Biochemical Pharmacology, Vol. 19, pp. 2937–2948. Pergamon Press, 1970. Printed in Great Britain

STUDIES ON TRIMETOQUINOL—I

DISTRIBUTION, EXCRETION AND METABOLISM OF TRIMETOQUINOL

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(*Received* 6 *November* 1969; *accepted* 16 *March* 1970)

Abstract—Distribution, excretion and metabolism of [^{3}H]trimetoquinol and [^{3}H]isoproterenol in the guinea pig and rat were studied. Ten min after [^{3}H]trimetoquinol was injected intravenously (i.v.) into a guinea pig, the highest concentration was found in kidney, moderate levels in spleen, lung and heart, and very low level in brain. The pattern of distribution of [^{3}H]isoproterenol was similar to that of [^{3}H]trimetoquinol. However, levels of [^{3}H]trimetoquinol in tissues other than liver and kidney were higher than were those of [^{3}H]isoproterenol. After [^{3}H]trimetoquinol administration, 42 per cent of the radioactivity was excreted in 48 hr in urine and 49 per cent in 48 hr in faeces; after [^{3}H]isoproterenol administration, 87 per cent of the radioactivity was excreted in the 48-hr urine. It was found that trimetoquinol, like isoproterenol, was metabolized by either *O*-methylation or conjugation with glucuronic acid. Of the radioactivity excreted in the 4-hr urine of guinea pigs given [^{3}H]trimetoquinol, the largest part (61·0 per cent) was the glucuronide of this drug, 11·5 per cent was unchanged and the remainder was *O*-methylated trimetoquinol and its glucuronide. When [^{3}H]isoproterenol was injected i.v. into guinea pigs, of the radioactivity in the 4 hr urine, the largest part (55·0 per cent) was the glucuronide of *O*-methylated isoproterenol, 10 per cent was unchanged and the remainder was either *O*-methylated isoproterenol or the glucuronide. Differential urinary and biliary excretion of trimetoquinol and its *d*-isomer was observed in both the rat and guinea pig. The *O*-methylation of the *l*- and *d*-isomers by liver homogenates of rat and guinea pig was relatively stereospecific for the *l*-isomer.

TRIMETOQUINOL [*l*-1-(3,4,5-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline][1] was shown by Iwasawa and Kiyomoto[2,3] to have very strong bronchodilating activity as compared with isoproterenol. The present investigation was undertaken to determine the distribution, excretion and metabolism of [^{3}H]trimetoquinol in the guinea pig.

Since the *l*-isomer of trimetoquinol is a potent pharmacological agent (β-sympathomimetic agent) while the *d*-isomer has little or no activity,[2] a difference in the metabolic fate of the stereoisomers may help to explain the biochemical processes that determine the drug's action. The differential metabolism and excretion of the stereoisomers in rats and guinea pigs were studied. Axelrod and Tomchick[4] reported that the rates of *O*-methylation of epinephrine by a liver-soluble fraction were the same for the two optical isomers. However, the present work showed that *O*-methylation of trimetoquinol in rat and guinea pig was greater for the *l*-isomer than for the *d*-isomer.

METHODS

Materials

[^{3}H]trimetoquinol, labeled with tritium as indicated in Fig. 1, was prepared by the catalytic hydrogenation of brominated trimetoquinol (obtained by bromination of trimetoquinol) with 2 c of tritium gas as described earlier.[5] [^{3}H]trimetoquinol has a specific activity of 0·66 mc/mg and was more than 97·0 per cent pure. The *d*-isomer of

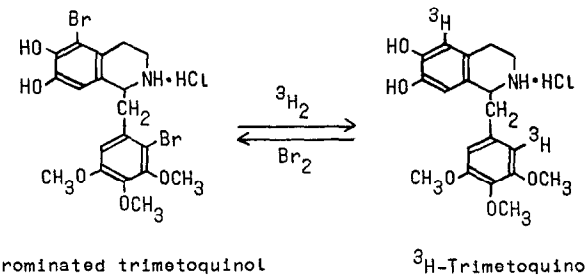

FIG. 1. [^{3}H]trimetoquinol and the nonradioactive brominated analog.

trimetoquinol was also labeled with tritium in the same manner. The [^{3}H]*d*-isomer has a specific activity of 1·55 mc/mg and was more than 98·0 per cent pure. [^{3}H]isoproterenol with a specific activity of 6·8 c/mM was obtained from New England Nuclear Corp.

Tissues distribution studies

Male Hartley guinea pigs weighing about 250 g were used for the distribution experiments. [^{3}H]trimetoquinol or [^{3}H]isoproterenol was injected into the femoral vein of guinea pigs in a dose of 250 mμ moles/kg. Guinea pigs were anesthetized with ether, and blood was drawn by cardiac puncture with heparinized syringes. The animals were then sacrificed and the entire brain, heart, lung, kidney, spleen, muscle, trachea and adrenal were removed. The tissues were rinsed, blotted, weighed and homogenized in 4 vol. of 0·1 N hydrochloric acid. The total radioactivity (which represents the sum of [^{3}H]trimetoquinol and its metabolites) in the supernatant fluid was determined; then it was alkalinized with 1 M K_2HPO_4 and extracted twice with 2 vol. of 10 per cent *n*-amyl alcohol in benzene. The extracts, which contained [^{3}H]trimetoquinol and *O*-methylated [^{3}H]trimetoquinol, were evaporated to dryness *in vacuo* and dissolved in methanol. Radioactivity in the methanol solution was assayed and aliquots were applied on Toyo roshi No. 51 filter paper. The chromatograms were developed ascending with chloroform–acetic acid–water (2:1:1, v/v). Two spots of radioactivity were obtained on the chromatograms after a scan of 1-cm strips. One spot had the same R_f value (0·24) as authentic [^{3}H]trimetoquinol; the other corresponded in R_f value (0·94) to [^{3}H]*O*-methylated trimetoquinol (synthesized enzymatically by incubation of [^{3}H]trimetoquinol with the rat liver enzyme preparation, S-adenosylmethionine and $MgCl_2$ according to the method of Axelrod and Tomchick[4]). Isotope dilution also confirmed that the radioactive compound in the former spot (R_f 0·24) was unchanged [^{3}H]trimetoquinol. Therefore, the isolation of unchanged [^{3}H]trimetoquinol from blood and tissue homogenates was accomplished by combining solvent extraction and paper chromatography. Controls were carried through

the whole procedure. The isolation of [^{3}H]isoproterenol from its metabolites was carried out by column chromatography using alumina and Dowex 50 according to the procedure of Hertting.[6]

Collection of bile, urine and faeces

Polyethylene catheters were inserted into the common bile ducts of guinea pigs anesthetized with sodium pentobarbital. After intravenous injection of [^{3}H]trimetoquinol or [^{3}H]isoproterenol, the bile was collected at various intervals up to 2 hr. The collected bile was diluted with water to a fixed volume and assayed for radioactivity. Guinea pigs injected intravenously with [^{3}H]trimetoquinol or [^{3}H]isoproterenol were placed in cages constructed to permit the separate collection of urine and faeces. Urine and faeces were collected at various intervals up to 48 hr. Faeces were homogenized in 10 vol. of 0·1 N HCl, and the supernatant was assayed for radioactivity. Urine was diluted with water to a fixed volume and measured for radioactivity. Biliary and urinary excretion of two optical isomers of trimetoquinol were studied comparatively in male guinea pigs and male Wistar strain rats weighing about 200 g.

Urinary and biliary metabolites

Urine samples were collected for 4 hr and bile samples were collected for 2 hr after intravenous injection of [^{3}H]trimetoquinol into guinea pigs, and both samples were analysed for metabolites. Solvent extraction and paper chromatography were used for the separation of urinary and biliary metabolites. Solvent extraction was done with 10 per cent *n*-amyl alcohol in benzene after the sample was alkalinized with aqueous ammonia. The solvent systems used for ascending paper chromatography were: (A) *n*-butanol–acetic acid–water (4:1:5, v/v); and (B) chloroform–acetic acid–water (2:1:1, v/v). After radioactive scanning of the chromatograms, the radioactive areas were cut off and eluted with water. The eluted materials were rechromatographed with the authentic sample in another solvent. Enzymatic hydrolysis of the conjugates was effected with β-glucuronidase (Sigma Chemical Company).

For studies of stereoselective metabolism, biliary and urinary metabolites of two optical isomers in guinea pigs and rats were also analysed quantitatively by combining solvent extraction and paper chromatography.

For paper chromatographic analyses of metabolites of [^{3}H]isoproterenol, a 4-hr urine sample of guinea pigs given [^{3}H]isoproterenol was used. Quantification of urinary metabolites of [^{3}H]isoproterenol was accomplished by column chromatography using alumina and Dowex 50 according to the procedure of Hertting.[6]

O-methylation of optical isomers by liver homogenates of rat and guinea pig

The livers of rat or guinea pig were homogenized in 3 vol. of ice-cold 1·15 per cent KCl and centrifuged at 9000 *g* for 20 min in a refrigerated centrifuge. The supernatant fraction was used for the metabolism of two optical isomers of trimetoquinol. The final composition of the incubation mixture was: 50 μmoles phosphate buffer, pH 7·8; 10 μmoles $MgCl_2$; 0·5 ml homogenate; 0·033–0·33 μmole substrate; 0·5 μmole S-adenosylmethionine, and sufficient water to make a final volume of 1 ml. The mixture was incubated for 10 min at 37° in an air. (The reaction was linear for at least 15 min.) After the incubation, 4 ml of 0·4 N perchloric acid was added to the mixture and centrifuged. The supernatant fluid was neutralized with 0·4 N potassium hydroxide

and extracted twice with 2 vol. of 10 per cent *n*-amyl alcohol in benzene. The extracts were analysed for unchanged trimetoquinol and its *O*-methylated derivative by paper chromatography in the solvent system, chloroform–acetic acid–water (2:1:1, v/v).

For analysis of enzyme kinetic data, reciprocal velocities were plotted against the reciprocals of substrate concentration, as described by Lineweaver and Burk.[7]

Measurement of radioactivity

An Aloka liquid scintillation spectrometer LSC-502 equipped with an automatic quenching monitor system was used for assay of radioactivity. Detection of the metabolites on paper chromatograms was carried out using an Aloka chromatogram scanner TRM-1B. Quantitative estimation of [^{3}H]trimetoquinol and its metabolites involved dividing the paper chromatograms into 10-mm sections, extracting each segment with 1 ml of 50 per cent methanol in a counting vial, and adding 15 ml of scintillation fluid to the extract.[8] The scintillation fluid used for aqueous samples was made by dissolving 7·0 g 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene in 1 l. of 50 per cent ethanol–toluene.

RESULTS

Tissue distribution of [^{3}H]trimetoquinol and [^{3}H]isoproterenol in the guinea pig

Table 1 shows the tissue levels of unchanged [^{3}H]trimetoquinol and [^{3}H]isoproterenol at various times after the intravenous injection of the corresponding compounds

TABLE 1. DISTRIBUTION OF [^{3}H]TRIMETOQUINOL AND [^{3}H]ISOPROTERENOL IN VARIOUS ORGANS OF THE GUINEA PIG AFTER INTRAVENOUS INJECTION OF THE CORRESPONDING COMPOUNDS (250 mμmoles/kg)*

	10 min		30 min		60 min		120 min	
	TMQ	Iso	TMQ	Iso	TMQ	Iso	TMQ	Iso
	(μμmoles/g)		(μμmoles/g)		(μμmoles/g)		(μμmoles/g)	
Blood	27	35	6	9	3·0	2·5	1·5	1·0
Liver	5	35	2	14	1·0	1·0	1·5	0·5
Kidney	704	1620	152	560	50	40	19	3·5
Muscle	71	68	53	43	27	14	16	3·5
Spleen	315	37	118	21	45	10	16	3·5
Heart	135	61	47	19	15	6	8	2·5
Lung	176	94	95	47	47	21	27	5·0
Adrenal gland	68	32	11	4	3·0	1·0	n.m.	n.m.
Trachea	70	33	11	4	4·0	1·5	n.m.	n.m.
Brain	0·1	0·1	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.

* The data represent the mean of three to four animals. TMQ, trimetoquinol; Iso, isoproterenol; n.m., not measurable.

to the guinea pig. Ten minutes after the injection of [^{3}H]trimetoquinol, the highest concentration was found in the kidney. Spleen, lung and heart had appreciable levels, whereas a very low level was present in the brain. The levels of [^{3}H]trimetoquinol in the blood were lower than those in tissues except the brain and liver. This indicates that the drug has a marked affinity to the tissues.

The pattern of the distribution of [^{3}H]isoproterenol was found to be essentially similar to that of [^{3}H]trimetoquinol. However, 10 min after the injection, the levels of [^{3}H]isoproterenol in tissues, except the blood, liver and kidney, were lower than those of [^{3}H]trimetoquinol.

Figure 2 shows the time course of [^{3}H]trimetoquinol and its metabolites in the blood after the intravenous injection of [^{3}H]trimetoquinol into the guinea pig. [^{3}H]trimetoquinol and its metabolites disappeared rapidly from the blood. The percentage

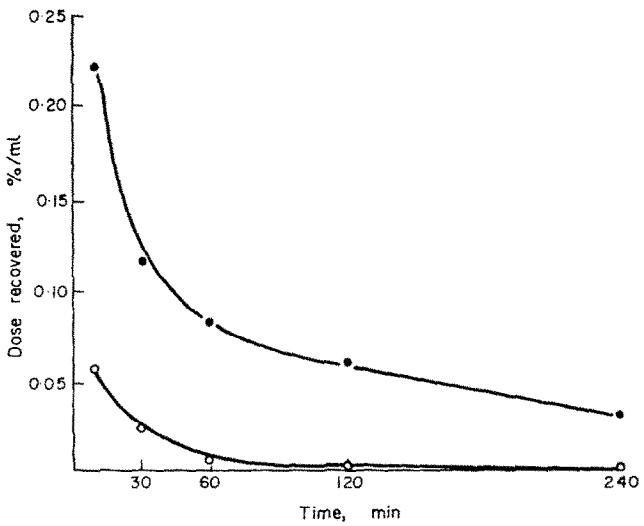

FIG. 2. Time course of the contents of [^{3}H]trimetoquinol and its metabolites in the blood of guinea pigs after i.v. injection of [^{3}H]trimetoquinol. —○—, [^{3}H]trimetoquinol; —●—, [^{3}H]metabolites. Each point indicates the mean of experiments with three to four animals.

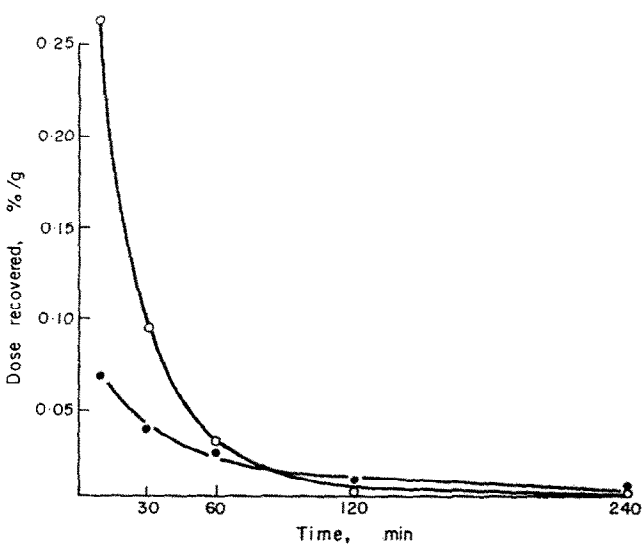

FIG. 3. Time course of the contents of [^{3}H]trimetoquinol and its metabolites in the heart of guinea pigs after i.v. injection of [^{3}H]trimetoquinol. —○—, [^{3}H]trimetoquinol; —●—, [^{3}H]metabolites. Each point indicates the mean of experiments with three to four animals.

of unchanged [^{3}H]trimetoquinol was about 20 per cent of the total radioactivity in the blood at 10 min and decreased to less than 5 per cent at 60 min. These data indicate that this drug is rapidly metabolized and excreted. Figure 3 shows the time course of [^{3}H]trimetoquinol and its metabolites in the heart. In the heart the percentage of [^{3}H]trimetoquinol was higher than that of the metabolites up to 60 min. The ratios of [^{3}H]trimetoquinol to its metabolites in the heart were apparently different from those in the blood. The time course of [^{3}H]trimetoquinol and its metabolites in the other tissues including trachea, adrenal gland, muscle and lung was similar to that in the heart.

Biliary, urinary and faecal excretion of tritium after injection of [^{3}H]trimetoquinol or [^{3}H]isoproterenol

Figure 4 shows the cumulative excretion of tritium in the bile up to 2 hr after the intravenous injection of [^{3}H]trimetoquinol or [^{3}H]isoproterenol into the guinea pig. When [^{3}H]trimetoquinol was administered, approximately 60 per cent of the radioactivity was excreted in the bile within 2 hr; when [^{3}H]isoproterenol was administered,

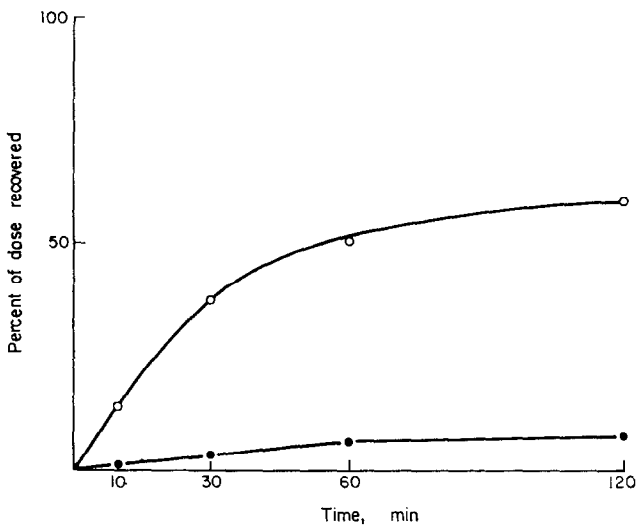

FIG. 4. Cumulative excretion of [^{3}H] in the bile of guinea pigs after i.v. injection of [^{3}H]trimetoquinol or [^{3}H]isoproterenol. —○—, [^{3}H]trimetoquinol; —●—, [^{3}H]isoproterenol. Each point indicates the mean of two to four animals.

only 8 per cent was excreted in the bile. Figure 5 shows the cumulative excretion of tritium in the urine and faeces up to 48 hr after intravenous injection of [^{3}H]trimetoquinol or [^{3}H]isoproterenol. Forty-two per cent of the radioactivity after the administration of [^{3}H]trimetoquinol was excreted in 48 hr in urine, and most of the urinary excretion occurred in the first 24 hr. Forty-nine per cent of the radioactivity was excreted in 48 hr in faeces. Consequently, about 90 per cent of the administered radioactivity was excreted in the urine and faeces within 48 hr. In contrast to [^{3}H]-trimetoquinol, 87 per cent of the radioactivity after the administration of [^{3}H]isoproterenol was excreted in the urine of guinea pigs within 48 hr. This reveals that the

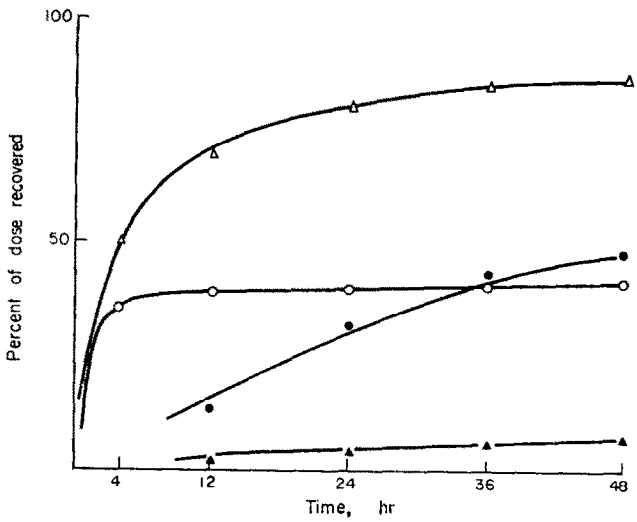

FIG. 5 Cumulative excretion of total ^3H in the urine and faeces of guinea pigs after i.v. injection of [^3H]trimetoquinol or [^3H]isoproterenol. [^3H]trimetoquinol: —○—, ^3H in urine; —●—, ^3H in faeces. [^3H]isoproterenol: —△—, ^3H in urine; —▲—, ^3H in faeces. Each point indicates the mean of two to four animals.

elimination of isoproterenol and its metabolites from the body of the guinea pig occurs mainly by way of the kidney into the urine.

Urinary metabolites of [^3H]trimetoquinol and [^3H]isoproterenol in the guinea pig

Table 2 shows the results of paper chromatographic analyses of the urine sample collected for 4 hr after the intravenous injection of [^3H]trimetoquinol. When the 4-hr urine sample of guinea pigs given [^3H]trimetoquinol was subjected to paper chromatography in solvent system A, four radioactive components usually were observed. When the urine sample was adjusted to pH 7·0 and then extracted with

TABLE 2. R_f VALUES OF URINARY METABOLITES OF [^3H]TRIMETOQUINOL*

	R_f values			
	Urine sample		β-Glucuronidase-treated sample†	
Peak No.	Solvent A	Solvent B	Solvent A	Solvent B
1	0·25	0·0	0·70	0·24
2	0·38	0·0	0·78	0·94
3	0·70	0·24	0·70	0·24
4	0·78	0·94	0·78	0·94

* Urine samples were collected for 4 hr after i.v. injection of [^3H]trimetoquinol into guinea pigs. Solvent system A was *n*-butanol–acetic acid–water (4:1:5, v/v); solvent system B was chloroform–acetic acid–water (2:1:1, v/v).

The R_f values of the authentic sample of [^3H]trimetoquinol were 0·70 in solvent system A and 0·24 in solvent system B respectively.

† The extracts from paper chromatograms were treated with 2000 Fishman units of β-glucuronidase in 0·2 M acetate buffer at pH 5·0 and rechromatographed.

10 per cent *n*-amyl alcohol in benzene, the radioactive components of peaks 3 and 4 were transferred into the organic solvent layer. Therefore, the radioactive materials of peaks 1 and 2 were assumed to be water-soluble metabolites of trimetoquinol. The radioactive materials of peaks 1 and 2 were eluted from paper chromatograms and incubated with β-glucuronidase in the presence or absence of saccharo-(1:4)-lactone, which is known to be a potent inhibitor of β-glucuronidase.[9] It was found that they were hydrolyzed only in the absence of the inhibitor. This fact indicated that the water-soluble metabolites of peaks 1 and 2 were glucuronide conjugates. The hydrolyzed compound of peak 1 had an R_f value (0·70) similar to that of peak 3 and that of peak 2 had an R_f value (0·78) similar to that of peak 4. The R_f value of peak 3 was identical to that of the authentic sample of [^{3}H]trimetoquinol. Co-chromatography in solvent system B and co-crystallization confirmed that the radioactive component of peak 3 was unaltered [^{3}H]trimetoquinol. Therefore, the radioactive material of peak 1 was assumed to be the glucuronide conjugate of [^{3}H]trimetoquinol.

Since the R_f value of peak 4 was higher than that of original [^{3}H]trimetoquinol in solvent systems A and B, the metabolite of peak 4 was thought to have a higher lipid solubility than trimetoquinol. The R_f value of the lipid-soluble metabolite was found to be similar to that of the *O*-methylated derivative of [^{3}H]trimetoquinol. It is well known that pyrogallol is a potent inhibitor of catechol *O*-methyl transferase (COMT).[10,11] Kopin *et al.*[12] reported that inhibition *in vivo* of COMT with pyrogallol blocked the *O*-methylation of epinephrine. Incidentally, when amounts of the metabolites of peak 4 and its glucuronide were compared in pyrogallol-treated and untreated guinea pigs, it was found that the percentage of the metabolite of peak 4 and its glucuronide in pyrogallol-treated guinea pigs decreased to about one-half of that in the untreated animals, as shown in Table 3. Therefore, it was assumed that the

TABLE 3. EFFECT OF PYROGALLOL UPON METABOLISM *in vivo* OF [^{3}H]TRIMETOQUINOL*

Peak no.	Corresponding compound	Percentage of urinary metabolites	
		Untreated	Pyrogallol-treated†
1	Glucuronide of trimetoquinol	22·0 ± 3·1 (61·0)	14·3 ± 2·0 (42·8)
2	Glucuronide of *O*-methylated trimetoquinol	7·5 ± 1·3 (21·3)	3·1 ± 0·4 (9·5)
3	Trimetoquinol	4·1 ± 0·5 (11·5)	14·5 ± 2·7 (44·3)
4	*O*-methylated trimetoquinol	2·3 ± 0·2 (6·2)	1·2 ± 0·2 (3·4)

* Urine samples were collected for 4 hr after i.v. injection of [^{3}H]trimetoquinol into guinea pigs and the samples were analysed by paper chromatography in solvent systems A and B. Results are expressed as the mean percentage of the administered radioactivity of unchanged [^{3}H]trimetoquinol and its metabolites found in the urine of each of four animals (± standard deviation). Figures in parentheses represent the per cent recovery of urinary radioactivity present as unchanged [^{3}H]trimetoquinol and its metabolites.

† Guinea pigs received 50 mg/kg of pyrogallol i.v. at 3 min before and at 15 and 30 min after [^{3}H]trimetoquinol (250 mμmoles/kg).

metabolite of peak 4 was an *O*-methylated derivative of trimetoquinol and hence the metabolite of peak 2 was a glucuronide of *O*-methylated trimetoquinol. As shown in Table 3, a major metabolite of trimetoquinol (61·0 per cent of the urinary radioactivity) in the urine sample of guinea pigs was the glucuronide conjugate of this

drug, and minor metabolites were *O*-methylated trimetoquinol and its glucuronide. The percentage of the unchanged drug in the 4-hr urine was 11·5 per cent.

When the 4-hr urine sample of guinea pigs given [^{3}H]isoproterenol was subjected to paper chromatography in solvent system A, three radioactive peaks were detected in the same positions as those reported by Hertting.[6] Peak 1 corresponded in R_f value (0·14) to a mixture of glucuronides of isoproterenol and *O*-methylated isoproterenol;

TABLE 4. R_f VALUES AND AMOUNTS OF URINARY METABOLITES OF [^{3}H]ISOPROTERENOL

R_f value†	Corresponding compound	Amount (%)
0·14	Conjugate of isoproterenol	17·2 (23·5)
0·14	Conjugate of *O*-methylated isoproterenol	41·2 (55·0)
0·61	Unchanged isoproterenol	7·5 (9·8)
0·70	*O*-methylated isoproterenol	11·3 (11·7)

* Urine samples were collected for 4 hr after i.v. injection of [^{3}H]isoproterenol into guinea pigs and the samples were analysed by column chromatography using alumina and Dowex 50. Results are expressed as the percentage of the administered radioactivity of unchanged [^{3}H]isoproterenol and its metabolites found in the urine of two animals. Figures in parentheses represent the per cent recovery of urinary radioactivity present as unchanged [^{3}H]isoproterenol and its metabolites.

† Paper chromatograms were developed in solvent system A, *n*-butanol–acetic acid–water (4:1:5, v/v).

peak 2 corresponded in R_f value (0·61) to unchanged isoproterenol; and peak 3 corresponded in R_f value (0·70) to *O*-methylated isoproterenol. Table 4 shows the percentages of radioactive metabolites in the 4-hr urine of guinea pigs given [^{3}H]isoproterenol. Of the radioactivity in the 4-hr urine sample, the largest part (55·0 per cent) was the glucuronide of *O*-methylated isoproterenol. The glucuronide (23·5 per cent) of isoproterenol was a minor metabolite. The percentage of the unchanged drug in the 4-hr urine was 9·8 per cent.

Stereoselectivity in excretion and metabolism of trimetoquinol

Table 5 shows the urinary excretion of unchanged isomers and their metabolites for 24 hr after intravenous administration of [^{3}H]trimetoquinol (*l*-isomer) or [^{3}H]*d*-isomer to rats and guinea pigs. The urinary excretion of total radioactivity was higher in rats than in guinea pigs; with the *l*-isomer, the former was 1·5 times higher than the latter, and with the *d*-isomer, the former was 2·5 times higher. The composition of the radioactivity recovered from urine was also significantly different with the two optical isomers; in the rat, slightly more radioactivity was excreted after administration of the *d*-isomer. By contrast, in the guinea pig, less radioactivity was excreted after administration of the *d*-isomer. These data indicate that both stereoselectivity and species differences exist in the urinary excretion of two optical isomers and their metabolites. As shown in Table 5, in the rat, the major metabolites of both optical isomers were glucuronides of the *O*-methylated compounds, while those in the guinea pig were glucuronides of unaltered isomers. This indicates that *O*-methylation of the two optical isomers occurs more readily in the rat than in the guinea pig.

TABLE 5. STEREOSELECTIVITY IN URINARY EXCRETION OF [^{3}H]TRIMETOQUINOL AND ITS METABOLITES BY THE RAT AND GUINEA PIG*

Drug and metabolites	Rat		Guinea pig	
	l-isomer (%)	*d*-isomer (%)	*l*-isomer (%)	*d*-isomer (%)
Total tritium recovered	58·4 ± 6·5	69·6 ± 7·8	40·4 ± 5·3	27·4 ± 3·1
Unchanged drug	0·5 ± 0·1†	5·2 ± 0·9	4·4 ± 0·6	5·6 ± 0·8
O-methylated drug	4·8 ± 0·7	10·8 ± 1·5	2·4 ± 0·3	1·7 ± 0·2
Conjugate of unchanged drug	17·5 ± 3·0	23·6 ± 3·9	24·9 ± 4·1	16·8 ± 2·9
Conjugate of *O*-methylated drug	35·6 ± 5·1	30·0 ± 4·6	8·7 ± 1·5	3·3 ± 0·4

* Urine samples were collected for 24 hr after i.v. injection of the [^{3}H]*l*-isomer or [^{3}H]*d*-isomer and the samples were analysed by paper chromatography. Results are expressed as the mean percentage of the administered radioactivity of the unchanged drug and its metabolites found in the urine of each of four animals (± standard deviation).

† $P < 0.01$ compared with the *d*-isomer.

In the rat, urinary excretion of the two unchanged isomers differed greatly; in the guinea pig, urinary excretion of the two isomers differed to a lesser extent. Urinary excretion of unaltered isomers in rats was significantly greater for the *d*-isomer than for the *l*-isomer.

Table 6 shows stereoselectivity in biliary excretion of metabolites of the two isomers. More than 99 per cent of the radioactivity excreted in the 2-hr bile was present as

TABLE 6. STEREOSELECTIVITY IN BILIARY EXCRETION OF [^{3}H]TRIMETOQUINOL AND ITS METABOLITES BY THE RAT AND GUINEA PIG*

Drug and metabolites	Rat		Guinea pig	
	l-isomer (%)	*d*-isomer (%)	*l*-isomer (%)	*d*-isomer (%)
Total tritium recovered	59·5 ± 7·1	54·1 ± 6·2	58·9 ± 6·3	69·8 ± 7·7
Unchanged drug	negligible	negligible	negligible	negligible
O-methylated drug	negligible	negligible	negligible	negligible
Conjugate of unchanged drug	10·0 ± 1·6	17·5 ± 2·8	33·0 ± 4·7	49·5 ± 6·3
Conjugate of *O*-methylated drug	49·5 ± 5·9†	36·6 ± 5·1	25·9 ± 4·3	20·3 ± 3·5

* Bile samples were collected for 2 hr after i.v. injection of the [^{3}H]*l*-isomers or [^{3}H]*d*-isomers and the samples were analyzed by paper chromatography. Results are expressed as the mean percentage of the administered radioactivity of the metabolites found in the bile of each of four animals (± standard deviation).

† Significantly different ($P < 0.05$) from the comparable value for the *d*-isomer.

glucuronides of unchanged and *O*-methylated isomers. In contrast to the urinary excretion of unchanged isomers, the biliary excretion of glucuronide conjugates of the *O*-methylated isomers in rats was significantly greater with the *l*-isomer than with the *d*-isomer.

O-methylation of [^{3}H]trimetoquinol and its isomers by liver homogenates of the rat and guinea pig

Table 7 shows kinetic data in the *O*-methylation of the two optical isomers by liver homogenates of the rat and guinea pig. *O*-methylation of the two optical isomers by

TABLE 7. K_m AND V_{max} VALUES OF *O*-METHYLATION OF [^{3}H]TRIMETOQUINOL AND ITS ISOMER BY LIVER HOMOGENATES OF THE RAT AND GUINEA PIG*

	Rat		Guinea pig	
	l-isomer	*d*-isomer	*l*-isomer	*d*-isomer
K_m (M × 10^{-4})	2·54 ± 0·42†	1·56 ± 0·38	0·98 ± 0·18†	0·66 ± 0·13
V_{max} (μmoles/g/hr)	5·51 ± 0·88†	3·63 ± 0·76	1·74 ± 0·23†	1·30 ± 0·17

* The reaction mixture contained liver homogenate (125 mg liver), 0·033 to 0·33 μmole [^{3}H]*l*-isomer or [^{3}H]*d*-isomer, 10 μmoles $MgCl_2$, 0·5 μmole S-adenosylmethionine, 50 μmoles phosphate buffer at pH 7·8, and water to make a final volume of 1 ml and was incubated for 10 min. Data represent the mean (± standard deviation) of five experiments.

† Significantly different ($P < 0·05$) from comparable values for the *d*-isomer.

liver homogenates took place more readily with rat than with guinea pig. This was in good agreement with the data *in vivo*.

It was noticed that values of the Michaelis constant (K_m) and maximal velocity (V_{max}) for the *l*-isomer and *d*-isomer were statistically different from each other in both animals. The data *in vitro* indicated that the *l*-isomer was *O*-methylated more readily than the *d*-isomer.

DISCUSSION

Hertting[6] demonstrated that isoproterenol was metabolized in the rat both by *O*-methylation and by conjugation with glucuronic acid. The results of the present study also showed that in the guinea pig isoproterenol was converted to the same metabolites as those identified in the rat. Since trimetoquinol, like isoproterenol, has a catechol moiety in its molecule, it was supposed that this drug might be metabolized similarly. Previously, Satoh *et al.*[13] demonstrated by spectroscopic analyses that a lipid-soluble metabolite of trimetoquinol was the *O*-methylated derivative and that the *O*-methylation occurred at either the 6- or 7-hydroxyl group. The present studies indicate that the metabolic pathways of trimetoquinol and isoproterenol are essentially the same, but that the extent of *O*-methylation and conjugation for trimetoquinol and isoproterenol are different. As shown in Tables 3 and 4, isoproterenol is *O*-methylated to a greater extent than is trimetoquinol in the guinea pig.

The elimination of trimetoquinol and isoproterenol also differed significantly in the guinea pig. The excretion of isoproterenol took place mainly by way of the kidney into urine, whereas the excretion of trimetoquinol occurred both by way of the kidney into the urine and by way of the liver into faeces. The great difference in elimination of the two drugs was attributed to the fact that about 60 per cent of the administered radioactivity was excreted in 2 hr in the bile for trimetoquinol, while only 8 per cent was excreted for isoproterenol.

From the results described above, it may be considered that there are two great differences in the fate of trimetoquinol and isoproterenol in guinea pigs: (1) the major metabolite of trimetoquinol is the glucuronide conjugate of the original drug, while that of isoproterenol is the glucuronide conjugate of the *O*-methylated drug; (2) the elimination of trimetoquinol from the body is accomplished by both urine and faeces, while isoproterenol and its metabolites are excreted mainly into the urine.

Axelrod and Tomchick[4] reported that the rats of *O*-methylation of epinephine were the same for the two optical isomers. However, as shown in Table 7, the *O*-methylation of trimetoquinol and its isomer by the liver homogenates was significantly greater for the *l*-isomer than for the *d*-isomer. Furthermore, the biliary excretion of *O*-methylated isomers was found to be higher in the *l*-isomer than in the *d*-isomer. From a consideration of these facts, it seems that *O*-methylation of the two optical isomers is relatively stereospecific for the *l*-isomer.

The present work, concerning the urinary excretion of unchanged trimetoquinol and its isomer, showed that less of the *l*-isomer was excreted in the urine of the rat, although the difference in the urinary excretion of the two optical isomers was very small in the guinea pig. Several mechanisms may account for the differences in excretion of the two isomers: there may be a selective uptake of the *l*-isomer from the blood into the tissues, combined with a stereospecific catabolism of the *l*-isomer, or a prolonged retention of the unmetabolized *l*-isomer, followed by a slower excretion at a later time. A stereospecific uptake of the *l*-isomer into tissues appears unlikely from results of the autoradiographic studies,[5] which showed that the uptake of radioactivity into the tissues of mice was the same for the two optical isomers. The fact that a great difference in the urinary excretion of the two unchanged isomers was observed in the rat rather than in the guinea pig might be explained by taking account of the fact that the stereospecific *O*-methylation occurred much more readily in the rat than in the guinea pig. Therefore, it seems most likely to assume that the lesser excretion of the *l*-isomer is due to stereospecific metabolism of the *l*-isomer.

From the results of excretion and metabolism, it is evident that the fate of the two optical isomers of trimetoquinol in the body of animals is not identical. How this is related to the difference in their pharmacological activity is not clear at present.

Acknowledgements—The authors wish to express their deep gratitude to Dr. K. Abe, Director of this Research Laboratory, for his interest and encouragement throughout this work. The technical assistance of Miss K. Node is gratefully recognized.

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