusions from these variations might not be valid because the doses used were varied for the convenience of estimation. However, it does appear that the strong acids in Table 2, e.g. the aminobenzenesulphonic acids, 4-nitrobenzoic acid and benzoic acid, have the lowest biliary excretion.

The compound of lowest molecular weight in Table 2 is benzene. By using [^{14}C]benzene, it was found that about 0·8% of the injected ^{14}C radioactivity appeared in the bile. Chromatography of this bile in three solvent systems (*A*, *B* and *E*; see Table 1) showed that there were four metabolites

Table 2. *Biliary excretion in the rat of some simple benzene derivatives*

Compounds in 1 ml. of solution were injected intraperitoneally into biliary-cannulated female rats and the bile was collected for 24 hr.; benzene and toluene were administered in propane-1,2-diol, quinol in aq. 50% (v/v) propane-1,2-diol, and aniline (hydrochloride), phenol, phenylthiourea, 2- and 4-aminophenol and the acids (as sodium salts) in water.

Compounds	Mol.wt.	Dose (mg./kg.)	Dose (m-mole/kg.)	% of dose in bile in 24 hr.*	Metabolites found in bile
[^{14}C]Benzene	78	50	0·64	0·8 (0·5–1·3)	Phenyl glucuronide (16%) and three others
[^{14}C]Toluene	92	50	0·54	< 2	Not examined
[^{14}C]Aniline	93	20	0·22	5·7 (4·3–8·1)	4-Aminophenol, 4-aminophenyl glucuronide (major), 2-aminophenol, 2-aminophenyl glucuronide (minor)
[^{14}C]Phenol	94	50	0·53	4·6 (1·7–7·9)	Phenyl glucuronide (54%) and three others
2-Aminophenol	109	50	0·46	7·5 (6·6–8·2)	2-Aminophenyl glucuronide
4-Aminophenol	109	50	0·46	7·6 (7·0–8·5)	4-Aminophenyl glucuronide
[^{14}C]Quinol	110	100	0·91	7·0 (2·8–11·5)	Not examined
[^{14}C]Benzoic acid	122	50	0·41	1·2 (1·1–1·4)	Hippuric acid (80%), benzoyl glucuronide (13%), benzoic acid (7%)
2-Aminobenzoic acid	137	18	0·13	3·0 (2·6–3·3)	2-Aminobenzoic acid and probably its glycine and glucuronic acid conjugates, and other minor metabolites
		101	0·74	5·3 (3·7–6·8)	
4-Aminobenzoic acid	137	18	0·13	5·8 (3·9–8·5)	4-Aminohippuric acid, 4-acetamidohippuric acid, 4-aminobenzoic acid and other minor metabolites
		105	0·77	2·5 (1·1–3·2)	
Salicylic acid	138	50	0·36	1·5 (1·4–1·9)	Salicylic acid only
[^{14}C]Phenylthiourea	152	5	0·03	1·3 (1·1–1·5)	Not examined
4-Nitrobenzoic acid	167	50	0·30	0·0	None
Sulphanilic acid	173	22	0·13	< 1	None
		50	0·29	0·0	
Metanilic acid	173	22	0·13	< 1	Not examined
Orthanilic acid	173	22	0·13	0·0	None
Conjugates					
[^{14}C]Hippuric acid	179	50	0·28	0·0	None
4-Acetamidobenzoic acid	179	23	0·13	< 1	Not examined
3-Aminophenyl sulphate	189	83	0·44	0·2 (0·1–0·3)	Unchanged compound
4-Aminohippuric acid	194	25	0·13	1·2 (1·0–1·5)	4-Acetamidohippuric acid (80%), unchanged compound (20%)
		134	0·69	1·5 (0·2–2·8)	
4-Acetamidohippuric acid	236	31	0·13	1·9 (< 1–4·1)	Unchanged compound
		150	0·64	2·1 (1·4–2·7)	
4-Aminophenyl glucuronide	285	100	0·35	0·1 (0·05–0·15)	Unchanged compound

* Mean values for three rats, with ranges in parentheses.

(R_F 0·02, 0·09, 0·41 and 0·56 in solvent *E*), the first two amounting to 75% of the ^{14}C radioactivity of the bile, the third 16% and the last 9%. The one of R_F 0·41 was identified as phenyl glucuronide. When [^{14}C]phenol was administered, about 5% of the ^{14}C radioactivity was found in the bile as the same four metabolites as found after benzene, but in different proportions, namely 9, 22, 54 and 15%. For phenol, the major metabolite in the bile is phenyl glucuronide (54%). Phenyl sulphate, which is a well-known metabolite of benzene and phenol in the urine in rabbits and rats, was not found in the bile. On feeding benzene (50 mg./kg.) to biliary-cannulated rats, the major metabolite found in the urine was phenyl sulphate (R_F 0·77 in solvent *E*), whereas with phenol the metabolites in the urine were phenyl glucuronide (R_F 0·41; 53%), phenyl sulphate (R_F 0·77; 36%) and a third metabolite, possibly a quinol derivative (R_F 0·21; 11% of the ^{14}C radioactivity in urine). The [^{14}C]toluene used

Table 3. *pK_a values and distribution ratios of some compounds between organic solvents and phosphate buffer*, pH 7·4

0·1 M-Phosphate buffer, pH 7·4, was used with chloroform or ethyl acetate. *C* means concentration in the solvent indicated by the subscript (EtAc refers to ethyl acetate).

Compound	pK_a*	C_{CHCl_3}/C_{buffer}	C_{EtAc}/C_{buffer}
[^{14}C]Aniline	4·6	0·41	1·6
[^{14}C]Phenol	10·0	—	—
[^{14}C]Quinol	9·9, 12·0	—	—
[^{14}C]Benzoic acid	4·2	0·0004	0·0008
[^{14}C]Hippuric acid	3·7	<0·0001	0·0001
Salicylic acid	3·0, 12·4	—	—
4-Nitrobenzoic acid	3·4	—	—
2-Aminophenol	4·7, 9·7	0·23	2·1
2-Aminophenyl glucuronide	3·3	0·005	0·011
3-Aminophenol	4·2, 9·9	0·03	3·2
3-Aminophenyl sulphate	4·1	<0·0003	0·0008
4-Aminophenol	5·5, 10·3	0·15	1·0
4-Aminophenyl glucuronide	3·1	<0·0001	<0·0001
2-Aminobenzoic acid	2·1, 5·0	<0·0001	0·0003
4-Aminobenzoic acid	2·4, 4·9	0·0004	0·0008
4-Acetamidobenzoic acid	4·3	<0·0001	<0·0001
4-Aminohippuric acid	4·0	0·002	0·015
4-Acetamidohippuric acid	—	0·0004	0·0006
2-Aminobenzenesulphonic acid	— 2·5	0·0003	<0·0001
3-Aminobenzenesulphonic acid	0·4, 3·7	<0·0001	<0·0001
4-Aminobenzenesulphonic acid	1·0, 3·2	<0·0001	0·0004

* Values taken from the literature.

Table 4. *Effect of ligation of the renal pedicles on biliary excretion*

Female rats were prepared as described in the text. Compounds were administered intraperitoneally as described in Table 2. The values quoted are the means for three rats with ranges in parentheses.

		% of dose excreted in bile in 3 hr.	
Compound administered	Dose (mg./kg.)	With renal pedicles tied	With renal pedicles not tied
[^{14}C]Benzoic acid	92	5·1 (4·6–5·4)	0·8 (0·6–1·1)
4-Aminohippuric acid	134	10·0 (4·1–21·0)	1·4 (1·3–1·6)
3-Aminophenyl sulphate	100	1·0 (0·5–1·5)	0·2 (0·1–0·3)
4-Acetamidohippuric acid	150	9·0 (3·9–12·0)	1·3 (0·6–1·9)
4-Aminophenyl glucuronide	100	0·2 (0·05–0·5)	0·1 (0·05–0·15)

was of very low specific activity, and it was only possible to say that less than 2% of the ^{14}C radioactivity of the administered toluene appeared in the bile (see Table 2).

Another point that Table 2 brings out is that the metabolites of most of the compounds studied that appear in the bile are conjugates and that little of the original compounds is found in the bile. Only for the strongly acidic salicylic acid is the compound administered the only substance excreted in the bile.

The general conclusions that can be drawn from Table 2 is that, in the rat, compounds of low molecular weight, i.e. less than 300, are poorly excreted in the bile and that the metabolites derived from these appearing in the bile are usually acidic conjugates.

Table 2 contains several acidic conjugates: three glycine conjugates, one glucuronide, two acetyl derivatives and one sulphate. These compounds are poorly lipid-soluble at pH 7·4 (see Table 3). It will be noted that all the conjugates with molecular weight below 300 are poorly excreted in the bile (0–2%) in the unchanged form, except 4-aminohippuric acid, which is further conjugated by acetylation to 4-acetamidohippuric acid but is still poorly excreted (about 1%).

A possible reason why compounds of small

molecular weight such as benzoic acid are poorly excreted in the bile is that they could be rapidly cleared by the kidney. To test this possibility, the biliary excretion of five compounds poorly excreted in the bile was examined in rats in which the renal pedicles had been tied so that clearance by the kidney was prevented. The results, shown in Table 4, indicate that when clearance by the kidney is prevented there is an increased biliary excretion, but that it is still below a mean value of 10% of the dose. This finding is interpreted as meaning that rapid clearance by the kidney does not necessarily explain the poor biliary excretion of these compounds and that other factors such as ability to penetrate into the liver may be involved.

One reason for the low biliary excretion of a compound may be that it has difficulty in entering the liver from the blood. For 4-aminophenyl glucuronide, which is 99·99% ionized as the anion at pH 7·4 and almost insoluble in organic solvents at this pH (see Table 3), distribution studies have shown that this compound does not readily enter the liver in the rat (M. M. Abou-El-Makarem, P. Millburn, R. L. Smith & R. T. Williams, unpublished work). In such studies, 4-aminophenyl glucuronide (100mg./kg. intraperitoneally) was administered to biliary-cannulated rats with ligated renal pedicles, and the concentrations of the compound in plasma, liver and bile were measured at various times. At all times the plasma concentration of 4-aminophenyl glucuronide was four to six times the liver concentration, whereas the bile concentration was of the same order as that of the liver. 4-Aminophenyl glucuronide therefore does not readily enter the liver of the rat, and this may explain its low biliary excretion (0·1%) compared with that of 4-aminophenol (7·6%), which is also excreted in the bile as 4-aminophenyl glucuronide. 4-Aminophenol, which is only 1% ionized at pH 7·4 and is soluble in organic solvents at this pH, can presumably readily enter the rat liver, where it is metabolized to its *O*-glucuronide before excretion in the bile. The low biliary excretion of 3-aminophenyl sulphate may also be partly explained in this way, for distribution studies, similar to the above, have shown that this sulphate has difficulty in entering the liver in the rat, since its concentration in the plasma is greater than in the liver

The findings described in this paper are examined further in conjunction with other data in the succeeding paper (Millburn, Smith & Williams, 1967).

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