Biliary Excretion of Some Diquaternary Ammonium Cations in the Rat, Guinea Pig and Rabbit

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

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1. The extent of the excretion in the bile and urine of the ^{14}C -labelled dications, diquat, paraquat, morfamquat, decamethonium and dimethyltubocurarine in bile-duct-cannulated rats, guinea pigs and rabbits was examined. 2. These compounds were excreted unchanged in bile and urine, except diquat, which was metabolized to a significant extent (18% of the dose) in the rabbit only. 3. The extent of the biliary excretion of diquat (mol wt. of ion 184), paraquat (186), decamethonium (258) and morfamquat (469) was less than 10% of the dose in the three species, whereas that of dimethyltubocurarine (653) was greater than 10% in the rat and rabbit but not in the guinea pig. 4. These results together with data from the literature suggest that the molecular weight at which the excretion of dications in the bile exceeds 10% of the dose is in the region of 500–600, which differs from the values for monocations (Hughes *et al.*, 1973) and anions (Millburn *et al.*, 1967; Hirom *et al.*, 1972).

From a study of the biliary excretion in the rat, guinea pig and rabbit of some monoquaternary ammonium or pyridinium cations, Hughes *et al.* (1973) suggested that there is a molecular-weight threshold of about 200 ± 50 for the significant (i.e. 10% or more of the dose) biliary excretion of monoquaternary nitrogen cations in these species. The biliary excretion in the same three species of five diquaternary ammonium or pyridinium cations with molecular weights between 184 and 653 has now been examined. Apart from the dication of highest molecular weight (i.e. 653), these compounds were poorly (less than 10% of dose) excreted in the bile of the three species examined and it appears that the molecular-weight threshold for the significant biliary excretion of diquaternary organic cations is different from that of monoquaternary ammonium cations and may be in the region of at least 500–600.

Materials and Methods

Compounds

Diquat dichloride, paraquat di-iodide, morfamquat dichloride dihydrate, [^{14}C]diquat dibromide ([1,1'- $^{14}\text{C}_2$]ethylene-2,2'-bipyridylium dibromide; $5.2 \mu\text{Ci/mg}$), [^{14}C]paraquat di-iodide (1,1'-di- ^{14}C]methyl-4,4'-bipyridylium di-iodide; $8.4 \mu\text{Ci/mg}$) and [^{14}C]morfamquat dibromide dihydrate [1,1'-bis-(3,5-dimethylmorpholino- ^{14}C]carbonylmethyl)-4,4'-bipyridylium dibromide dihydrate; $0.57 \mu\text{Ci/mg}$] were the gift of the Industrial Hygiene Research Laboratories, Imperial Chemical Industries Ltd., Alderley Park, Cheshire, U.K. Deca-

methonium iodide was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K., and [^{14}C]decamethonium bromide [decane-1,10-bis-(monomethyl- ^{14}C]trimethylammonium bromide); $36 \mu\text{Ci/mg}$] and [^{14}C]dimethyltubocurarine iodide (OO'-di- ^{14}C]methyl-*d*-tubocurarine iodide, but see the Discussion section; $123 \mu\text{Ci/mg}$) were from The Radiochemical Centre, Amersham, Bucks., U.K. Dimethyltubocurarine iodide was a gift from Professor R. Creese, of the Department of Physiology of this Medical School. Each of these ^{14}C -labelled compounds ran as one spot on paper or thin-layer chromatograms. Their R_F values and the colour tests used for their detection are given in Table 1.

Animals

Wistar albino rats (170–225 g body wt.), Dunkin Hartley albino guinea pigs (425–600 g), English guinea pigs (435–475 g) and Dutch rabbits (1.8–3.2 kg) were used. The animals, which were all females, were anaesthetized and the common bile ducts cannulated as described in the preceding paper (Hughes *et al.*, 1973). In experiments with diquat, paraquat and morfamquat, the rats were maintained for 24 h and the guinea pigs and rabbits for 3 h as described by Hughes *et al.* (1973). With decamethonium and dimethyltubocurarine, all the animals were kept anaesthetized for the duration (3 h) of bile collection by injection of small amounts of anaesthetic when necessary. Bile-duct-cannulated rats with ligated renal pedicles were prepared as previously described (Hughes *et al.*, 1973).

Table 1. R_F values of some diquatery organic compounds

Whatman no. 1 paper and the descending technique were used with the following solvent systems (proportions by vol.): A, butan-1-ol-acetic acid-water (4:1:2); B, butan-1-ol-98% (v/v) formic acid-water (6:1:2); C, propan-2-ol-ethanol-M-HCl (3:3:2). T.l.c. was carried out on silica gel G (E. Merck A.G., Darmstadt, W. Germany; 0.25 mm thick) on glass plates (ascending technique) with the following solvent systems: D, propan-2-ol-1 M-HCl (1:1, v/v); E, acetone-1 M-HCl (1:1, v/v). Paper chromatograms were run for 12-18 h and t.l.c. plates for 2 h at room temperature. The compounds were detected with the iodoplatinate reagent (Hughes *et al.*, 1973) and also with 2% (w/v) sodium dithionite in 1 M-NaOH for diquat, paraquat and morfamquat.

Compound Solvent ...	R_F					Colour	
	Paper chromatography			T.l.c.		With iodoplatinate*	With dithionite†
	A	B	C	D	E		
Diquat dichloride	0.14	0.10	0.16	—	—	Dark grey	Green
Paraquat di-iodide	0.14	0.10	0.20	—	—	Dark grey	Blue
Morfamquat dichloride	0.44	0.28	0.57	—	—	Mauve-grey	Purple
Decamethonium iodide	0.49	0.32	0.61	—	—	Mauve	—
Dimethyltubocurarine iodide‡	0.62	0.48	0.56	0.04	0.03	Brown	—

* On a pink background.

† The colours obtained result from the reduction of the compounds to coloured free radicals, which readily reoxidize in air; the colours are transient, lasting for only about 15-20s.

‡ See the Discussion section for structure.

Measurement of ^{14}C

The radioactivity in bile (0.1-0.5 ml) and urine (0.1-1.0 ml) samples was determined with a Packard Tri-Carb liquid-scintillation spectrometer (model 3214 or 3320) by using a dioxan-based scintillator (see Bridges *et al.*, 1967) for all the compounds except [^{14}C]dimethyltubocurarine, which was counted in a scintillator consisting of 2,5-diphenyloxazole (0.5%, w/v) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.01%, w/v) dissolved in toluene. This scintillator (2 parts) was added to Triton X-100 (Koch-Light) (1 part), and the bile or urine (1 ml) containing [^{14}C]dimethyltubocurarine was added to 10 ml of this mixture and counted by using an internal standard (see Patterson & Greene, 1965). The bile and urine samples were counted in glass vials except with those containing [^{14}C]decamethonium, for which polythene vials were used since such samples did not give reproducible counts in glass vials.

Chromatograms of radioactive bile and urine were scanned in a Packard radiochromatogram scanner (model 7200). Bile (0.01-0.2 ml) or urine (0.01-0.5 ml) was chromatographed on thin strips (5 cm wide) of paper (Whatman no. 1) or on silica gel G (E. Merck, A.G., Darmstadt, Germany; 0.25 mm thick) on glass plates and the R_F values of the ^{14}C -labelled peaks were compared with those of authentic compounds (see Table 1). In some experiments, the paper chromatograms were cut into small pieces (1-2.5 cm by 5 cm) and placed in the appro-

priate scintillator fluid (see above) and the associated ^{14}C was counted.

Separation of diquat and paraquat from urine

[^{14}C]Diquat or [^{14}C]paraquat was separated from urine samples by column chromatography on a cation-exchange resin (Dowex 50W, X8; British Drug Houses, Poole, Dorset, U.K.; 1 ml of resin was used to prepare a column of 5 mm internal diameter) essentially as described by Daniel & Gage (1966). The amount of [^{14}C]diquat or [^{14}C]paraquat in the eluate from the column was determined both from the ^{14}C content of portions (0.2 ml) of the eluate and by colorimetric analysis as described by Daniel & Gage (1966).

Results

Biliary and urinary excretion

The extent of excretion in bile and urine of five diquatery ammonium or pyridinium cations with molecular weights in the range 184-653 by bile-duct-cannulated female rats, guinea pigs and rabbits is shown in Table 2. These compounds were administered intraperitoneally in doses (see Table 2) at which the cannulated animals showed no overt toxic symptoms, apart from dimethyltubocurarine iodide administered to rats at a dose of 0.03 mg/kg. In this case, the rats had to be artificially respired

Table 2. *Biliary and urinary excretion of some diquaternary nitrogen cations*

The ^{14}C -labelled compounds (5mg/ml in water unless otherwise stated) were injected intraperitoneally into bile-duct-cannulated female animals (see the text). For diquat dichloride, paraquat di-iodide and morfamquat dichloride the doses were: rats, 15mg/kg, guinea pigs and rabbits, 5mg/kg. Doses of decamethonium iodide: rats, 0.3mg/kg, guinea pigs, 0.2mg/kg and rabbits, 0.1mg/kg. Doses of dimethyltubocurarine iodide (see the Discussion section for structure): rats, 0.01 or 0.03mg/kg, guinea pigs, 0.02mg/kg and rabbits, 0.01mg/kg. Bile and urine were collected for 3h and analysed as described in the text. The results are the averages for three animals, unless otherwise indicated by numerals in brackets, with ranges in parentheses.

Compound (mol.wt. of cation)	Dose (μ mol of cation/kg)				^{14}C excreted in 3h (% of dose)		
	Rat	Guinea pig	Rabbit		Rat	Guinea pig	Rabbit
Diquat dichloride (184)	40	13	14	Bile	1.4 (1.2–1.8)*	4.8 (3.8–6.3)‡	2.9 (2.2–3.2) [4]
				Urine	82 (74–90)†	45 (33–53)	64 (44–78)
				Total	84 (76–92)†	50 (39–57)	67 (46–81)
Paraquat di-iodide (186)	13	5	5	Bile	0.4 (0.3–0.4)*	1.9 (1.2–2.4)	1.2 (1.1–1.4)
				Urine	84 (73–95)†	71 (70–72)	55 (41–74)
				Total	85 (74–95)†	73 (71–74)	56 (42–75)
Decamethonium bromide§ (258)	0.41	0.27	0.17	Bile	1.0 (0.4–2.2)	0.7 (0.5–0.9)	0.5 (0.2–0.6)
				Urine	44 (41–47)	46 (39–50)	50 (45–54)
				Total	45 (43–47)	47 (40–51)	51 (46–55)
Morfamquat dichloride (469)	32	9	9	Bile	0.2 (0.1–0.2)*	0.6 (0.3–0.8)	0.6 (0.3–1.0) [5]
				Urine	91 (83–97)†	62 (49–73)	55 (47–67)
				Total	91 (83–97)†	63 (50–74)	56 (47–67)
Dimethyltubocurarine iodide (653)	0.006	0.015	0.007	Bile	17 (9.5–22)	1.8 (1.4–2.0)	16 (4.8–25)
				Urine	65 (48–79)	28 (21–33)	29 (14–40)
				Total	82 (58–101)	30 (23–34)	45 (19–60)
	0.02	—	—	Bile	18 (13–25)	—	—
				Urine	62 (51–74)	—	—
				Total	80 (76–87)	—	—

* The values for 24h were: diquat, 2.2 (1.8–2.6); paraquat, 0.6 (0.5–0.7); morfamquat, 0.3 (0.2–0.3).

† These are figures for 24h.

‡ English guinea pigs were used for this compound only; Dunkin Hartley guinea pigs were used for the others.

§ The injected solution contained 0.2mg/ml.

|| The injected solution contained 0.04mg/ml.

by mechanically pumping air in and out of the lungs by means of a polythene tube inserted into the trachea. Although this dose of dimethyltubocurarine depressed respiration, the drug did not appear to affect the excretory ability of the liver and the kidneys, since the proportions of dimethyltubocurarine appearing in bile and urine after a dose of 0.03mg/kg did not differ from those excreted at the lower dose of 0.01mg/kg, which did not depress respiration (see Table 2).

The first four dications in Table 2, with molecular weights between 184 and 469, were poorly (less than 5% of the dose) excreted in the bile and were eliminated mainly in the urine (44–91% of the dose) of all three species. With the fifth compound (dimethyltubocurarine, mol.wt. 653) in Table 2, the rat and

rabbit excreted more (16–18% of the dose) in the bile than did the guinea pig (1.8%). These dications were excreted almost entirely unchanged, except diquat, which appeared to be metabolized to some extent, especially in the rabbit (see below).

Paraquat, decamethonium and dimethyltubocurarine. In the urine of bile-duct-cannulated rats, guinea pigs and rabbits, these compounds were found by paper chromatography and t.l.c. to be present in the unchanged state, and for paraquat this was confirmed by column chromatography (see Table 3). In the bile, decamethonium was excreted unchanged by rats and dimethyltubocurarine by rats and rabbits. The concentrations of ^{14}C in the bile of rats, guinea pigs and rabbits receiving paraquat, in the bile of

Table 3. *Unchanged diquat and paraquat in urine estimated colorimetrically and radiometrically*

Urine samples from bile-duct-cannulated animals given [^{14}C]diquat or [^{14}C]paraquat intraperitoneally (see Table 2) were analysed colorimetrically and radiometrically for diquat or paraquat after separation of the compounds from the urine by column chromatography with a cation-exchange resin as described in the text. Values for two animals are given.

Compound	Assay method	Urinary material present as unchanged compound		
		Rat (% in 24 h)	Guinea pig (% in 3 h)	Rabbit (% in 3 h)
Diquat	Colorimetric	91, 101	93, 84	62, 68
	^{14}C	97, 101	92, 87	68, 76
Paraquat	Colorimetric	93, 98	91, 92	85, 94
	^{14}C	96, 98	91, 105	89, 93

Table 4. *Effect of renal ligation on the biliary excretion of some diquatertiary organic cations in the rat*

Female rats were bile-duct-cannulated and some had their renal pedicles ligated as described in the text. The ^{14}C -labelled compounds were administered intraperitoneally at the doses given in Table 2. Bile was collected for 3 h and analysed as described in the text. The results are the average values for three animals with ranges in parentheses.

Compound	Mol.wt. of cation	^{14}C in bile in 3 h (% of dose)	
		Non-ligated renal pedicles	Ligated renal pedicles
Diquat dichloride	184	1.4 (1.2–1.8)	6.1 (3.8–7.8)
Paraquat di-iodide	186	0.4 (0.3–0.4)	1.7 (1.4–1.8)
Decamethonium bromide	258	1.0 (0.4–2.2)	0.4 (0.4–0.4)
Morfamquat dichloride	469	0.2 (0.1–0.2)	1.3 (0.8–2.2)
Dimethyltubocurarine iodide*	653	17 (9.5–22)†	30 (21–35)†
		18 (13–25)‡	29 (21–35)‡

* See the Discussion section for the structure of this compound.

† Dose of dication, $0.006\ \mu\text{mol/kg}$.

‡ Dose of dication, $0.02\ \mu\text{mol/kg}$.

guinea pigs and rabbits receiving decamethonium and in the bile of guinea pigs receiving dimethyltubocurarine were too low for chromatographic analysis.

Diquat. Most of the ^{14}C excreted by bile-duct-cannulated rats receiving [^{14}C]diquat was found in the urine (see Table 2), which on paper chromatography showed only one ^{14}C peak corresponding to unchanged diquat. Column chromatography of the urine on a cation-exchange resin showed that 91–101% of the material present was unchanged diquat (see Table 3). Chromatography of rat bile, which contained only 1.4% of the dose (Table 2), showed three ^{14}C peaks, one of which corresponded to unchanged diquat (54% of the biliary ^{14}C or 0.8%

of the dose), but the other two, which had R_f 0.24 (28 or 0.4%) and 0.66 (18 or 0.2%) in solvent B (Table 1), were not identified. The main product in the bile in the rat was thus unchanged diquat.

Paper chromatography of the urine of guinea pigs and rabbits receiving [^{14}C]diquat also showed a large ^{14}C peak corresponding to unchanged diquat, which amounted to 89 and 72% respectively of the urinary ^{14}C or 40 and 46% respectively of the dose. These values were confirmed by column chromatography, which showed that in the guinea pig 84–93% and in the rabbit 62–76% of the material excreted in urine was unchanged diquat. The radiochromatograms of guinea pig and rabbit urine showed a second small ^{14}C peak, R_f 0.25 in guinea pig and 0.27 in

rabbit urine in solvent B, accounting for 11 and 28% respectively of the urinary ^{14}C or 5 and 18% of the dose. The R_F value of this second peak was similar to that of one of the two unidentified ^{14}C peaks in rat bile. The amount of ^{14}C in guinea-pig and rabbit bile was too low for chromatographic analysis. It was concluded that diquat was excreted largely unchanged in the rat and guinea pig, only <1 and 5% of the dose respectively being unidentified, whereas in the rabbit there was an appreciable metabolism, amounting to some 18% of the dose, to an unidentified product.

Morfamquat. In the rat, [^{14}C]morfamquat was excreted unchanged in the bile (0.3% of the dose), but the main bulk of the dose (91%) was excreted in the urine (see Table 2). In solvents B and C, the urine showed on paper chromatograms one ^{14}C peak, which corresponded to morfamquat, but in solvent A two peaks of R_F 0.44 and 0.59 were found, the former corresponding to morfamquat itself (and equivalent to 89% of the urinary ^{14}C or 81% of the dose), but the latter (11% of urinary ^{14}C or 10% of the dose) was an unknown compound. It was found, however, that the amount of the unknown increased at the expense of morfamquat when the radioactive urine was kept. If the urine was kept for a week at room temperature, the morfamquat decreased to 44% of the urinary ^{14}C , whereas the unknown increased to 56%. It was concluded that the unknown was an artifact formed in rat urine from morfamquat.

In guinea pigs and rabbits [^{14}C]morfamquat was largely excreted unchanged in the urine. Little ^{14}C was found in the bile, which was consequently not chromatographed.

Effect of renal ligation. The effect of renal ligation on the extent of excretion in the bile of the five diquaternary organic cations in bile-duct-cannulated rats is shown in Table 4. Apart from decamethonium, the general effect is to increase biliary excretion. With diquat, paraquat and morfamquat (mol.wts. 184, 186 and 469 respectively) the increase is small and the biliary excretion is still below 10% of the dose. With decamethonium (mol.wt. 258) less (0.4%) is excreted in the bile after the renal pedicles are tied than in rats with functioning kidneys (1.0%). With dimethyltubocurarine, which has the highest molecular weight (653), there is, on renal ligation, a marked

increase in biliary excretion from 17–18% to 29–30% of the dose.

Discussion

Metabolism

Highly polar compounds are not readily metabolized in the body (see Williams, 1959) and the five diquaternary cations used in this study were not expected to be metabolized to any great extent by the tissues. In fact, paraquat (I), morfamquat, decamethonium (III) and dimethyltubocurarine when injected into bile-duct-cannulated rats, guinea pigs and rabbits were excreted unchanged. Diquat (II), however, was metabolized to an appreciable extent (18% of the dose) in the cannulated rabbit, but not in the cannulated rat or guinea pig. Studies on the metabolism of all these compounds, except morfamquat, have been reported in the literature. Daniel & Gage (1966) found only unchanged paraquat and diquat in the urine of intact rats given these compounds subcutaneously, and decamethonium has been reported to be excreted unchanged in the urine by rabbits (Broen Christensen, 1967), cats (Lüthi & Waser, 1965) and humans (see Paton & Zaimis, 1952). Dimethyltubocurarine labelled with ^{14}C in four of its methyl groups has been examined by Marsh (1952), whose results suggest that the compound is largely excreted unchanged in the urine after injection into guinea pigs, but when injected into rats the compound is excreted in the urine largely as unidentified metabolites, with a small amount of ^{14}C appearing in the expired air in an unidentified form. It is possible that the latter was $^{14}\text{CO}_2$ arising by demethylation, but Marsh (1952) makes no comment on this. In the present work, [^{14}C]dimethyltubocurarine was excreted unchanged on injection into bile-duct-cannulated rats. In such animals contact between the drug and the gut flora is avoided and it is thus possible that in Marsh's (1952) experiments dimethyltubocurarine was metabolized in the intact rat by the gut flora. It is of interest that the structure of *d*-tubocurarine has been questioned, and Everett *et al.* (1970) and Codding & James (1972) have shown that *d*-tubocurarine is not a diquaternary compound but a monoquaternary compound containing a tertiary nitrogen. Sobell *et al.* (1972), however, have shown that

(I)

(II)

(III)

the compound described as *OO'*-dimethyl-*d*-tubocurarine is actually *OO'N*-trimethyl-*d*-tubocurarine, which is a diquaternary nitrogen cation.

Molecular weight and the biliary excretion of dications

Hughes *et al.* (1973) have suggested that for the biliary excretion of monoquaternary nitrogen cations in amounts greater than 10% of the dose, the cation should have a molecular weight of not less than about 200 ± 50 . For anions this minimum molecular weight was for the rat about 325 ± 50 (Millburn *et al.*, 1967; Hirom *et al.*, 1972). The data for diquaternary nitrogen cations are less extensive than for the mono-cations and anions, but are sufficient to make the tentative suggestion that this minimum molecular weight for dications is different from that for mono-cations and anions and is probably in the region of 500–600. Our own data (see Table 2) show that the four dications with molecular weights 184–469 are poorly (less than 5% of the dose) excreted in the bile, whereas the fifth, dimethyltubocurarine, with mol. wt. 653, has a biliary excretion in the rat and rabbit which is greater than 10%, i.e. 17 and 16% of the dose respectively. Hexamethonium (mol.wt. 202) and five related dications with molecular weights ranging from 206 to 355 were found by Levine (1960) to be excreted in the bile of rats to the extent of not more than 4% in 3 h. Prothidium (mol.wt. 438), a dication used as a prophylactic in cattle against trypanosomiasis, was found by MacGregor & Clarkson (1972; see also Taylor, 1960) to be excreted in the bile by rats to an extent of less than 10% of the dose even with their renal pedicles ligated. Thus 11 dications with molecular weights less than 500 were excreted in the bile by rats to the extent of less than 10% of the dose.

Three dications of molecular weight greater than 500 have been examined, namely dimethyltubocurarine (mol.wt. 653) studied here, hexafluorenium (mol.wt. 503), a muscle relaxant, which has a biliary excretion in rats of 34% of the dose in 1 h and in man of 27–45% in 18 h (Meijer *et al.*, 1971), and *d*-tubocurarine (mol.wt. 611; see above for its new structure), which in the isolated perfused rat liver is excreted in the bile to the extent of 38% of the dose (Meyer & Scaf, 1968). The biliary excretion of *d*-tubocurarine in the dog was found by Cohen *et al.* (1967) to be 11% of the dose, and after

kidneys had been tied off, 39%. These three dications have molecular weights greater than 500 and a biliary excretion greater than 10% of the dose. At the present time there is insufficient information to decide whether the molecular weight threshold for more than 10% biliary excretion of these dications varies with species, but it appears to be unlikely (see Table 2).

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References

- Bridges, J. W., Davies, D. S. & Williams, R. T. (1967) *Biochem. J.* **105**, 1261–1267
- Broen Christensen, C. (1967) *Acta Pharmacol. Toxicol.* **25**, 465–472
- Codding, P. W. & James, M. N. G. (1972) *Chem. Commun.* 1174–1175
- Cohen, E. N., Brewer, H. W. & Smith, D. (1967) *Anesthesiology* **28**, 309–317
- Daniel, J. W. & Gage, J. C. (1966) *Brit. J. Ind. Med.* **23**, 133–136
- Everett, A. J., Lowe, L. A. & Wilkinson, S. (1970) *Chem. Commun.* 1020–1021
- Hirom, P. C., Millburn, P., Smith, R. L. & Williams, R. T. (1972) *Biochem. J.* **129**, 1071–1077
- Hughes, R. D., Millburn, P. & Williams, R. T. (1973) *Biochem. J.* **136**, 967–978
- Levine, R. R. (1960) *J. Pharmacol. Exp. Ther.* **129**, 296–304
- Lüthi, U. & Waser, P. G. (1965) *Arch. Int. Pharmacodyn. Ther.* **156**, 319–347
- MacGregor, J. T. & Clarkson, T. W. (1972) *Biochem. Pharmacol.* **21**, 1679–1696
- Marsh, D. F. (1952) *J. Pharmacol. Exp. Ther.* **105**, 299–316
- Meijer, D. K. F., Vermeer, G. A. & Kwant, G. (1971) *Eur. J. Pharmacol.* **14**, 280–285
- Meyer, D. K. F. & Scaf, A. H. J. (1968) *Eur. J. Pharmacol.* **4**, 343–346
- Millburn, P., Smith, R. L. & Williams, R. T. (1967) *Biochem. J.* **105**, 1275–1281
- Paton, W. D. & Zaimis, E. (1952) *Pharmacol. Rev.* **4**, 219–253
- Patterson, M. S. & Greene, R. C. (1965) *Anal. Chem.* **37**, 854–857
- Sobell, H. M., Sakore, T. D., Tavale, S. S., Canepa, F. G., Pauling, P. & Petcher, T. J. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 2212–2215
- Taylor, A. E. R. (1960) *Brit. J. Pharmacol.* **15**, 235–242
- Williams, R. T. (1959) *Detoxication Mechanisms*, 2nd edn., pp. 730–732, Chapman and Hall, London