Molecular Weight as a Factor in the Excretion of Monoquaternary Ammonium Cations in the Bile of the Rat, Rabbit and Guinea Pig

By ROBIN D. HUGHES, PETER MILLBURN and R. TECWYN WILLIAMS
Department of Biochemistry, St. Mary's Hospital Medical School, London W2 1PG, U.K.

(Received 13 July 1973)

1. The excretion in the bile and urine of intraperitoneally injected ^{14}C -labelled monoquaternary ammonium or pyridinium cations was measured in bile-duct-cannulated rats (ten compounds) and in guinea pigs and rabbits (six compounds). 2. Seven of these, namely *N*-methylpyridinium, tetraethylammonium, trimethylphenylammonium, diethylmethylphenylammonium, methylphenyldipropylammonium, dibenzyltrimethylammonium and tribenzylmethylammonium, were excreted largely unchanged in the bile and urine. 3. 3-Hydroxyphenyltrimethylammonium, 3-bromo-*N*-methylpyridinium and cetyltrimethylammonium were metabolized to an appreciable extent in the rat. 4. In intact rats intraperitoneally injected trimethylphenylammonium (mol.wt. 136) was excreted mainly in the urine, dibenzyltrimethylammonium (mol.wt. 226) was excreted in roughly equal amounts in the urine and faeces, and tribenzylmethylammonium (mol.wt. 302) was excreted mainly in the faeces. The faecal excretion of these compounds corresponded to their biliary excretion in bile-duct-cannulated rats. About 3–4% of tribenzyl[^{14}C]methylammonium was eliminated as $^{14}\text{CO}_2$. 5. In rats the extent of biliary excretion of four cations with molecular weights in the range 94–164 was less than 10% of the dose, whereas that of five cations with molecular weights 173–302 was greater than 10%. These results and other data from the literature suggested that the molecular weight needed for the biliary excretion of such cations to an extent of 10% or more of the dose was about 200 ± 50 . Studies with six cations in guinea pigs and rabbits suggest that this value applies also to these species. 6. The results suggest that the threshold molecular weight for the appreciable (>10%) biliary excretion of monoquaternary cations is different from that for anions (Millburn *et al.*, 1967a; Hirom *et al.*, 1972b). With rats, guinea pigs and rabbits, no significant species difference was noted, whereas with anions there is a marked species difference.

It has been shown that the extent to which organic anions are excreted in bile is influenced by physico-chemical factors such as molecular weight, polarity, molecular structure and lipid solubility (Millburn *et al.*, 1967a,b; Hirom *et al.*, 1972a) and by biological factors such as species, metabolic transformations (Abou-El-Makarem *et al.*, 1967b; Millburn *et al.*, 1967a; Hirom *et al.*, 1972b) and, in rats, sex (Gregson *et al.*, 1972; Bertagni *et al.*, 1972). However, relatively little is known of the influence, if any, of these factors on the elimination in the bile of organic cations (see Millburn, 1970; Smith, 1971).

The extent of biliary excretion of ten monoquaternary ammonium or pyridinium cations with molecular weights in the range 94–302 has therefore been examined in female rats, guinea pigs and rabbits. As with organic anions, molecular weight was found to have an effect and it appears that the molecular weight at which the biliary excretion of these cations becomes appreciable, that is 10% or more of the dose (see Hirom *et al.*, 1972b), is less than for anions, but species variation in this parameter does not appear to

occur to any great extent. The results obtained here, together with others recorded in the literature for rats, suggest a threshold value of about 200 ± 50 for the excretion in the bile of these cations to an extent of more than 10% of the dose. Studies with six of these cations in rats, guinea pigs and rabbits indicated that there is no marked species difference in the extent of their biliary excretion. This is in contrast with organic anions, which show a species variation in the threshold molecular weight for appreciable (more than 10% of dose) biliary excretion, which is about 325 ± 50 in the rat, 400 ± 50 in the guinea pig and 475 ± 50 in the rabbit (Millburn *et al.*, 1967a; Hirom *et al.*, 1972b).

Materials and Methods

Compounds

Methyl iodide (b.p. 42°C , redistilled and kept over copper wire), pyridine (b.p. $114\text{--}116^\circ\text{C}$), *NN*-dimethylaniline (b.p. $191\text{--}195^\circ\text{C}$), *NN*-diethylaniline

(b.p. 212–216°C), dibenzylamine (b.p. 300°C, decomp.), tribenzylamine (m.p. 91–92°C), tetraethylammonium bromide (m.p. 270°C, decomp.), cetyltrimethylammonium bromide (m.p. 235–240°C, after recrystallization from dry acetone) (all from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K.), 3-bromopyridine (b.p. 170°C; Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.) and *NN*-dipropylaniline (b.p. 245°C; Eastman Kodak Co., Rochester, N.Y., U.S.A.) were purchased and used without further purification unless otherwise stated.

Tetra[1-¹⁴C]ethylammonium bromide (14.3 μCi/mg) (New England Nuclear Corp., Boston, Mass., U.S.A.), [1-¹⁴C]methyl iodide (382 μCi/mg), 3-hydroxyphenyl[monomethyl-¹⁴C]trimethylammonium iodide (36.5 μCi/mg) and [1-¹⁴C]cetyltrimethylammonium bromide (41.2 μCi/mg) (The Radiochemical Centre, Amersham, Bucks., U.K.) were purchased.

[1-¹⁴C]Methyl-labelled quaternary ammonium compounds (Table 1) were synthesized by the direct addition of [1-¹⁴C]methyl iodide to the corresponding tertiary, or in one case secondary, amine dissolved in a suitable solvent. For each radiochemical synthesis approximately 150 μCi of [1-¹⁴C]methyl iodide in 2 ml of methanol was used. This solution was added to sufficient non-labelled methyl iodide to provide a 10% excess of methyl iodide in each reaction.

Preparations

The following compounds were prepared (for yields and melting points, see Table 1).

N-[1-¹⁴C]Methylpyridinium iodide. Pyridine (0.14 ml) was mixed with the above amount of [1-¹⁴C]methyl iodide solution, kept at 4°C for 24 h and then heated under reflux for 1 h. On cooling, *N*-[1-¹⁴C]methylpyridinium iodide separated as white needles. It was purified by dissolving in a little hot ethanol, and then adding ether to cloudiness, heating until clear and cooling. The hygroscopic iodide was kept in a vacuum desiccator (cf. Prescott, 1896).

[monomethyl-¹⁴C]Trimethylphenylammonium iodide. Dimethylaniline (0.096 ml) was heated under reflux with [1-¹⁴C]methyl iodide solution for 1 h. After cooling, dry ether was added until no further precipitation of white crystals occurred. These were recrystallized from ethanol (cf. Pass & Ward, 1933).

Diethyl[1-¹⁴C]methylphenylammonium iodide. *NN*-Diethylaniline (0.16 ml) was mixed with [1-¹⁴C]methyl iodide solution, kept overnight at 4°C and then refluxed for 10 h. After cooling, ether was added and an oil separated, which formed crystals from dry acetone (cf. Claus & Howitz, 1884).

3-Bromo-*N*-[1-¹⁴C]methylpyridinium iodide. 3-Bromopyridine (0.2 ml) was mixed with [1-¹⁴C]methyl iodide solution and kept at 4°C for 48 h. The cream-

Table 1. Properties of some quaternary nitrogen compounds

No.	Compound	Yield on weight basis (%)	Specific radioactivity (μCi/mg)	Melting point* (°C)	Molecular weight	
					Found†	Calculated
1	<i>N</i> -Methylpyridinium iodide	32	0.26	114 (117)	225	221
3	Trimethylphenylammonium iodide	53	0.28	220 (decomp.) (228)	263	263
5	Diethylmethylphenylammonium iodide	37	0.49	103 (102)	297	291
6	3-Bromo- <i>N</i> -methylpyridinium iodide	17	0.19	158–161 (164–165)	306	300
7	Methylphenyldipropylammonium iodide	13	0.41	162–165 (156)	326	319
8	Dibenzylmethylammonium iodide	12	0.20	189–191 (191)	353	353
10	Tribenzylmethylammonium iodide	13	0.31	184 (184)	431	429

* Literature values are given in parentheses and the references are in the text.

† Determined by the Volhard method (see Vogel, 1961) for the iodide content of non-labelled samples.

coloured crystals which separated were recrystallized from ethanol (cf. McElvain & Goese, 1943).

[^{14}C]Methylphenyldipropylammonium iodide. *NN*-Dipropylaniline (0.2ml) mixed with [^{14}C]methyl iodide solution was kept at room temperature for 48h and then refluxed for 24h. After cooling, dry ether was added and the oil which separated was crystallized from ethanol-ether (cf. Jones, 1903).

Dibenzyl[^{14}C]methylammonium iodide. Dibenzylamine (0.33ml) mixed with [^{14}C]methyl iodide solution was kept overnight at 4°C and then refluxed for 1h. The methanol solvent was removed under reduced pressure and the yellow residue washed with water (3ml) and then ether (10ml). The dibenzyl[^{14}C]methylammonium iodide was separated from the more soluble dibenzylamine hydriodide by crystallization from ethanol (cf. Emde, 1909).

Tribenzyl[^{14}C]methylammonium iodide. Tribenzylamine (0.288g) and [^{14}C]methyl iodide solution were heated in a sealed tube at 100°C for 4h. On cooling the tribenzyl[^{14}C]methylammonium iodide was precipitated with dry ether, washed with ether and recrystallized from water (cf. Marquardt, 1886).

Non-radioactive preparations. Unlabelled samples of all the above compounds for chromatography and isotope-dilution procedures were prepared similarly.

Each labelled compound ran as one spot on paper chromatograms and their R_F values and the colour test used for detection are given in Table 2.

Animals

Female Wistar albino rats (200–250g body wt.),

Dunkin Hartley albino guinea pigs (350–800g), English guinea pigs (250–450g) and Dutch rabbits (1.5–3.0kg) were used. They were anaesthetized by intraperitoneal injection of thiopentone sodium (Pentothal; Abbott Laboratories Ltd., Queenborough, Kent, U.K.; 70mg/kg for rats and 50mg/kg for guinea pigs) or intravenous injection of pentobarbitone sodium (Nembutal; Abbott Laboratories Ltd.; 30mg/kg for rabbits). The common bile duct was cannulated as described by Abou-El-Makarem *et al.* (1967b) and in the guinea pig and rabbit the cystic duct was ligated to ensure that only hepatic bile was collected. The cannulated rats were kept for 24h, bile and urine being collected as described by Abou-El-Makarem *et al.* (1967a). The cannulated guinea pigs and rabbits were kept anaesthetized for the duration (3h) of bile collection by the injection of small amounts of anaesthetic when necessary, and then killed at the end of the experiment with an overdose of the barbiturate. The abdominal cavity was opened and the urine removed from the bladder by a syringe. In some experiments with rats the excretion of urine was prevented, and they were anaesthetized while the bile was collected (3h). In these animals the common bile duct was cannulated (Abou-El-Makarem *et al.*, 1967a) and both renal pedicles (i.e. renal artery, vein and ureter) were ligated to prevent urine formation.

The urinary and faecal excretion of ^{14}C -labelled compounds in intact rats was studied for a period of 3 days. The compounds were injected intraperitoneally and the animals kept in metabolism cages (Metabowl; Jencons (Scientific) Ltd., Hemel Hempstead, Herts., U.K.), which permitted the urine,

Table 2. R_F values of some monoquaternary organic compounds

Descending chromatography on Whatman no. 1 paper was used. Solvent systems (proportions by vol.) were: A, butan-1-ol-acetic acid-water (4:1:2); B, propan-1-ol-aq. NH_3 (sp.gr. 0.88) (7:3); C, ethanol-aq. NH_3 (sp.gr. 0.88) (19:1). Chromatograms were run for 12–18h at room temperature. Iodoplatinate reagent was used to detect the cations on paper, which was sprayed with a mixture of chloroplatinic acid (5%, w/v, in water) (5ml), KI solution (10%, w/v, in water) (45ml) and water (100ml) (Smith, 1969). The colours obtained were on a pink background.

No.	Compound	Solvent ...	R_F			Colour with iodoplatinate
			A	B	C	
1	<i>N</i> -Methylpyridinium iodide		0.45	0.34	0.62	Grey
6	3-Bromo- <i>N</i> -methylpyridinium iodide		0.56	0.36	0.29	Dark grey
2	Tetraethylammonium bromide		0.62	0.72	0.66	Purple
9	Cetyltrimethylammonium bromide		0.93	0.88	0.84	Dark red
4	3-Hydroxyphenyltrimethylammonium iodide*		0.68	—	—	—
3	Trimethylphenylammonium iodide		0.63	0.51	0.69	Blue-grey
5	Diethylmethylphenylammonium iodide		0.76	0.79	0.77	Blue-grey
7	Methylphenyldipropylammonium iodide		0.87	0.83	0.79	Dark red
8	Dibenzylmethylammonium iodide		0.87	0.80	0.71	Blue-grey
10	Tribenzylmethylammonium iodide		0.92	0.88	0.83	Faint red

* This compound had R_F 0.56 in solvent D (butan-1-ol-water-ethanol-acetic acid, 32:12:8:1, by vol.) (see Somani *et al.*, 1970).

faeces and expired CO₂ to be collected daily. The CO₂ was trapped in 400ml of either 4M-NaOH (cations nos. 3 and 10; see Table 6) or a solution of ethanolamine (33%, v/v) in ethylene glycol monomethylether (cation no. 8).

Measurement of radioactivity

The ¹⁴C in bile (0.1–0.5ml), urine (0.1–1.0ml) and aqueous faecal-homogenate (0.2ml) samples was determined with a Packard Tri-Carb liquid-scintillation spectrometer (model 3214 or 3320) by using a dioxan-based scintillation fluid (see Bridges *et al.*, 1967). The samples from the collection of ¹⁴CO₂ (see above) were counted for radioactivity as follows. A portion (20ml) of the NaOH solution used to trap the ¹⁴CO₂ was added to 25ml of saturated BaCl₂ solution. The precipitated Ba¹⁴CO₃ was filtered off, dried and a 0.1 g sample counted by using the dioxan-based scintillator. In those experiments where the ethanolamine solution was used to trap the ¹⁴CO₂, a portion (2.0ml) of this was counted in a toluene scintillator as described by Jeffay & Alvarez (1961). Bile (0.01–0.2ml) or urine (0.01–0.5ml) was chromatographed on thin strips (5cm wide) of Whatman no. 1 paper and the chromatograms were scanned in a Packard radiochromatogram scanner (model 7200). The *R_f* values of the ¹⁴C peaks were compared with those of authentic reference compounds. In some experiments the chromatograms were cut into small pieces (1–2.5cm by 5cm) and counted in the Packard liquid-scintillation spectrometer as described above.

Detection of glucuronic acid conjugates

Glucuronides on paper chromatograms were detected as blue spots by a spray of naphtharesorcinol (see Capel *et al.*, 1972). Bile and urine samples (0.5ml), adjusted to pH5 with 0.2M-sodium acetate buffer, were incubated at 37° for 12–18h with an equal volume of Ketodase (ox liver β-glucuronidase preparation; Warner-Chilcott Laboratories, Eastleigh, Hants., U.K.). Samples (0.01–0.5ml) of this hydrolysed bile or urine were then chromatographed as described above. Control samples were prepared containing sufficient saccharo-1,4-lactone [Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.] to inhibit the β-glucuronidase.

Reverse isotope-dilution analysis

The non-radioactive quaternary ammonium compound (0.5–1.0g) was added to bile (1–2ml) or urine (10ml), and the mixture dissolved in hot ethanol (50ml) and then filtered. The filtrate was evaporated to dryness under reduced pressure on the rotary evaporator. The residue was then recrystallized to constant specific radioactivity by using the appro-

priate solvent (see above under 'Compounds'). Samples (5 or 10mg) of recrystallized material were added to dioxan scintillator fluid (15–20ml), which turned yellow. Saturated sodium thiosulphate solution was added dropwise to the yellow solution until the scintillator became colourless. (Failure to take up the free iodine with sodium thiosulphate gave samples with poor counting efficiency.) The ¹⁴C was then determined as described above. In some cases the compounds were poorly soluble in the dioxan scintillator and were therefore dissolved in 1–2ml of water before being added to the scintillator.

Results

Biliary excretion in rats

Effect of molecular weight. The extent of excretion in the bile and urine of ten monoquaternary ammonium or pyridinium cations with molecular weights in the range 94–302 by bile-duct-cannulated female rats is shown in Table 3 (in this table the cations are numbered and are referred to in the text by number). These compounds were administered intraperitoneally at a dose of 15mg/kg, except cations nos. 4 and 10, which were administered at 0.6mg/kg (intravenously) and 10mg/kg respectively. They were found to be less toxic when given intraperitoneally than when given intravenously and at the doses used the cannulated animals showed no overt toxic symptoms. To check that these cations did not interfere with the ability of the liver and kidney to excrete foreign compounds, the effect of trimethylphenylammonium (no. 3) and tribenzylmethylammonium (no. 10) on the biliary and urinary excretion of tartrazine by bile-duct-cannulated female rats was examined. The results in Table 4 show that cations nos. 3 and 10 did not significantly (*P* > 0.05 by Student's *t* test) modify the extent of biliary or urinary excretion of tartrazine during a 3h period. Most of these highly polar compounds were excreted almost entirely unchanged, except cations nos. 4, 6 and 9, which are discussed below.

The first five compounds in Table 3 have molecular weights below 170 and it is to be noted that apart from cation no. 4 (see below) their biliary excretion is less than 10% of the dose in 24h. These compounds are largely (60–85%) excreted in the urine. The next five compounds (nos. 6–10) have molecular weights above 170 and their biliary excretion is greater than 10% of the dose, ranging from 15% for no. 6 (mol.wt. 173) to 51% for no. 10 (mol.wt. 302), and it is apparent that the extent of biliary excretion is increasing and that of urinary excretion decreasing with molecular weight. The total recovery in bile and urine in 24h of the injected ¹⁴C compounds is high, being in the range 67–86% of the dose for eight of the compounds. Cations nos. 8 (57%

Table 3. *Biliary and urinary excretion of some monoquaternary organic cations in the rat*

The ^{14}C -labelled compounds dissolved in water (5 mg/ml unless otherwise stated) were injected intraperitoneally into bile-duct-cannulated female Wistar albino rats. The dose was 15 mg/kg intraperitoneally except for no. 4, for which it was 0.6 mg/kg given intravenously, and no. 10 (10 mg/kg intraperitoneally). Bile and urine were collected for 24 h and analysed as described in the text. Results are mean values for three animals unless otherwise indicated in brackets, with ranges in parentheses.

No.	Compound*	Mol.wt. of cation	Dose (μmol of cation/kg)	^{14}C excreted in 24 h (% of dose)		
				Bile	Urine	Total
1	<i>N</i> -Methylpyridinium iodide	94	29	0.8 (0.6–1.0)	85 (70–93)	86 (71–94)
2	Tetraethylammonium bromide	130	44	0.5 (0.3–0.8)	72 (52–85)	73 (52–85)
3	Trimethylphenylammonium iodide	136	29	0.7 (0.5–0.8)	70 (63–84)	71 (64–85)
4	3-Hydroxyphenyltrimethylammonium iodide	152	1.1	Females 14 (7.8–17) [4] Males 11 (9.2–14) 7.6 (5.9–9.1)	55 (49–65) [4] 61 (56–65) 59 (45–73)	69 (57–79) [4] 72 (65–79) 67 (53–79)
5	Diethylmethylphenylammonium iodide	164	29	15 (3.2–22) [5]	60 (37–94) [5]	75 (59–97) [5]
6	3-Bromo- <i>N</i> -methylpyridinium iodide	173	29	17 (14–21) [5]	65 (60–70) [5]	82 (74–85) [5]
7	Methylphenyldipropylammonium iodide	192	28	36 (24–48) [6]	21 (13–29) [6]	57 (42–75) [6]
8	Dibenzyltrimethylammonium iodide†	226	27	36 (33–40)	12 (11–13)	48 (44–53)
9	Cetyltrimethylammonium bromide	285	32	51 (32–64) [5]	16 (6.5–31) [5]	67 (59–77) [5]
10	Tribenzylmethylammonium iodide‡	302	16			

* These compounds were excreted almost entirely unchanged except nos. 4, 6 and 9 (see the text).

† Concentration given was 3 mg/ml.

‡ Concentration given was 2 mg/ml.

recovery in 24h) and 9 (48%) were more slowly excreted.

Reverse isotope dilution and paper chromatography showed that cations nos. 2, 3, 5 and 7 were excreted in the bile and urine unchanged.

Compounds slightly metabolized. *N*-Methylpyridinium (no. 1), dibenzyltrimethylammonium (no. 8)

Table 4. *Excretion of tartrazine in the presence of quaternary ammonium cations*

The control bile-duct-cannulated rats were injected intraperitoneally with tartrazine (50 μ mol/kg in water). The other rats were injected intraperitoneally simultaneously with the same amount of tartrazine and with trimethylphenylammonium iodide (cation no. 3), 15mg/kg in water, or tribenzylmethylammonium iodide (cation no. 10, see Table 3), 10mg/kg in water. Urine and bile were collected for 3h and analysed for tartrazine as described by Gregson *et al.* (1972). Results are given as averages with the ranges in parentheses and the number of animals indicated in brackets.

Excretion	Tartrazine excreted in 3h (% of dose)	
	Controls	Cation no. 3 present*
Bile	23 (16–37) [4]	25 (15–35) [3]
Urine	48 (42–55) [4]	41 (29–54) [3]
Total	71 (66–79) [4]	68 (63–77) [3]
Excretion	Tartrazine excreted in 3h (% of dose)	
	Controls	Cation no. 10 present*
Bile	30 (15–24) [9]	26 (16–45) [8]
Urine	41 (32–50) [9]	35 (20–48) [8]
Total	71 (48–85) [9]	61 (49–81) [8]

* Not significantly different from the control group $P > 0.05$.

and tribenzylmethylammonium (no. 10) were slightly metabolized, but largely excreted unchanged. Cation no. 1, as shown by chromatography, was excreted unchanged in the urine, but chromatography of the bile, which contained only 0.8% of the dose, showed two ^{14}C peaks, one corresponding to the unchanged cation (58% of the biliary ^{14}C or 0.5% of the dose) and an unknown metabolite of R_F 0.08 in solvent B (see Table 2), which amounted to 0.3% of the dose. Cation no. 8 was excreted unchanged in the urine, but chromatography of the bile, which contained 36% of the dose, showed two ^{14}C peaks, one large (97% of the ^{14}C of the bile) and corresponding to unchanged no. 8 (proved by isotope dilution, which gave a value of 99%) and one small, of R_F 0.30 in solvent B and accounting for about 1% of the dose. The latter was not identified. Cation no. 10 was also excreted unchanged in the urine, but the biliary ^{14}C (51% of the dose) consisted mainly of unchanged no. 10 (91% of the biliary ^{14}C ; checked by isotope dilution, which gave 88%) and a minor unidentified metabolite (5% of the dose) of R_F 0.26 in solvent B.

Cations appreciably metabolized. 3-Hydroxyphenyltrimethylammonium (no. 4), 3-bromo-*N*-methylpyridinium (no. 6) and cetyltrimethylammonium (no. 9) were appreciably metabolized. Cation no. 4 was examined in both male and female rats but, as Table 3 indicates, there was no sex difference in the excretion of this compound. Some 11–14% of the dose of ^{14}C appeared in the bile and 55–61% in the urine in 24h after an intravenous dose. The biliary ^{14}C consisted of two metabolites, one of R_F 0.02–0.09 in solvent D (see Table 2) and accounting for 93–97% of the biliary ^{14}C and the other of R_F 0.19–0.31 accounting for 3–6%. The bile contained only traces of the unchanged 3-hydroxyphenyltrimethylammonium. On prolonged incubation (48h) at 37°C of bile samples with β -glucuronidase, the above two metabolites disappeared and were replaced by peaks at R_F 0.61 and 0.86 in solvent D. The peak of R_F 0.61

Table 5. *Effect of renal ligation on the biliary excretion of cations in the rat*

Female rats were used. They were all bile-duct-cannulated and some also had their renal pedicles ligated as described in the text. The ^{14}C -labelled compounds were administered intraperitoneally at the doses given in Table 3. Bile was collected for 3h and analysed as described in the text. The results are the average values for three animals unless otherwise indicated in brackets, with ranges in parentheses.

No.	Compound	Mol.wt. of cation	^{14}C in bile in 3h (% of dose)	
			Renal pedicles not tied	Renal pedicles tied
1	<i>N</i> -Methylpyridinium iodide	94	0.6 (0.5–0.8)	2.0 (1.6–2.8)
2	Tetraethylammonium bromide	130	0.4 (0.2–0.7)	3.7 (3.5–4.1)
5	Diethylmethylphenylammonium iodide	164	5.8 (4.8–7.0)	16 (15–16)
7	Methylphenyldipropylammonium iodide	192	12 (8.7–15) [5]	29 (27–30)
8	Dibenzyltrimethylammonium iodide	226	27 (15–39) [6]	31 (22–46) [4]
10	Tribenzylmethylammonium iodide	302	39 (17–53) [5]	39 (34–43)

corresponded to cation no. 4 and it would appear that the main radioactive compound in the bile (10–14% of the dose) was the glucuronide of 3-hydroxyphenyltrimethylammonium (mol.wt. 327). The minor biliary metabolite of R_F 0.19–0.31 is probably the *O*-glucuronide of 3-dimethylaminophenol, as shown by Somani *et al.* (1970). The latter workers record an R_F of 0.87 for 3-dimethylaminophenol in solvent D, which is close to the value of 0.86 found above for the second biliary metabolite after β -glucuronidase hydrolysis. The urinary ^{14}C was made up of three compounds, of R_F 0.02–0.04 (53% of urinary ^{14}C), 0.27–0.29 (1.3%) and 0.59–0.60 (45%) in solvent D. The first two of these corresponded to the biliary metabolites, whereas the last corresponded to the unchanged cation.

The urine of rats which had received cation no. 6 intraperitoneally showed three peaks on chromatography in solvent C. These had R_F 0.07 (12% of urinary ^{14}C), 0.31 (45%) and 0.66 (43%). The second peak, R_F 0.31, corresponded to unchanged 3-bromo-*N*-methylpyridinium. The peak at R_F 0.07, which gave a positive naphtharesorcinol test for glucuronic acid, disappeared on incubation with β -glucuronidase but did not disappear if saccharo-1,4-lactone was present in the incubation mixture. However, tests for phenols in the hydrolysate were negative and the material was not further identified. The peak at R_F 0.66 was not identified. The bile contained 15% of the dose of ^{14}C and the same three ^{14}C peaks in solvent C as did the urine, and their proportions were R_F 0.02, 76% of biliary ^{14}C , R_F 0.29, 19% (unchanged cation no. 6) and R_F 0.65, 5%. The main biliary metabolite (11% of the dose) of 3-bromo-*N*-methylpyridinium was therefore an unidentified glucuronide.

The bile from rats receiving cetyltrimethylammonium (cation no. 9) contained 36% of the dose of ^{14}C and showed only one ^{14}C peak, whose R_F in solvents B and C corresponded exactly to that of unchanged cation no. 9. In solvent A, however, two peaks were found, one of R_F 0.92 (79% of biliary ^{14}C) and corresponding to the unchanged cation and one of R_F 0.73 (21% of biliary ^{14}C or 8% of the dose). The latter was not identified. Chromatography of the urine showed several ^{14}C peaks in solvent B, of R_F 0.30 (17% of urinary ^{14}C), 0.41 (22%), 0.59 (19%), 0.71 (15%) and 0.79 (27%). These were not identified, but they could be products of the ω -oxidation followed by β -oxidation of the cetyl group. However, it was clear that the main biliary product was the unchanged cetyltrimethylammonium ion.

Excretion of cations in rats

Effect of renal ligation on the biliary excretion of cations in the rat. The effect of renal ligation on the extent of excretion in the bile of six cations in bile-duct-cannulated rats is shown in Table 5. The general

Table 6. Excretion of ^{14}C by intact rats receiving ^{14}C -labelled quaternary ammonium cations

No.	Compound	Dose of cation ($\mu\text{mol/kg}$)	Time (days)	^{14}C recovered (% of dose)			
				Urine	Faeces	CO_2	Total
3	Trimethylphenylammonium iodide	29	1	74 (70–79)	7.2 (3.7–12)	0.3 (0–0.5)	82 (79–83)
			3	79 (75–85)	8.7 (6.1–13)	0.3 (0–0.5)	88 (86–91)
8	Dibenzyltrimethylammonium iodide	27	1	32 (25–37)	31 (25–39)	0.5 (0.3–0.7)	64 (55–77)
			3	34 (26–40)	37 (33–42)	0.8 (0.5–0.9)	72 (60–83)
10	Tribenzyltrimethylammonium iodide	16	1	14 (12–15)	59 (54–61)	2.4 (2.1–2.7)	75 (71–78)
			3	17 (15–18)	76 (73–80)	3.6 (3.4–4.1)	97 (92–102)

The ^{14}C -labelled compounds dissolved in water (see Table 3) were injected intraperitoneally into intact Wistar albino rats. Urine, faeces and expired CO_2 were collected for 3 days and analysed as described in the text. Results are mean values for three animals, with ranges in parentheses.

Table 7. *Biliary excretion of some monoquaternary ammonium ions in different species*

Aqueous solutions of the ^{14}C -labelled compounds (5 mg/ml unless otherwise stated) were injected intraperitoneally into bile-duct-cannulated female animals (Wistar albino rats, Dunkin Hartley albino or English guinea pigs, Dutch rabbits). The doses were; rats, 15 mg/kg (except cation no. 10; 10 mg/kg), guinea pigs, 10 mg/kg, and rabbits, 5 mg/kg. Bile and urine were collected for 3 h and analysed as described in the text. The results are the averages for three animals, unless otherwise indicated in brackets, with ranges in parentheses.

No.	Compound* (mol.wt. of cation)	Dose (μmol of cation/kg)			^{14}C excreted in 3 h (% of dose)		
		Rat	Guinea pig	Rabbit	Rat†	Guinea pig	Rabbit
2	Tetraethylammonium bromide (130)	44	29	15	0.4 (0.2-0.7) 72 (52-85) 72 (52-85)	0.5 (0.2-0.6)‡ 75 (73-78) 76 (74-79)	2.4 (1.9-3.2) 43 (28-63) 45 (30-65)
3	Trimethylphenylammonium iodide (136)	29	20	9.8	0.5 (0.4-0.6) 70 (63-84) 71 (64-84)	<0.1 17 (3.4-26) 17 (3.4-26)	0.4 (0.1-0.5) 32 (22-49) 32 (22-49)
5	Diethylmethylphenylammonium iodide (164)	29	19	9.6	5.8 (4.8-7.0) 59 (45-73) 65 (51-78)	1.3 (0.7-2.4) 33 (18-52) 34 (19-53)	5.5 (2.7-8.7) 41 (37-48) 47 (41-57)
7	Methylphenyldipropylammonium iodide (192)	28	19	9.3	12 (8.7-15) [5] 65 (60-70) 77 (70-80)	27 (21-32) [5]‡ 19 (9.7-37) 46 (36-63)	13 (3.8-23) [4] 36 (17-47) 49 (36-56)
8	Dibenzyltrimethylammonium iodide§ (226)	27	18	9.0	27 (15-39) [6] 21 (13-29) 48 (33-66)	41 (36-48) 5.1 (4.0-7.4) 46 (40-55)	29 (16-43) [4] 20 (13-24) 49 (40-61)
10	Tribenzylmethylammonium iodide (302)	16	16	8.1	39 (17-53) [5] 16 (6.5-31) 55 (48-66)	38 (28-46) 2.3 (0.6-5.3) 40 (33-47)	41 (32-45) 10 (7.8-11) 51 (43-55)

* These compounds were excreted largely unchanged (see the text).

† The urine values for rats are for 24 h.

‡ English guinea pigs were used for these two compounds and Dunkin Hartley strain for the other compounds.

§ The injected solution of this compound contained 3 mg/ml.

|| The injected solution of this compound contained 2 mg/ml.

effect is to increase biliary excretion. With cations nos. 1 and 2, whose molecular weights are 94 and 130 respectively, the extent of biliary excretion is increased from less than 1% to about 2–4%, which is still less than the arbitrary threshold value of greater than 10% of the dose suggested above. With cations nos. 5 and 7, whose molecular weights are 164 and 192 respectively, there is a marked increase in biliary excretion on renal ligation, from 6 to 16% with no. 5 and from 12 to 29% with no. 7. With the cations of highest molecular weights in Table 5, namely nos. 8 and 10, with molecular weights 226 and 302 respectively, renal ligation causes little change in the extent of biliary excretion.

Urinary and faecal excretion of some cations in intact rats. Three ^{14}C -labelled compounds, trimethylphenylammonium iodide (no. 3), dibenzyl dimethylammonium iodide (no. 8) and tribenzylmethylammonium iodide (no. 10) were administered intraperitoneally to rats and the excretion of ^{14}C in the urine, faeces and expired air was measured daily for 3 days (see Table 6). With cation no. 3 (mol.wt. 136), the main route of excretion was the urine (79% of the dose or 90% of the ^{14}C recovered). With cation no. 8, which has a higher molecular weight (226), roughly equal amounts were excreted in the urine (34%) and faeces (37%). With cation no. 10, which has the highest molecular weight (302) of the three compounds examined, the main route of excretion is via the faeces (76% of the dose or 78% of the ^{14}C recovered). Some $^{14}\text{CO}_2$ was eliminated in the expired air, amounting to less than 1% of the dose with cations nos. 3 and 8 and about 3–4% with cation no. 10.

Biliary excretion of cations in guinea pigs and rabbits

The biliary and urinary excretion of six cations with molecular weights ranging from 130 to 302 were examined in bile-duct-cannulated guinea pigs and rabbits and the results compared with those for rats as shown in Table 7. These six cations were excreted mainly unchanged in the bile and urine of all three species. The first two, nos. 2 and 3, with molecular weights of 130 and 136 respectively, were poorly (about 0.5–2% of the dose) excreted unchanged in the bile in all three species. With cation no. 5 (mol.wt. 164), some 5–6% of the dose was excreted unchanged in the bile in rats and rabbits and about 1–2% in guinea pigs. Thus the first three cations in Table 7, which have molecular weights below 170, have a biliary excretion of less than 10% of the dose in all three species, and their main route of elimination is the urine, in which they were excreted unchanged.

With cations no. 7 (mol.wt. 192), no. 8 (226) and no. 10 (302) biliary excretion exceeds 10% of the dose in all three species. The guinea pig appears to excrete rather more of cations nos. 7 and 8 in the bile than do the rat and rabbit, but with cation no. 10 the extent

of its biliary excretion (about 40% of the dose) is practically the same in the three species. Cations nos. 7, 8 and 10 were eliminated in bile and urine virtually unchanged (i.e. only 0–1% of dose as metabolites) by rats and guinea pigs, but were metabolized to an appreciable extent (i.e. 10–14% of dose) in rabbits. Thus for each cation about 90% of the biliary ^{14}C in rabbits was associated with the unchanged compound and the remaining 10% was not identified. For cations nos. 7 and 10 about 80% of the urinary ^{14}C in the rabbit was present as the unchanged compound. However, with cation no. 8 only about half of the urinary ^{14}C was present as the unchanged compound, the remainder being present in five unidentified metabolites.

Discussion

Metabolism

The compounds studied are strongly polar in that they contain a quaternary ammonium or pyridinium group and thus exist as cations at physiological pH values. These compounds were selected because they were not expected to be extensively metabolized, for if they were the study would be complicated through the formation of metabolites of different molecular weights, structure and polarity. It was found that eight (nos. 1, 2, 3, 5, 7, 8, 9 and 10; see Table 3) of the ten cations studied in the rat were excreted in the bile largely unchanged. The main biliary product of 3-hydroxyphenyltrimethylammonium (no. 4) and 3-bromo-*N*-methylpyridinium (no. 6) was in each case a glucuronic acid conjugate. The conjugate of no. 4 would probably be the zwitterion

(mol.wt. 327), and it is possible that the conjugate of no. 6 is also a glucuronic acid-containing zwitterion.

Six of the above cations were also investigated in the guinea pig and rabbit. The guinea pig was found to handle these compounds (nos. 2, 3, 5, 7, 8 and 10) like the rat in that they were excreted in bile and urine virtually unchanged. In the rabbit, however, cations nos. 2, 3, and 5 were not metabolized, whereas nos. 7, 8 and 10 were transformed to the extent of 10–14% of dose, although the metabolites were not identified. The rabbit thus had some capacity for metabolizing these monoquaternary cations. Other work in this laboratory (Hughes *et al.*, 1973) has shown that the rabbit can also metabolize the diquaternary cation diquat to an appreciable extent (18% of the dose), whereas in the rat and guinea pig diquat is excreted largely unchanged, the extent of its metabolism in these species being <1% and 5% of the dose respectively.

When cations nos. 3, 8 and 10 were injected into

intact rats, a small amount of $^{14}\text{CO}_2$ was found in the expired air and it was suspected that this may have arisen as a result of bacterial *N*-demethylation of the methyl- ^{14}C -labelled compounds after their excretion into the intestine via the bile. Studies in this laboratory (G. M. Hawksworth, R. D. Hughes, P. Millburn & R. T. Williams, unpublished work) have shown that these cations can be demethylated *in vitro* by rat caecal contents, cation no. 10, which gave the most $^{14}\text{CO}_2$ *in vivo*, being demethylated *in vitro* by caecal contents to a greater extent than cations nos. 3 and 8.

Molecular-weight threshold for biliary excretion

Millburn *et al.* (1967a) and Hirom *et al.* (1972b) showed that for the biliary excretion in the rat of organic anions to occur to a significant extent, which was defined as 10% or more of the dose, the anion or its conjugate should have a minimum molecular weight of about 325 ± 50 . Table 3 suggests that a minimum molecular weight is also necessary for the significant biliary excretion of monoquaternary organic cations and that this minimum is in the region of 170 and therefore lower than for anions. Thus cations nos. 1–3 (mol.wt. 94–136) are poorly excreted (about 1% of the dose) in the bile of rats and the extent of excretion begins to increase with cation no. 5 (mol.wt. 164; 6–9% of the dose in bile). The first cation in Table 3 to show a biliary excretion of greater than 10% and which is excreted mainly unchanged is no. 7, with mol.wt. 192 and a biliary excretion of 17%. Cations no. 4 (mol.wt. 152) and no. 6 (mol.wt. 173) also show a biliary excretion of greater than 10%, but in both cases the main metabolite in bile is a glucuronide (mol.wt. 327 for cation no. 4, but unidentified for cation no. 6). The remaining cations (nos. 8 and 10) in Table 3, which have molecular weights over 225, are extensively (greater than 30% of the dose) eliminated largely unchanged in the bile.

To obtain a better definition of the threshold molecular weight for 10% or more biliary excretion of monoquaternary organic cations, the extent of biliary excretion of the relevant compounds in Table 3 and of cations studied by other workers (see Millburn, 1970; Hwang & Schanker, 1970; Kuntzman *et al.*, 1970; Meijer *et al.*, 1970; Nakai *et al.*, 1970; Hespe & Wieriks, 1971; MacGregor & Clarkson, 1971; Suga *et al.*, 1971; Lavy *et al.*, 1972) has been plotted against molecular weight to produce a diagram (see Fig. 1) similar to that given by Hirom *et al.* (1972b) for anions. In Fig. 1, results for 32 cations where the nature of the material in the bile is known are included. The points on the diagram are made up of data from rats with functioning kidneys (20 results) and rats with ligated renal pedicles (12 results). Fig. 1 suggests that the threshold molecular weight in the

Fig. 1. Threshold molecular weight for appreciable biliary excretion (>10% of dose) of monoquaternary organic cations in the rat

The extent of biliary excretion of 32 monoquaternary organic cations in rats is plotted against molecular weight. The vertical box shows the region of the molecular weight threshold of 200 ± 50 required for their biliary excretion to the extent of 10% or more of the dose.

rat for the biliary excretion of monoquaternary organic cations to the extent of 10% or more of the dose is in the region of 200 ± 50 . In Fig. 1, there are five compounds with molecular weights below 150 whose biliary excretion is less than 5%. There are nine compounds in the proposed threshold region of 200 ± 50 and, of these, three have a biliary excretion below 10% and six between 15 and 40%. There are 18 compounds whose molecular weights are greater than 250, and their biliary excretion is greater than 10% of the dose and in most cases more than 30%. In constructing Fig. 1 results from renally ligated rats

have been included. This appears to be justified, since Table 5 suggests that the biliary excretion of cations of molecular weight less than 150 is not increased to over 10% by renal ligation. It would appear that renal ligation may increase the biliary excretion of cations if their molecular weights are in the threshold region, 150–250 (see Table 5).

Relationship between excretion in the urine, bile and faeces

In bile-duct-cannulated animals, biliary and urinary excretion of cations are complementary in that the extent of urinary excretion is greatest for those organic cations of lowest molecular weight and decreases as the molecular weight increases and biliary excretion becomes more extensive (see Tables 3 and 7). This is a similar situation to that for organic anions (Hirom *et al.*, 1972b). Cations nos. 3, 8 and 10 were also investigated in intact rats, and the amounts of ^{14}C appearing in the faeces and urine (Table 6) are similar to the extent of biliary and urinary excretion respectively of these compounds in bile-duct-cannulated rats (Table 3). This suggests that, at least for these cations, the material excreted in the bile is not reabsorbed to any appreciable extent from the intestine (if absorption took place the compound might undergo enterohepatic circulation or be excreted in the urine) but passes directly into the faeces.

For trimethylphenylammonium (cation no. 3) the amount of ^{14}C appearing in the faeces in intact rats, although small (about 7% of the dose in day 1), is about ten times that (0.7% of dose) appearing in the bile of cannulated rats. Some of this faecal ^{14}C could have arisen by contamination of the faeces by radioactive urine. However, the tenfold difference between the values for bile and faeces suggests that most of the faecal radioactivity from cation no. 3 arrives there by a route other than that of bile, possibly through the intestinal or pancreatic secretions.

Species

Hirom *et al.* (1972b) showed that there was a species difference in the threshold molecular weight for the significant biliary excretion of organic anions. Table 6 suggests that this may not be true for monoquaternary organic cations as far as the rat, guinea pig and rabbit are concerned. There might be some variation within the threshold value of 200 ± 50 , i.e. cations nos. 5, 7 and 8, since the guinea pig differs from the rat and rabbit in the extent of biliary excretion of these cations. But the difference is inconsistent, since the biliary excretion of cation no. 5 (mol.wt. 164) is lower in the guinea pig than in the rat and rabbit, whereas for nos. 7 (192) and 8 (226) the reverse is true, and more data in different species are obviously needed.

In conclusion, the results for biliary elimination in Table 7 suggest that the threshold molecular weight for appreciable biliary excretion of monoquaternary organic cations is similar in the rat, guinea pig and rabbit, this being about 200 ± 50 . This is in marked contrast to organic anions, for which this molecular weight varies with species, being about 325 ± 50 for the rat, 400 ± 50 for the guinea pig and 475 ± 50 for the rabbit (Millburn *et al.*, 1967a; Hirom *et al.*, 1972b). Schanker & Solomon (1963) have suggested that the liver has an active secretory process for the transport of quaternary ammonium ions from blood to bile which is different from that for organic anions. It is therefore not surprising that the molecular-weight criteria defined above for organic anions are different from those for cations.

This work was supported by the Medical Research Council, to whom R. D. H. is grateful for a maintenance grant.

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