Biliary Excretion of Foreign Compounds

SPECIES DIFFERENCES IN BILIARY EXCRETION

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(Received 21 June 1967)

1. The biliary excretion of injected [^{14}C]aniline, [^{14}C]benzoic acid, 4-aminohippuric acid and 4-acetamidohippuric acid in six or eight species of animal (rat, dog, hen, cat, rabbit, guinea pig, rhesus monkey and sheep) was studied. 2. These compounds, with molecular weights in the range 93–236, are poorly excreted in the bile in all the species examined and, in effect, there is little significant species difference in the extent of their biliary excretion. 3. Compounds of higher molecular weight (355–495) were also studied, namely succinylsulphathiazole, [^{14}C]stilboestrol glucuronide, sulphadimethoxine N^1 -glucuronide and phenolphthalein glucuronide. 4. With these compounds a clear species difference in the extent of biliary excretion was found, the rat, dog and hen being good excretors, the rabbit, guinea pig and monkey poor excretors, and the cat and sheep taking an intermediary position. 5. There was a general trend for biliary excretion to be higher in all species when the compounds were of higher molecular weight. 6. These results are discussed in their relation to species differences in drug metabolism.

In the preceding papers (Abou-El-Makarem, Millburn, Smith & Williams, 1967*b*; Millburn, Smith & Williams, 1967*a,b*) some of the factors that affect the extent of biliary excretion of foreign compounds in the rat were elucidated. It was shown that a minimum molecular weight and the possession of a strongly polar anionic group were important factors in appreciable or extensive biliary excretion. A compound may possess these properties as such or may acquire them by metabolism. However, the pattern of biliary excretion in the rat may, or may not, apply to other species. Therefore we examined the extent of biliary excretion of eight selected compounds, namely aniline (mol.wt. 93), benzoic acid (mol.wt. 122), 4-aminohippuric acid (mol.wt. 194), 4-acetamidohippuric acid (mol.wt. 236), succinylsulphathiazole (mol.wt. 355), stilboestrol glucuronide (mol.wt. 445), sulphadimethoxine N^1 -glucuronide (mol.wt. 487) and phenolphthalein glucuronide (mol.wt. 495), in eight species of animals, namely cat, dog, guinea pig, hen, monkey (rhesus), sheep, rabbit and rat. The first three compounds are of low molecular weight and are extensively metabolized, but their biliary excretion is low in the rat (Abou-El-Makarem *et al.* 1967*b*). 4-Acetamidohippuric acid is a polar conjugate of low molecular weight that is not metabolized and is poorly excreted in the bile in the rat (Abou-El-Makarem *et al.* 1967*b*). The last four compounds are of relatively high

molecular weight and on injection into rats are excreted in the bile in appreciable amounts (Millburn *et al.* 1967*a,b*). It is shown below that the dog and hen are similar to the rat, but that the cat and sheep, and the rabbit, guinea pig and rhesus monkey are different from the rat in the extent of biliary excretion of foreign compounds.

MATERIALS AND METHODS

Chemicals. [carboxy- ^{14}C]Benzoic acid (100 $\mu\text{C}/\text{mg.}$), [U- ^{14}C]aniline hydrogen sulphate (194 $\mu\text{C}/\text{mg.}$) (both from The Radiochemical Centre, Amersham, Bucks.), 4-aminohippuric acid, m.p. 197°, phenolphthalein glucuronide sodium salt (both from Koch-Light Laboratories Ltd., Colnbrook, Bucks.) and succinylsulphathiazole, m.p. 186–188° (May and Baker Ltd., Dagenham, Essex), were purchased. 4-Acetamidohippuric acid, m.p. 227°, was prepared from 4-aminohippuric acid, sulphadimethoxine N^1 -glucuronide was synthesized by the method of Bridges, Kibby & Williams (1965) and [monoethyl- ^{14}C]stilboestrol glucuronide sodium salt was prepared by Dr L. J. Fischer (Fischer, Millburn, Smith & Williams, 1966).

Preparation of animals with biliary fistulae. Details of the body weights of the animals used, the dose level of anaesthetic and the sizes of the polythene cannulae used in these experiments are shown in Table 1.

The biliary-cannulation procedure was as follows. The abdominal cavity of the animal was opened under anaesthesia and the bile duct cannulated with the appropriate size polythene or rubber tubing. The cystic duct was tied off to ensure that hepatic bile only was collected. For the

Table 1. *Species, body weights, dose of anaesthetic and size of cannula*

i.p., Intraperitoneal; i.v., intravenous; i.m., intramuscular.

Species	Body wt. (kg.)	Dose of pentobarbital (mg./kg.)	Diameter of cannula (mm.)	
			Internal	External
Rat, female (Wistar albino)	0.2 ± 0.02	70 (i.p.)	0.4	0.8
Guinea pig, female (English)	0.75 ± 0.25	40 (i.p.)	1.14	1.57
Rabbit, female (New Zealand White)	2.5 ± 0.5	40 (i.v.)	1.0	2.0
Cat, female (domestic)	3.0 ± 0.3	40 (i.p.)	1.0	2.0
Dog, female (mongrel)	7.0 ± 1.5	30 (i.v.)	1.5	2.7
Hen, female (Light Sussex)	2.0 ± 0.2	50 (i.m.)	1.55	1.9
Rhesus monkey, male and female (Bengal)	4.0 ± 0.1	40 (i.v.)	1.14	1.57
Sheep, male and female (Kent)	25 ± 10	30 (i.v.)	7.0	11.0

Table 2. *Biliary excretion of [¹⁴C]benzoic acid in various species*

Females were used. The dose of [¹⁴C]benzoic acid was 50 mg./kg. (10 µC/kg.) injected as the sodium salt in 1–5 ml. of water. Bile was collected for 3 hr. For determination of metabolites the bile samples were chromatographed in an appropriate solvent (see the text) and the radioactivity associated with each metabolite was determined by scintillation counting. i.p., Intraperitoneal; i.v., intravenous.

Metabolites in bile as % of ¹⁴C of bile†

Species	Route of administration	% of dose of ¹⁴ C in bile*	Metabolites in bile as % of ¹⁴ C of bile†			
			Benzoic acid	Hippuric acid	Benzoyl glucuronide	Ornithuric acid
Rat	i.p.	1.2 (1.1–1.4)	7	80	13	0
Guinea pig	i.p.	1.7 (1.5–2.0)	1	98	1	0
Rabbit	i.v.	0.7 (0.7–0.8)	1	99	1	0
Dog	i.v.	0.8 (0.8–0.8)	59	8	33	0
Cat	i.v.	1.2 (0.8–1.7)	15	68	17	0
Hen	i.p.	0.5 (0.3–0.8)	20	0	< 1	80

* Average values for three animals, with the ranges in parentheses.

† Values for samples of pooled bile from three animals.

chicken, which has two bile ducts, both were cannulated. The abdominal cavity and skin incision of operated animals were closed and warming blankets or a heated operating table were used to help maintain body temperature throughout the experiment. Bile was collected for 3 hr., during which time the animal was kept anaesthetized.

Analytical methods. Bile samples were analysed colorimetrically, chromatographically and radiochemically according to the methods described for the compounds listed above in the preceding papers (Abou-El-Makarem *et al.* 1967b; Millburn *et al.* 1967a,b). The only compound used here whose properties were not described in the above papers is ornithuric acid, which was found to be a major metabolite of benzoic acid in hen bile. The L-(+)-ornithuric acid used had m.p. 182° and was a sample previously prepared in the Laboratory (Baldwin, Robinson & Williams, 1960). It is readily distinguished chromatographically from benzoic acid, hippuric acid and benzoyl glucuronide by its R_F value in a solvent system consisting of butan-1-ol saturated with a mixture of equal volumes of 1.5N-NH₃ and 1.5M-(NH₄)₂CO₃ (Fewster & Hall, 1951). With chromatography on Whatman no. 1 paper for 12–14 hr. by the descending technique, the R_F values found were: ornithuric acid, 0.83; benzoic acid, 0.49; hippuric acid, 0.35; benzoyl glucuronide, 0.15. Ornithuric acid was

located on the paper by u.v. light, under which it appeared as a dark spot, and by its radioactivity when [¹⁴C]benzoic acid was administered.

RESULTS AND DISCUSSION

Compounds of low molecular weight

The extent of biliary excretion, in six species, of compounds of low molecular weight, i.e. benzoic acid (mol.wt. 122), aniline (mol.wt. 93), 4-aminohippuric acid (mol.wt. 194) and 4-acetamidohippuric acid (mol.wt. 236), are shown in Tables 2, 3, 4 and 5. The Tables show that these compounds are poorly excreted in the bile in all six species (eight species for 4-acetamidohippuric acid). The extent of biliary excretion of benzoic acid is less than 2% of the dose (Table 2) in the six species, and the only differences between the species are in the nature of this small amount of metabolites in the bile. In the rat, guinea pig, rabbit and cat the main metabolite is hippuric acid, in the dog benzoic acid and benzoyl glucuronide, and in the hen ornithuric acid.

Table 3. *Biliary excretion of [¹⁴C]aniline in various species*

Females were used. [¹⁴C]Aniline (20 mg./kg.; 5 μ C/kg.) as the hydrogen sulphate was injected in 1–5 ml. of water. Bile was collected for 3 hr. The metabolites were identified chromatographically. i.p., Intraperitoneal; i.v., intravenous.

Species	Route of administration	% of dose of ¹⁴ C in bile*	Metabolites detected in bile
Rat	i.p.	5.7 (4.3–8.1)	4-Aminophenol and its glucuronide (major), 2-aminophenol and its glucuronide (minor)
Guinea pig	i.p.	5.6 (4.7–7.4)	
Rabbit	i.v.	2.6 (2.2–3.3)	
Hen	i.p.	2.7 (2.2–3.3)	2- and 4-Aminophenol and glucuronides 2-Aminophenol only.
Dog	i.v.	0.3 (0.3–0.3)	
Cat	i.v.	1.6 (1.2–1.9)	

* Mean values for three animals, with the ranges in parentheses.

Table 4. *Biliary excretion of 4-aminohippuric acid in various species*

The acid (134 mg./kg.) was injected as the sodium salt in 1–5 ml. of water. Free and acetylated 4-aminohippuric acid were determined as given in the text. Bile was collected for 3 hr. after dosing. i.p., Intraperitoneal; i.v., intravenous.

Species	Route of administration	% of dose in bile*	% of material in bile*	
			Free	Acetylated
Rat	i.p.	1.4 (1.3–1.6)	14 (10–18)	86 (82–90)
	i.v.	3.3 (2.4–4.1)	—	—
Guinea pig	i.p.	6.7 (6.1–6.9)	7 (4–12)	93 (88–96)
Rabbit	i.p.	3.0 (2.2–3.6)	8 (7–10)	92 (90–93)
Dog	i.v.	3.4 (1.8–6.3)	100	0
Cat	i.p.	0.7 (0.5–0.9)	42 (40–45)	58 (55–60)
Hen	i.p.	0.5 (0.3–0.6)	95 (93–97)	5 (0–7)

* Mean values for three animals, with the ranges in parentheses.

The biliary excretion of aniline (Table 3) is somewhat higher than that of benzoic acid. In the rat, guinea pig, rabbit and hen the excretion is 3–6% and the main metabolites in the bile are 4-aminophenol and its glucuronide (mol.wt. 285) with small amounts of the 2-isomer. In the dog and cat the output in the bile is much lower, the cat producing only unconjugated 2-aminophenol and the dog both 2- and 4-aminophenol and their glucuronides.

In Table 4, the results for a conjugate, 4-aminohippuric acid, which can be further conjugated, are shown. The extent of biliary excretion is similar (1–7%) in four species, i.e. rat, guinea pig, rabbit and dog, and rather lower (0.5 and 0.7%) in the hen and cat. The acid is, in effect, poorly excreted in the bile of all six species. In the rat, guinea pig and rabbit the main metabolite in the bile is 4-acetamidohippuric acid; in the dog 4-aminohippuric acid is excreted in the bile unchanged and this is not unexpected, since the dog is unable to acetylate aromatic amino groups; the cat excretes roughly equal amounts of the free and acetylated compound; the hen produces mainly the unchanged

acid with a small amount (5%) of the acetylated acid.

Table 5 shows that the conjugate, 4-acetamidohippuric acid, is excreted unchanged in the bile at a low level (0.4–4% of the dose) in eight species, which now include the rhesus monkey and the sheep.

From these Tables it can be concluded that the compounds of small molecular weight, whether they are polar or can undergo conjugation to form polar compounds, are poorly excreted in the bile in all species examined. It appears that there is little significant species difference in the extent of biliary excretion of these compounds although there may be a species difference in the nature of the metabolites in the bile.

Compounds of higher molecular weight

The results for the biliary excretion of four compounds, succinylsulphathiazole (mol.wt. 355), stilboestrol glucuronide (mol.wt. 445), sulphadimethoxine *N*¹-glucuronide (mol.wt. 487) and phenolphthalein glucuronide (mol.wt. 495), in

eight species are shown in Table 6. The compounds were chosen for study because they are not metabolized to any great extent and are excreted in the bile mainly unchanged. In the rat, as already shown by Millburn *et al.* (1967*a,b*), these compounds are excreted in the bile in large amounts. Table 6 shows that there is a distinct species difference in

the extent of biliary excretion of these compounds. There is, however, a general trend in that, in all the species examined, biliary excretion tends to increase with increasing molecular weight, although this is obviously not the only factor involved. Let us examine each of the four compounds separately.

Succinylsulphathiazole. This is a polar compound of molecular weight 355 which is excreted in the bile unchanged. Table 6 shows that, as far as this compound is concerned, the eight species examined can be divided into three groups. The first group contains the rat, dog and hen, in which the biliary excretion is about 10–15% of the dose when given intraperitoneally and 20–30% when given intravenously. The second group contains the cat and sheep, in which the biliary excretion is 6–7%. The third group contains the rabbit, guinea pig and rhesus monkey, in which the excretion is less than about 1% of the dose. The material excreted in the bile in all the species is the same, namely unchanged succinylsulphathiazole.

[¹⁴C]Stilboestrol glucuronide. This compound has a higher molecular weight (445) than the preceding compound and the species can be divided into two groups. The rat, dog, hen and cat excrete this compound in large amounts (60–100%) in the bile, whereas the guinea pig and rabbit excrete 20–30%. It is to be noted that, though the guinea pig and rabbit excrete appreciable amounts of this com-

Table 5. *Biliary excretion of 4-acetamidohippuric acid in various species*

The compound (150 mg./kg.) was administered to biliary-cannulated female animals unless otherwise indicated (m, male; f, female) as the sodium salt dissolved in 1–5 ml. of water. Bile was collected for 3 hr. and analysed. The results are expressed as means for three animals, with the ranges in parentheses, except for the sheep and the monkey, where two animals were used. i.p., Intraperitoneal; i.v., intravenous.

Species	Route of administration	% of dose in bile in 3 hr.
Rat	i.p.	1.3 (0.6–1.9)
Dog	i.p.	3.5 (2.0–5.0)
Hen	i.p.	0.4 (0.2–0.6)
Cat	i.p.	0.9 (0.7–1.1)
Sheep	i.v.	3.8 (m), 4.6 (f)
Rabbit	i.p.	1.5 (1.3–1.8)
Guinea pig	i.p.	0.4 (0.2–0.7)
Monkey	i.v.	1.2 (m), 3.4 (f)

Table 6. *Biliary excretion in various species of some polar compounds of molecular weights in the range 350–500*

The animals used were females unless otherwise indicated (m, male; f, female). Compounds were injected as sodium salts (ammonium salt for sulphadimethoxine glucuronide) in 1–5 ml. of water. Results are expressed as mean values for three animals, with the ranges in parentheses, except for sheep and monkeys, where two animals were used. i.p., Intraperitoneal; i.v., intravenous.

Compound		Succinylsulphathiazole	[¹⁴ C]Stilboestrol glucuronide	Sulphadimethoxine N ¹ -glucuronide	Phenolphthalein glucuronide
Mol.wt.		355	445	487	495
Dose (mg./kg.)		20	10	15	10
Species	Route of administration	% of dose in bile in 3 hr.			
Rat (Wistar albino)	i.p.	14 (10–17)	—	43 (37–50)	54 (47–61)
	i.v.	29 (23–39)	95 (82–102)	—	—
Dog (mongrel)	i.p.	11 (10–12)	—	34 (one animal)	—
	i.v.	20 (19–22)	65 (63–67)	43 (41–44)	81 (67–95)
Hen (Light Sussex)	i.p.	10 (8–13)	—	—	—
	i.v.	25 (18–32)	93 (90–97)	—	71 (62–82)
Cat (domestic)	i.p.	6 (4–8)	—	—	—
	i.v.	7 (4–10)	77 (71–82)	—	34 (25–43)
Sheep (Kent)	i.v.	7, 16	—	—	32 (m), 43 (f)
Rabbit (New Zealand White)	i.p.	0.8 (0.5–1.1)	—	9 (8–10)	—
	i.v.	1.1 (0.8–1.4)	32 (24–40)	10 (7–13)	13 (10–16)
Guinea pig (English)	i.p.	0.2 (0.1–0.3)	—	12 (10–14)	6 (5–7)
	i.v.	0.9 (0.3–1.4)	20 (11–38)	—	—
Rhesus monkey (Bengal)	i.v.	0.2, 0.3	—	—	5 (f), 13 (m)

pound in the bile, these amounts are only about one-third to one-quarter of those excreted by the other four animals. In all the species, except the dog and cat, stilboestrol glucuronide is excreted in the bile largely in the unchanged state.

Sulphadimethoxine N¹-glucuronide. This glucuronide (mol.wt. 487) was examined in four species only since its supply was limited. Again the rat and dog, in which biliary excretion was about 40%, can be distinguished from the rabbit and guinea pig, in which the excretion was about 10%.

Phenolphthalein glucuronide. Of the four compounds examined, this one had the highest molecular weight (495). Again, the eight species can be divided into three groups, as for succinylsulphathiazole. In the rat, dog and hen the biliary excretion is about 50–90%, in the cat and sheep about 30% and in the rabbit, guinea pig and rhesus monkey about 5–15%.

The results obtained (Tables 2–6) suggest that compounds of low molecular weight are poorly excreted in the bile in all the species examined, but, when the molecular weight of a compound reaches or exceeds a minimum value, which is about 325 ± 50 , or when such a value can be attained by a metabolic change, then biliary excretion becomes appreciable and its extent depends on species. Of the eight species examined, it appears that they can be divided into three groups. The good biliary excretors of compounds of higher molecular weight are the rat, dog and hen, and the relatively poor biliary excretors are the rabbit, guinea pig and rhesus monkey. There is an intermediary group that are neither good nor poor biliary excretors, and the cat and sheep are in this group. The reasons for these species differences in the extent of biliary excretion are now being examined and a preliminary report has been published (Abou-El-Makarem, Millburn, Smith & Williams, 1967a).

Species differences in the metabolism of foreign compounds frequently occur and are of considerable importance in the testing of the safety and activity

of drugs, food additives, pesticides etc. for use by man. The choice of chemicals for safe use in man depends largely on tests in animals. Some of the reasons for species differences in drug metabolism, especially those of an enzymic nature, have been discussed by Williams (1967). Differences in biliary excretion can also be an important reason for species differences in drug metabolism, for, in those species in which biliary excretion is extensive, high faecal excretion, enterohepatic circulation and metabolism by gut bacteria can be contributory causes of differences in drug metabolism (Smith, 1966).

This work was in part supported by grants from Ciba Laboratories Ltd., Horsham, Sussex, and Lilly Research Laboratories Ltd., Bromborough Port, Cheshire. M.M. A.-E.-M. participated during the tenure of a scholarship from the U.A.R. Education Bureau. We are grateful to the Medical Research Council and the National Institutes of Health, Bethesda, Md., U.S.A., for grants to purchase equipment.

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