The Metabolism of *N*-Triphenylmethylmorpholine in the Dog and Rat

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1. Rats were given *N*-triphenyl[^{14}C]methylmorpholine, triphenyl[^{14}C]carbinol, *N*-triphenylmethyl[$\text{G-}^3\text{H}$]morpholine or [$\text{G-}^3\text{H}$]morpholine as single oral doses; the routes of excretion were examined. 2. Dogs were given single oral doses of *N*-triphenyl[^{14}C]methylmorpholine. 3. ^{14}C -labelled metabolites were excreted mainly in the faeces in both rats and dogs; no $^{14}\text{CO}_2$ was expired and less than 3% remained in the carcass and skin after 96 hr. 4. ^3H -labelled metabolites were excreted rapidly in urine; part of the label was found in the expired gases and over 10% remained in the carcass and skin after 96 hr. 5. Differences in excretion pattern between the sexes were noticed in rats but not in dogs. 6. *N*-Triphenylmethylmorpholine was rapidly hydrolysed to form triphenylcarbinol and morpholine in the stomach; morpholine was absorbed rapidly and excreted largely unchanged, though some was degraded, since some of the ^3H was found in water. 7. Triphenylcarbinol was absorbed only slowly and was oxidized to *p*-hydroxyphenyldiphenylcarbinol. 8. Both triphenylcarbinol and its *p*-hydroxy derivative were found in urine, bile and faeces in the free form and conjugated with glucuronic acid. The proportion of conjugates was higher in rat bile than in faeces. 9. Traces of *o*-hydroxyphenyldiphenylcarbinol and *m*-hydroxyphenyldiphenylcarbinol were detected as metabolites both free and conjugated.

N-Triphenylmethylmorpholine (I) [also known as trityl morpholine, WL 8008 and Frescon (Registered Trade Mark of Shell International Chemical Co. Ltd.)] (Boyce & Manis, 1962) is a very powerful and specific molluscicide that is used for the control of some aquatic and semi-aquatic snails, the intermediary hosts of *Schistosoma* species (Boyce, Crossland & Shiff, 1966; Crossland, 1967) and of the liver fluke (*Fasciola hepatica*). The compound is applied to pasture either as a granule or as an emulsifiable concentrate, and to water as a spray of the concentrate or as a drip-feed into the headwaters of an irrigation scheme.

This paper describes some investigations into the metabolic fate of this compound in dogs and rats.

MATERIALS

All melting points, which were determined on a Kofler block, are uncorrected.

N-Triphenylmethylmorpholine. This was synthesized by refluxing triphenylmethyl chloride and morpholine in benzene. It was washed with water to remove morpholine hydrochloride and was crystallized, m.p. 185–186°, from ethanol.

Triphenylcarbinol and morpholine. These compounds were obtained from British Drug Houses Ltd., Poole, Dorset.

Triphenyl[^{14}C]carbinol. Triphenyl[^{14}C]carbinol (sp. radioactivity 9.2 $\mu\text{C}/\text{mg.}$) was synthesized via a Grignard reaction from [carboxy- ^{14}C]methyl benzoate and was purified by column chromatography on silica gel.

N-Triphenyl[^{14}C]methylmorpholine. This material (sp. radioactivity 7.3 $\mu\text{C}/\text{mg.}$) was synthesized by condensing morpholine with triphenyl[^{14}C]methyl chloride, and was purified by column chromatography on alumina.

[$\text{G-}^3\text{H}$]Morpholine. [$\text{G-}^3\text{H}$]Morpholine hydrochloride, supplied by The Radiochemical Centre, Amersham, Bucks., was converted into the free base by making a solution strongly alkaline, and was then extracted into benzene. When diluted with carrier it had a sp. radioactivity of 1 $\mu\text{C}/\text{mg.}$

N-Triphenylmethyl[$\text{G-}^3\text{H}$]morpholine. This compound (sp. radioactivity 47 $\mu\text{C}/\text{mg.}$) was prepared by the reaction of triphenylmethyl chloride with [$\text{G-}^3\text{H}$]morpholine.

o-Hydroxyphenyldiphenylcarbinol. This compound was synthesized from methyl salicylate and phenylmagnesium

bromide by the method of Baeyer (1907). The solid product was dissolved in ether and was washed with 5% (w/v) Na_2CO_3 , boiled with Norit charcoal and filtered. The filtrate, dried with CaCl_2 , was concentrated to obtain the carbinol, which crystallized from light petroleum (b.p. 60–80°). The crystals melted at 141.5° with decomposition.

m-Hydroxyphenyldiphenylcarbinol. This compound was synthesized by the method of Baeyer (1907) from methyl *m*-hydroxybenzoate and phenylmagnesium bromide. The crude product was dissolved in benzene, boiled with Norit charcoal and filtered. Light petroleum (b.p. 60–80°) was added to the filtrate and, on standing for several days, *m*-hydroxyphenyldiphenylcarbinol, m.p. 146–148°, crystallized.

p-Hydroxyphenyldiphenylcarbinol. This compound was prepared from benzophenone chloride and phenol by the method of Gomberg & Jickling (1915). The crude product was dissolved in ethanol containing a few drops of aq. NH_3 (sp.gr. 0.88) (6ml. of ethanol/g. of the carbinol). Insoluble material (dihydroxytetraphenylmethane) was filtered off. Water was added to the filtrate until a permanent turbidity was obtained, and, on standing, the carbinol crystallized (m.p. 160°).

Bovine liver β -glucuronidase. This enzyme [lg.; 560000 Fishman units (Fishman, Springer & Brunetti, 1948)] was obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Reagents and solvents. All reagents and solvents were of A.R. grade.

METHODS

Experiments with animals. Young adult rats (approx. 2 months old, 150–200g. body wt., C.F.E. strain maintained as a specific pathogen-free colony in this Laboratory) were used. During the metabolic experiments they were housed in all-glass metabolism cages as described by Wright, Akintonwa, Crowne & Hathway (1965) and were given unlimited food and water [the food was prepared in stick form by baking Dixon 86 power diet (90g.) with one egg for 1½ hr.]. Radioactive compounds were given orally by using a syringe fitted with an intubation needle, and the animals were not starved beforehand. The closed cages were ventilated at 300–400ml./min. and the exhaled CO_2 was collected in 4*N*-NaOH. Exhaled H_2O was collected in water. Urine and faeces and expired gases were collected every 24 hr. and processed for measurement of radioactivity immediately. After 4 days the rats were killed and the skin (plus muzzle, feet and tail), alimentary canal, and carcass and remaining viscera were examined for radioactivity.

Adult beagle hounds, 6–14kg. body wt., maintained for 7 years as a closed colony in this Laboratory, were also used for metabolic experiments. They were housed in stainless-steel metabolism cages designed to separate urine and faeces, and were fed once a day on a proprietary meat dog-food and given a bowl of water. The animals were acclimatized to their cages for 7 days and were dosed with radioactive compounds in gelatin capsules. Urine and faeces were collected every 24 hr. and the experiment was terminated after 96 hr.

The following sets of experiments were carried out.

Expt. 1. Six male and six female rats were each given 1.2mg. of *N*-triphenyl[^{14}C]methylmorpholine (7.3 μC /mg.) in 1ml. of arachis oil.

Expt. 2. Three male and three female rats were each given

0.61mg. of triphenyl[^{14}C]carbinol (9.2 μC /mg.) in 1ml. of arachis oil.

Expt. 3. Six male and six female rats were each given 0.67mg. of *N*-triphenylmethyl[$\text{G-}^3\text{H}$]morpholine (47 μC /mg.) in 1ml. of arachis oil.

Expt. 4. Three male and three female rats were each given 50mg. of [$\text{G-}^3\text{H}$]morpholine (1 μC /mg.) in 1ml. of water.

Expt. 5. Three male and three female beagle hounds were each given a gelatin capsule containing 1.2mg. of *N*-triphenyl[^{14}C]methylmorpholine (7.3 μC /mg.) in 1ml. of arachis oil.

Expt. 6. One male rat was given 0.67mg. of *N*-triphenylmethyl[$\text{G-}^3\text{H}$]morpholine in 1ml. of arachis oil and was placed in a restraining cage (Bollman, 1948) mounted so that the urine passed through a funnel into the tubes of a fraction collector. Urine was collected every 45 min. and monitored for radioactivity.

Expt. 7. Two male rats were each given 1ml. of $^3\text{H}_2\text{O}$ (5 μC /ml.) and were treated as in Expt. 6.

Expt. 8. Two rats (one male and one female) were each dosed orally with 4mg. of *N*-triphenyl[^{14}C]methylmorpholine in 2ml. of arachis oil. Biliary fistulae were established in both animals (Boyland, Ramsey & Sims, 1961) and after recovery from the operation the animals were placed in individual restraining cages with access to food and water. Bile and urine were collected for 24 hr.; the animals were then killed and the alimentary tracts and carcasses were examined for radioactivity.

Expt. 9. Biliary fistulae were established in a further two rats (one male and one female) and these were each dosed intraperitoneally with 1.6mg. of *N*-triphenyl[^{14}C]methylmorpholine in 1ml. of *NN*-dimethylformamide. The animals were treated as in Expt. 8 except that the radioactivity in the alimentary tracts was not measured.

Expt. 10. Two rats (one male and one female) were anaesthetized and each pylorus was ligated by the method of Shay, Sun & Gruenstein (1954). After recovery from the operation the animals were dosed with 0.54 μC of *N*-triphenyl[^{14}C]methylmorpholine in 1ml. of arachis oil and were placed in restraining cages with access to food and water. After 6 hr. the animals were killed and the contents of the stomach were washed out with water. This was then extracted twice with benzene and monitored for radioactivity.

Extraction of metabolites. Two separate solvent extracts of the pooled days 1 and 2 urines from rats given *N*-triphenyl[^{14}C]methylmorpholine were made by continuous extraction with ether at pH 7 and pH 2. A further extract of the acid urine was made with butan-1-ol. Approx. 30% of the ^{14}C was extracted at pH 7 and 85% at pH 2. A further 3% was extracted with butanol. The extracts were evaporated under reduced pressure, and the residues of each were taken up in a small volume of acetone. Similar proportions of total ^{14}C (40% and 82%) were extracted with ether from the urine of rats dosed with triphenyl[^{14}C]carbinol. About 52% of the radioactivity could be extracted at pH 7 from the urine of the dogs given *N*-triphenyl[^{14}C]methylmorpholine.

Urine from rats dosed with *N*-triphenylmethyl[$\text{G-}^3\text{H}$]morpholine was extracted with ether on separate occasions at pH 2, pH 7 and pH 13: 2.3% of the radioactivity was extracted at pH 2, 12.4% at pH 7 and 20.7% at pH 13. About 75% of the ^3H could be extracted into toluene when NaOH was added to the urine at a final concentration of

30% (w/v); 80% of the ^3H could be extracted in this way from the urine of rats given [$\text{G-}^3\text{H}$]morpholine. Samples of the urine from rats dosed with *N*-triphenylmethyl[$\text{G-}^3\text{H}$]morpholine were acidified to pH 1 and then distilled at 100° .

Bile samples from rats dosed orally and intraperitoneally with *N*-triphenyl[^{14}C]methylmorpholine were extracted with ether at pH 7 and with ether-ethanol (3:1, v/v), at pH 2; 18% of the ^{14}C was extracted at pH 7 and 86% at pH 2. Other samples of bile were freeze-dried and the residue was extracted with methanol.

Samples of faeces from rats dosed orally with *N*-triphenyl[^{14}C]methylmorpholine were homogenized and extracted in a Soxhlet apparatus with acetone for 16 hr. The residue from the extraction was monitored for radioactivity. The acetone was evaporated under reduced pressure and the residue after evaporation was suspended in a small volume of light petroleum (b.p. $40\text{--}60^\circ$).

Measurement of radioactivity. A Packard Tri-Carb model 3003 liquid-scintillation spectrometer (Packard Instrument Ltd., Wembley, Middlesex) was used for measuring ^{14}C and ^3H in solid and liquid samples. Paper chromatograms were scanned with a Nuclear-Chicago Actigraph II gas-flow counter having a helium + *n*-butane (98.5:1.5) gas mixture. Thin-layer plates were scanned with a Desaga Radiochromatogram Scanner with a windowless gas-flow counter that used an argon + methane (90:10) gas mixture. Thin-layer plates were also examined by radioautography with Ilford Industrial G X-Ray film, ID 19 or PQX-1 developer and IF 9 fixer. The liquid scintillator used was based on that of Dobbs (1963) and was prepared from 1 l. of methanol, 1 l. of toluene, 1 l. of phenethylamine, 350 g. of naphthalene (molecular-weight-determination grade), 18 g. of 2,5-diphenyloxazole and 0.35 g. of 1,4-bis-(5-phenyloxazol-2-yl)-benzene. In this scintillator, ^{14}C was measured with a maximum efficiency of 70% and ^3H with an efficiency of 15%. Efficiency of radioactivity measurements was determined by the external-standard method.

Diluted urine was added directly to the scintillator. Faeces, gut and contents, skin, and carcass were homogenized with their own weight of water. For ^{14}C measurement, samples of the homogenates were dried and burnt in Schöniger flasks by the method of Dobbs (1963) and the $^{14}\text{CO}_2$ produced was absorbed in scintillator. For ^3H

measurements the wet homogenates were burnt at 750° in a tube furnace in a stream of O_2 (Griffiths & Mallinson, 1968). The $^3\text{H}_2\text{O}$ formed was collected in a chloroform-solid CO_2 -cooled trap and was washed out with scintillator. The $^{14}\text{CO}_2$ in the expired gases was absorbed successively in 4*N*-NaOH and in scintillator. The $^3\text{H}_2\text{O}$ in the expired gases was collected in a water trap and the water added directly to scintillator.

Paper chromatography. Labelled compounds in the solvent extracts of urine and bile from animals dosed with *N*-triphenyl[^{14}C]methylmorpholine and triphenyl[^{14}C]carbinol were separated by descending paper chromatography on Whatman no. 1 filter-paper strips at room temperature. Whatman no. 3MM paper was used for preparative chromatography. Butan-1-ol saturated with aq. 2*N*- NH_3 was used as developing solvent. The papers were dried and scanned for radioactivity. Phenolic marker compounds were run at the same time and were detected by spraying with Gibbs reagent [0.1% (w/v) 2,6-dichloro-*p*-benzoquinone-4-chloroimine in ethanol] and exposing the paper to NH_3 vapour. *N*-Triphenylmethylmorpholine and triphenylcarbinol were detected by spraying with 5% (w/v) trichloroacetic acid in acetone and heating to 115° for 5 min.

Thin-layer chromatography. Kieselgel G (E. Merck A.-G., Darmstadt, Germany) was layered on to standard 20 cm. $\times$ 20 cm. glass plates. The layer was 0.25 mm. thick for analytical work and 1 mm. thick for preparative chromatography. The R_F values of marker compounds, in those solvents found to be most suitable, are shown in Table 1. When plates were run in a reverse-phase system they were pre-run in 5% (v/v) liquid paraffin in ether. Fractions eluted from paper chromatograms with methanol were concentrated and marker compounds added to the concentrated material. The mixtures were then applied to the plates. After development, the marker compound was located by using the spray reagents described in the section on paper chromatography, and its position was compared with that of the radioactive peak. The fuchsone (II), the oxidation product of *p*-hydroxyphenyldiphenylcarbinol, was detected by its u.v. absorption and a bright-yellow colour when heated. The partial conversion of *p*-hydroxyphenyldiphenylcarbinol into the fuchsone by heating the

Table 1. R_F values of *N*-triphenylmethylmorpholine and some potential metabolites on thin-layer chromatography

Marker compounds were run in the solvents specified on thin-layer plates, thickness 0.25 mm., made from Kieselgel G. Compounds were: 1, *N*-triphenylmethylmorpholine; 2, triphenylcarbinol; 3, *o*-hydroxyphenyldiphenylcarbinol; 4, *m*-hydroxyphenyldiphenylcarbinol; 5, *p*-hydroxyphenyldiphenylcarbinol; 6, fuchsone.

Solvent ... Compound	R_F					Reverse phase: acetone-water (1:1, v/v)
	Ether-hexane (1:9, v/v)	Chloroform	Methanol- benzene (1:19, v/v)	Acetone- benzene (1:9, v/v)	Methanol- chloroform (3:97, v/v)	
1	0.48	0.69	—	—	—	0.31
2	0.35	0.64	0.80	—	—	0.35
3	0.15	0.53	0.58	0.65	0.85	0.41
4	0.05	0.11	0.25	0.45	0.60	0.56
5	0.05	0.11	0.25	0.45	0.60	0.69
6	—	0.25	0.45	0.60	—	0.59

(II)

thin-layer plate after one run was used as another method of identification. The plate was run a second time to separate the newly formed fuchson.

Solvent extracts of urine from animals dosed with *N*-triphenylmethyl[G - 3H]morpholine and [G - 3H]morpholine were also separated on Kieselgel G. Butan-1-ol-acetic acid-water (12:3:5, by vol.) and formic acid-methanol (1:49, v/v) were used as developing solvents and unlabelled morpholine, [G - 3H]morpholine and 3H_2O were used as marker compounds. Morpholine was detected by spraying with 0.05% eosin in ethanol and examining the plate under u.v. light. In the first solvent system morpholine had R_F 0.37 and in the second R_F 0.48; 3H_2O ran at the solvent front in formic acid-methanol (1:49, v/v).

Column chromatography. The urines from animals dosed with 3H -labelled compounds were chromatographed on 8 cm. $\times$ 1 cm. diam. columns of Bio-Rad AG 50W-X8 ion-exchange resin (H^+ form; 100–200 mesh). Metabolites were separated by elution with increasing concentrations of NaCl in water. Fractions from the column were monitored for radioactivity.

The light-petroleum suspension resulting from the extraction of faeces was applied to a silica-gel column [100 g. of silicic acid boiled in light petroleum (b.p. 40–60°) to remove air]. The radioactive metabolites were eluted with increasing concentrations of ether in light petroleum (b.p. 40–60°) followed by methanol in ether. Fractions were evaporated to a small volume under reduced pressure and portions were applied to thin-layer plates. The fractions were also monitored for radioactivity.

Low-voltage electrophoresis. Electrophoresis was carried out on strips of Whatman no. 1 paper at 15 v/cm. in 25 mm-potassium hydrogen phthalate buffer adjusted to pH 5.9 with 0.2N-NaOH. D-Glucose was used as a marker for the correction of electro-endosmotic flow. After electrophoresis the papers were dried in air at room temperature and then dried at 80°.

Hydrolysis of metabolites. Slow-moving fractions from preparative paper chromatography were eluted with methanol, evaporated to dryness and redissolved in 1 ml. of water. Then 5 ml. of 0.2M-sodium acetate buffer, pH 5, containing 10000 Fishman units of β -glucuronidase and 500 i.u. of penicillin was added and the mixture was incubated at 37° for 24 hr. The incubation mixture was adjusted to pH 2 and extracted with ether, and the extracts were subjected to either paper or thin-layer chromatography.

Similar slow-moving fractions were taken up, after evaporation, in 2N-HCl and sealed in boiling-tubes. These were maintained at 100° for 24 hr. and the tube contents extracted with ether. The extracts were chromatographed.

Isotope-dilution analysis for *p*-hydroxyphenyldiphenyl- ^{14}C carbinol. The fast-moving fractions from preparative

paper chromatography of extracts of urine from rats dosed with ^{14}C -labelled compounds were eluted with methanol. The resulting solutions, and also extracts of bile, were concentrated and chromatographed on preparative thin-layer plates in chloroform, and the bands of silica containing the *p*-hydroxy compound were scraped off the plate and eluted with methanol. In each case 100 mg. of unlabelled compound was added to the methanol. The solution was then evaporated to dryness, the residue taken up in acetic acid and 100 mg. of zinc dust added. After refluxing for 2 hr. the acid solution was poured into 20 ml. of ice-cold water, and the resulting precipitate was centrifuged. The precipitate was dissolved in benzene and dried with Na_2SO_4 , and the benzene was evaporated. The *p*-hydroxyphenyldiphenylmethane was recrystallized from light petroleum to constant specific radioactivity and constant melting point. The crystals were colourless needles and had m.p. 112° [Bistrzycki & Herbst (1903) give m.p. 110°].

Isotope-dilution analysis for [G - 3H]morpholine. Unlabelled morpholine was added to the toluene extract of urine from rats given 3H -labelled compounds. The morpholine was extracted into 1.0N-HCl, and sodium gold chloride in 1.0N-HCl was added until no further precipitation occurred. The precipitate was filtered off and recrystallized from warm water to constant specific radioactivity and melting point. The crystals (morpholine gold chloride) were long bright-yellow needles (m.p. 204° with decomposition). The same derivative was made from steam-distillates of the same urine, which had been adjusted to pH 13 with NaOH (Maller & Heidelberger, 1957b).

Unlabelled morpholine was added to samples of urine from the same rats and the urine was adjusted to pH 1 with 1.0N-HCl. Water was then distilled from the solution. On cooling, the residue solidified and was dissolved in hot ethanol. The solution was filtered and, on addition of ether, morpholine hydrochloride crystallized as white needles (m.p. 180°). The compound was recrystallized to constant specific radioactivity and melting point [Knorr (1898) gives m.p. 174–177°].

RESULTS

Excretion and retention of radioactivity in animals dosed with *N*-triphenyl- ^{14}C methylmorpholine. Rats given one oral dose of *N*-triphenyl- ^{14}C methylmorpholine excreted between 12.0 and 35.4% of the label in the urine and between 51.3 and 77.0% in the faeces in 4 days. No $^{14}CO_2$ was found in the expired gases over this period. A small part (1–3%) of the label was found in the carcass after 4 days; the skin of females contained more than twice the radioactivity found in the skin of males. Over 90% of the total radioactivity administered was recovered. Table 2 shows the distribution of the radioactivity.

Table 3 shows the rate of excretion. The slowness of this elimination, with some rats excreting more in the second day than the first, and the presence of measurable amounts of radioactivity in the gut after 96 hr. were commensurate with enterohepatic circulation. A difference in the pattern of excretion between male and female rats was observed.

Table 2. *Excretion and retention of radioactivity in rats after oral administration of N-triphenyl[¹⁴C]methylmorpholine*

Male and female rats (150–200 g. body wt.) were dosed orally with 1.2 mg. of *N*-triphenyl[¹⁴C]methylmorpholine (7.3 μ C/mg.) in 1 ml. of arachis oil. Results are mean values $\pm$ S.E.M. for five males and six females. Significance of difference between means of males and females: * $P < 0.05$; ** $P < 0.01$.

		Recovery of ¹⁴ C (% of dose) in 4 days					
	Faeces	Urine	Expired gases	Gut and contents	Skin and hair	Carcass plus remaining viscera	Total
Males	72.3* ± 3.29	17.8* ± 3.37	< 0.05	0.9 ± 0.47	0.9** ± 0.18	1.4 ± 0.30	93.3 ± 1.12
Females	59.1* ± 3.00	28.6* ± 3.08	< 0.05	1.7 ± 0.43	2.2** ± 0.17	1.4 ± 0.27	93.0 ± 1.03

Table 3. *Rates of faecal and urinary elimination of radioactivity from rats after oral administration of N-triphenyl[¹⁴C]methylmorpholine*

Male and female rats were treated with *N*-triphenyl[¹⁴C]methylmorpholine as in Table 2. Each result is the mean (for five males and six females) $\pm$ S.E.M.

		Elimination of ¹⁴ C (% of dose)							
Time (hr.)		0–24		24–48		48–72		72–96	
		Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
Males		37.7 $\pm$ 8.12	9.8 $\pm$ 1.54	23.8 $\pm$ 2.94	5.9 $\pm$ 2.11	7.6 $\pm$ 2.82	1.4 $\pm$ 0.80	3.1 $\pm$ 1.72	0.7 $\pm$ 0.26
Females		23.1 $\pm$ 7.42	13.6 $\pm$ 1.41	28.6 $\pm$ 2.68	10.9 $\pm$ 1.93	4.1 $\pm$ 2.58	2.7 $\pm$ 0.73	3.1 $\pm$ 1.57	1.4 $\pm$ 0.24

Table 4. *Excretion in the bile and retention of radioactivity in rats after administration of N-triphenyl[¹⁴C]methylmorpholine*

Rats 1 and 2 were dosed orally with 4 mg. of *N*-triphenyl[¹⁴C]methylmorpholine (7.3 μ C/mg.) in 2 ml. of arachis oil. Biliary fistulae were then established. Rats 3 and 4 had biliary fistulae established first and were then dosed intraperitoneally with 1.6 mg. of *N*-triphenyl[¹⁴C]methylmorpholine (7.3 μ C/mg.) in 1 ml. of *NN*-dimethylformamide. Urine and bile were collected for 24 hr.

		Recovery of ¹⁴ C (% of dose)				
Rat no.	Sex	Urine	Bile	Gut	Carcass	Total
1	F	0.80	21.49	59.68	7.37	89.34
2	M	1.35	8.36	86.77	5.02	101.5
3*	F	0.12	25.73	—	9.64	—
4	M	0.72	37.83	—	9.93	—

* This animal was alive 12 hr. after dosing but was found dead 11 hr. later.

The excretion of *N*-triphenyl[¹⁴C]methylmorpholine in the bile is shown in Table 4. It is clear that the compound is slowly absorbed when given orally, since 60 and 87% of the label was still in the gut after 24 hr. Over 56% of the radioactivity absorbed in this time was excreted in the bile. When the compound was injected intraperitoneally, over 72% of the absorbed radioactivity was excreted in the bile.

When two rats were dosed with *N*-triphenyl[¹⁴C]methylmorpholine and the pylorus was ligated, 96% of the activity could be recovered from the stomach after 6 hr. despite the fact that in one rat

the ligature had leaked and material had reached the duodenum.

Dogs given a single oral dose of *N*-triphenyl[¹⁴C]methylmorpholine excreted between 73.3 and 100% of the label in 4 days, 19.8–30.0% in the urine and 52.4–83.4% in the faeces (Table 5). Excretion in the urine was faster in dogs than in rats in that over 65% of the radioactivity that appeared in the urine was excreted in the first 24 hr. There was no apparent difference due to sex in dogs, nor was any obvious species difference between rats and dogs found except the sex difference present in rats and absent in dogs.

Table 5. *Rates of faecal and urinary elimination of radioactivity in dogs after oral administration of N-triphenyl[¹⁴C]methylmorpholine*

Each dog swallowed a gelatin capsule containing a solution of 1.2 mg. of *N*-triphenyl[¹⁴C]methylmorpholine (7.3 μ C/mg.) in 1 ml. of arachis oil. Each result is the mean $\pm$ S.E.M. for three animals.

Time (hr.)	Elimination of ¹⁴ C (% of dose)							
	0-24		24-48		48-72		72-96	
	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
Males	26.53 $\pm$ 11.72	14.40 $\pm$ 2.14	34.09 $\pm$ 12.26	4.81 $\pm$ 0.95	5.28 $\pm$ 4.06	1.83 $\pm$ 0.21	2.56 $\pm$ 0.47	0.88 $\pm$ 0.11
Females	26.54 $\pm$ 11.72	17.73 $\pm$ 2.14	24.23 $\pm$ 12.26	5.11 $\pm$ 0.95	7.62 $\pm$ 4.06	1.40 $\pm$ 0.21	1.98 $\pm$ 0.47	0.81 $\pm$ 0.11

Table 6. *Excretion and retention of radioactivity in rats after oral administration of N-triphenylmethyl[G-³H]morpholine*

Male and female rats (150-200 g. body wt.) were dosed orally with 0.67 mg. of *N*-triphenylmethyl[G-³H]-morpholine (47 μ C/mg.) in 1 ml. of arachis oil. Each result is the mean $\pm$ S.E.M. for six animals. Significance of difference between means of males and females: ** $P < 0.01$.

	Recovery of ³ H (% of dose) in 4 days						
	Faeces	Urine	Expired gases	Gut and contents	Skin and hair	Carcass plus remaining viscera	Total
Males	9.35 $\pm$ 1.59	67.30 $\pm$ 7.75	3.53** $\pm$ 0.18	1.68** $\pm$ 0.14	2.15 $\pm$ 0.26	9.78** $\pm$ 0.31	93.79 $\pm$ 1.40
Females	10.11 $\pm$ 1.59	74.17 $\pm$ 7.75	1.94** $\pm$ 0.18	1.06** $\pm$ 0.14	2.26 $\pm$ 0.26	6.73** $\pm$ 0.31	96.27 $\pm$ 1.40

Table 7. *Rates of faecal and urinary elimination of radioactivity in rats after oral administration of N-triphenylmethyl[G-³H]morpholine*

Male and female rats were treated with *N*-triphenylmethyl[G-³H]morpholine as in Table 6. Each result is the mean $\pm$ S.E.M. for six animals.

Time (hr.)	Elimination of ³ H (% of dose)							
	0-24		24-48		48-72		72-96	
	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
Males	4.66 $\pm$ 1.37	57.07 $\pm$ 4.13	1.55 $\pm$ 1.51	5.15 $\pm$ 1.93	0.96 $\pm$ 0.40	2.44 $\pm$ 0.37	2.18 $\pm$ 0.58	2.13 $\pm$ 0.21
Females	4.37 $\pm$ 1.37	59.30 $\pm$ 4.13	3.87 $\pm$ 1.51	9.25 $\pm$ 1.93	1.11 $\pm$ 0.40	3.37 $\pm$ 0.37	0.76 $\pm$ 0.58	2.25 $\pm$ 0.21

Excretion and retention of radioactivity in rats dosed with N-triphenylmethyl[G-³H]morpholine. Rats given a single oral dose of *N*-triphenylmethyl[G-³H]-morpholine excreted 62.0-90.9% of the label in the urine and 4.6-16.9% in the faeces (Table 6). Between 1.3 and 3.9% of the ³H was expired and between 7.5 and 13.7% remained in the carcass after 4 days despite the initially high excretion in the first day's urine (up to 80.4%; Table 7). The presence of 1.4-2.8% of the label in the fourth-day urine and the radioactivity in the carcass and the expired gases suggest that a metabolite of the morpholine part of the molecule is tritiated water. A difference between the two sexes was apparent in the excretion of radioactivity in the expired gases and in the retention in the carcass.

When the urine was collected on a fraction

collector from a rat given a single dose of *N*-triphenylmethyl[G-³H]morpholine, over 90% of the label appeared in 24 hr. When the logarithm of the radioactivity remaining in the body was plotted against time the resulting curve had two distinct straight-line sections (Fig. 1), the first representing a half-life (t_1) of approx. 4 hr. and the second part representing a t_1 of 6.5 days. When tritiated water was administered to two rats the curves were single straight lines and the values of t_1 were 4 days for one rat and 16 days for the other.

Excretion and retention of radioactivity in rats dosed with triphenyl[¹⁴C]carbinol and [G-³H]morpholine. The differences in excretion pattern between the triphenylmethyl and the morpholine portions of the *N*-triphenylmethylmorpholine molecule suggest that the compound is rapidly hydrolysed

in vivo. Because of this, the excretion of labelled forms of the chemical breakdown products, triphenylcarbinol and morpholine, was examined.

When triphenyl[¹⁴C]carbinol was administered as

Fig. 1. Decrease with time in the amount of radioactivity remaining in rats after oral administration of *N*-triphenylmethyl[G-³H]morpholine or ³H₂O (Expts. 6 and 7; see the Methods section). ●, One animal dosed with *N*-triphenylmethyl[G-³H]morpholine; ○, two animals dosed with ³H₂O.

a single oral dose, between 81.6 and 100% of the ¹⁴C was excreted in 4 days, of which 27.5–71.9% was in the faeces and 25.9–54.1% was in the urine (Table 8). Administration of a single oral dose of [G-³H]morpholine resulted in 72.0–83.2% of the ³H appearing in the urine, mainly on day 1 (57.7–73.5%), and only 1.2–1.9% in the faeces (Table 9). Between 2.4 and 5.9% of the ³H was eliminated in the expired gases.

Identification of metabolites from animals dosed with N-triphenyl[¹⁴C]methylmorpholine and triphenyl[¹⁴C]carbinol. Continuous extraction of the acidified urine of rats given *N*-triphenyl[¹⁴C]methylmorpholine resulted in 85% of the radioactivity being transferred to ether solution. When this extract was chromatographed on paper, five radioactive peaks were detected (Table 10). Only the fast-moving compounds were present in the extracts of neutral urine, and there was a lower proportion of the slow-moving compounds in extracts of acidified dog urine.

When the extract was hydrolysed either with β-glucuronidase or with hydrochloric acid, chromatography revealed that the slow-moving peaks were decreased in size, the radioactivity appearing in the fast-moving peaks (Table 10). Because the slow-moving peaks did not separate efficiently, they were combined, as were the two fast-moving peaks for the same reason. A triphenylcarbinol marker ran very near the solvent front and the three mono-hydroxyphenyldiphenylcarbinols ran slightly behind (*R_F* 0.8). The peaks resulting from the hydrolysis ran with the markers.

Table 8. Rates of faecal and urinary elimination of radioactivity in rats after oral administration of triphenyl[¹⁴C]carbinol

Elimination of ¹⁴ C (% of dose)								
Time (hr.)	0–24		24–48		48–72		72–96	
	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
Males	29.4*±4.10	20.9*±1.77	23.1±6.03	4.9±2.90	7.1±1.59	1.6±1.01	2.4±1.31	1.2±0.57
Females	6.2*±4.10	31.0*±1.77	24.3±6.03	12.5±2.90	9.0±1.59	3.8±1.01	3.1±1.31	2.3±0.57

Table 9. Rates of faecal and urinary elimination of radioactivity in rats after oral administration of [G-³H]morpholine

Elimination of ³ H (% of dose)								
Time (hr.)	0–24		24–48		48–72		72–96	
	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine
Males	0.58±0.40	62.21±3.79	0.41±0.27	8.45±2.05	0.25±0.07	1.67±0.53	0.15±0.07	1.11±0.26
Females	0.53±0.40	66.95±3.79	0.47±0.27	7.53±2.05	0.23±0.07	1.79±0.53	0.08±0.07	1.29±0.26

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Elution of the fast-moving peaks followed by thin-layer chromatography showed that the radioactive peaks coincided with unlabelled marker compounds in all solvents tested. The R_F values of the marker compounds in these solvents are shown in Table 1. No solvent mixture effected a satisfactory separation of the *m*- and *p*-hydroxyphenyldiphenylcarbinols and the latter was therefore converted on the plate into the fuchson. Table 11 gives the proportions of the metabolites in the ether extracts of urine from rats and dogs

given *N*-triphenyl[^{14}C]methylmorpholine. The amount of *m*-hydroxyphenyldiphenylcarbinol was recorded by assuming a 50% conversion of the *p*-hydroxy compound into the fuchson in a given heat treatment (borne out by repeated heat treatment of marker compound) and subtracting the calculated radioactivity of the *p*-isomer from that remaining in the position of the *m*-hydroxy compound on the plate. The radioactivity extracted from urine with butanol was chromatographed on thin layer and, on scanning, peaks were detected that ran more slowly [R_F 0.2–0.3 in methanol-chloroform (3:97, v/v)] than the *p*-hydroxyphenyldiphenylcarbinol. It is suggested that these could be polyhydric derivatives of triphenylcarbinol.

Low-voltage electrophoresis at pH 5.9 of the ether extracts of acidified urine from the rats dosed with *N*-triphenyl[^{14}C]methylmorpholine failed to show any ether sulphate conjugates as fast-moving peaks (Williams, Meikle & Redemann, 1964).

Bile from rats dosed orally and intraperitoneally with *N*-triphenyl[^{14}C]methylmorpholine was extracted and chromatographed, and the proportions of the metabolites are also shown in Table 11. The glucuronide conjugates were identified in all cases by hydrolysis with β -glucuronidase, and the *p*-hydroxyphenyldiphenylcarbinol was further identified in urine and bile by isotope-dilution analysis.

The radioactive components in the acetone extract of rat faeces (from rats dosed with *N*-triphenyl[^{14}C]methylmorpholine) were separated on a silicic acid column and were identified by thin-layer chromatography. The relative amounts are shown in Table 11.

Table 10. Paper chromatography of an ether extract of urine from rats dosed with *N*-triphenyl[^{14}C]methylmorpholine

Rat urine (days 1 and 2, pooled) was extracted at pH 2 and the extract run in the descending mode on Whatman no. 1 paper with butan-1-ol saturated with aq. 2*N*- NH_3 as solvent. A portion of the extract was hydrolysed with β -glucuronidase as described in the Methods section.

R_F	% of radioactivity	
	Original extract	Hydrolysed extract
0.0–0.11	4.7	2.4
0.11–0.23	5.9*	3.3
0.23–0.34	16.8*	3.9*
0.34–0.45	19.5*	5.1*
0.45–0.57	2.9	3.4
0.57–0.68	1.7	2.9
0.68–0.86	10.1*	16.6
0.86–1.00	38.4*	62.4*

* Individual peaks on the radioactive scan.

Table 11. Proportion of ^{14}C -labelled compounds in the urine, faeces and bile of rats and the urine of dogs dosed with *N*-triphenyl[^{14}C]methylmorpholine

Combined day 1 and day 2 urines from rats or from dogs were extracted with ether at pH 7 or pH 2 as described in the text. Rat faeces were extracted with acetone, and rat bile was freeze-dried and extracted with methanol. The values represent the percentages of ^{14}C label that ran with synthetic marker compounds on thin-layer plates in a series of solvents. The compounds were: 1, glucuronides; 2, *m*-hydroxyphenyldiphenylcarbinol; 3, *p*-hydroxyphenyldiphenylcarbinol and fuchson (these are combined because, to differentiate between *m*- and *p*-hydroxyphenyldiphenylcarbinol, the *p*-hydroxy compound was converted into the fuchson by heating); 4, *o*-hydroxyphenyldiphenylcarbinol; 5, triphenylcarbinol; 6, *N*-triphenylmethylmorpholine.

Compound	% of ^{14}C in each peak				
	Rat urine (pH 7)	Rat urine (pH 2)	Rat bile	Rat faeces	Dog urine (pH 2)
1*	—	47.0	57.3	27.1	37.0
2†	8.9	4.9	3.0	3.0	20.4
3	70.8	37.5	20.6	26.5	26.6
4	2.4	1.5	0.6	—	5.1
5	15.2	9.1	15.9	42.3	10.9
6	2.7	—	2.6	1.1	—

* Glucuronides were identified solely by their hydrolysis with β -glucuronidase.

† Only for dog urine is this value considered significant, since it represents the radioactivity remaining in this position after conversion of the *p*-hydroxyphenyldiphenylcarbinol into the fuchson.

A benzene extract of material washed from the intestine of rats whose pylorus had been ligated was chromatographed on thin-layer plates and the radioactive material was shown to consist wholly of triphenyl[^{14}C]carbinol.

Identification of metabolites from rats dosed with N-triphenylmethyl[G- ^3H]morpholine or [G- ^3H]morpholine. Efficient extraction of radioactive material (>75%) into an organic phase from the urine of rats dosed with either *N*-triphenylmethyl[G- ^3H]morpholine or [G- ^3H]morpholine was achieved only when the urine was made strongly alkaline [30% (w/v) sodium hydroxide]. Thin-layer chromatography of both sets of extracts and of the urines themselves showed that two radioactive components were present, one of which ran with morpholine in two different systems. The other component ran at the solvent front in formic acid-methanol (1:49, v/v), as did tritiated water.

When urine from rats dosed with *N*-triphenylmethyl[G- ^3H]morpholine was applied to an ion-exchange column, a proportion of the radioactivity (10–30%) was not retained even when applied to successive columns, and the remainder could only be eluted with 1.0M-sodium chloride. The fraction of the radioactivity that was not retained could be removed from the urine either by drying or by distilling from acidified urine, and it is identified as tritiated water. Urine that was collected on the fraction collector after the change of slope (Fig. 1) had a much higher proportion (>90%) of tritiated water.

The basic component that was held on the ion-exchange column was further identified as morpholine by isotope-dilution analysis of two salts of morpholine. When both were prepared from the same sample of urine, their specific radioactivities were in the inverse ratio of their molecular weights.

DISCUSSION

Ingestion of *N*-triphenylmethylmorpholine by rats is followed by rapid hydrolysis in the stomach to form triphenylcarbinol and morpholine. Absorption of the former is a slow process but the latter is rapidly absorbed and is excreted in a largely unchanged form in the urine, up to 90% being excreted within 24hr. The remainder of the morpholine is totally metabolized and the ^3H appears as tritiated water.

Part of the triphenylcarbinol is metabolized to *p*-hydroxyphenyldiphenylcarbinol and to traces of the *m*-hydroxy and *o*-hydroxy compounds and these, and the triphenylcarbinol, are conjugated with glucuronic acid. There appear to be three glucuronide fractions (R_F 0.17, 0.28 and 0.40 on paper), and it is possible that these are respectively the diglucuronides of the hydroxytriphenylcarbinols,

carbinol-group-derived glucuronides and phenolic-hydroxyl-group glucuronides. The major proportion of the ^{14}C -labelled metabolites leaves the body via the bile duct and is excreted in the faeces. The slow excretion by this route suggests that the metabolites can be reabsorbed before being excreted. The lower proportion of glucuronides in faeces compared with bile means that some hydrolysis occurs in the lower intestine.

The overall similarity of the excretion pattern of *N*-triphenyl[^{14}C]methylmorpholine in the dog to that in the rat and the fact that the same metabolites occur in the urine in each suggest that the same metabolic pathway exists in the dog, the only difference being that there appears to be more *m*-hydroxyphenyldiphenylcarbinol formed, and possibly less conjugation.

Most of the ^{14}C has left the body in 4 days, but a small amount (1–3%) remains after this time and this may be present in the fatty tissues since the skin of the female, with its higher amount of subcutaneous fat, contains more of the label than does male skin.

The results presented here confirm and extend the work of Cornish, Zamora & Babor (1964), who dosed rabbits with triphenylcarbinol and found the compound unchanged in the faeces and a hydroxy derivative in the urine.

Similarly, these results confirm a study on morpholine metabolism (Maller & Heidelberger, 1957a) with [^{14}C]morpholine. These workers found that this compound was excreted rapidly and largely unchanged.

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