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METABOLISM OF CHLORPHENIRAMINE-^{3}H BY THE RAT AND DOG*

JEROME J. KAMM,† CHARLES R. FERULLO, DAISY MILLER and EDWARD J. VAN LOON‡

Research and Development Division, Smith Kline & French Laboratories, Philadelphia, Pa., U.S.A.

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Abstract—Chlorpheniramine-^{3}H is rapidly and completely absorbed from the gastrointestinal tract of the rat and the dog. About 70–80 per cent of the administered radioactivity contained in a single acute dose is excreted in urine and feces in 4 days with most of the radioactivity being recovered in urine in 24 hr. When rats, but not dogs, are pretreated with nonradioactive chlorpheniramine and then given the labeled compound, the recovery of tritium in urine and feces is essentially complete in 4 days. Maximum radioactivity in blood in both species was observed 30–60 min after oral drug administration and the blood level fell to half its maximum value in 24 hr. Radioactivity was detected in rat tissues 15 min after dosing and reached peak levels in 30–60 min. About 50 per cent of an oral dose of chlorpheniramine-^{3}H is excreted in rat bile in 24 hr. *N*-dealkylation is a major pathway for the metabolism of chlorpheniramine-^{3}H in both the rat and the dog. Didesmethylchlorpheniramine is the major dealkylated metabolite detected in urine of both species after the doses of chlorpheniramine investigated. Only 1–3 per cent of chlorpheniramine-^{3}H is excreted unchanged in the urine.

CHLORPHENIRAMINE [2-*p*-chloro-α-(2-dimethylamino-ethyl) benzyl pyridine] is a potent antihistaminic often employed to alleviate the symptoms of the common cold and of allergic reactions. Considering the widespread use of this drug in both proprietary and ethical formulations, surprisingly little is known about its fate in either man or laboratory animals. Haley and Bassin[1] studied the tissue distribution of chlorpheniramine in the guinea pig 1 hr after the s.c. administration of 10 and 50 mg/kg and reported that the highest concentration is found in the lungs, spleen and brain. More recently, Lee[2] compared the rate of absorption and the degradation by liver of chlorpheniramine in young and old rats. He has shown that no differences exist in the degradation of the drug by liver slices from young or from old rats, but he observed that the percentage of the dose absorbed is greater in young than in old animals. He did not attempt to identify any metabolites of chlorpheniramine.

Studies of the fate of chlorpheniramine in man have mainly been confined to an estimation of the urinary excretion of the unchanged drug. Belles and Sievert[3] have reported that 50–70 per cent of an oral 50-mg dose is excreted in urine in 24 hr, depending upon the method of analysis employed. Cavallito *et al.*[4] showed that four human subjects excreted 12–57 per cent of an oral 10·2-mg dose of chlorpheniramine in urine in 24 hr. In contrast, Beckett and Wilkinson[5] reported that 4–11 per cent of

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† Present address: Hoffmann-La Roche, Inc., Nutley, N.J. 07110.

‡ Present address: Food and Drug Administration, Washington, D.C.

an oral 10-mg dose of chlorpheniramine was excreted in urine by normal subjects in 24 hr. These investigators also demonstrated that the renal excretion of unchanged drug by man is dependent upon both pH and the rate of urine flow.

Recently, Kabasakalian *et al.*[6, 7] have investigated the nature of the urinary excretion products in a single human subject after a single acute dose of chlorpheniramine and after chronic treatment with the drug. By utilizing the techniques of quantitative gas-liquid chromatography and mass spectroscopy, these workers showed that, under conditions of chronic treatment, man excretes daily in his urine chorpheniramine (12·6%), desmethylchlorpheniramine (13·2%), and didesmethylchlorpheniramine (5·8%). About 68% of the daily dose was unaccounted for.

This report will describe studies concerned with the absorption, excretion and tissue distribution of tritium contained in tritiated chlorpheniramine maleate administered to rats and dogs. In addition, it will describe studies which indicate that, in both species, the bulk of urinary radioactivity is comprised of dealkylated metabolites of chlorpheniramine.

METHODS

Materials

Chlorpheniramine maleate-^{3}H, labeled with tritium as indicated in Fig. 1, was prepared for us by Dr. W. Mendelson of our Organic Chemistry Section. The compound, which had a specific activity of 112·4 μc/mg, was > 99 per cent pure as determined by gas and thin-layer chromatography. The nonradioactive mono- and didealkylated derivatives were also prepared by Dr. Mendelson.

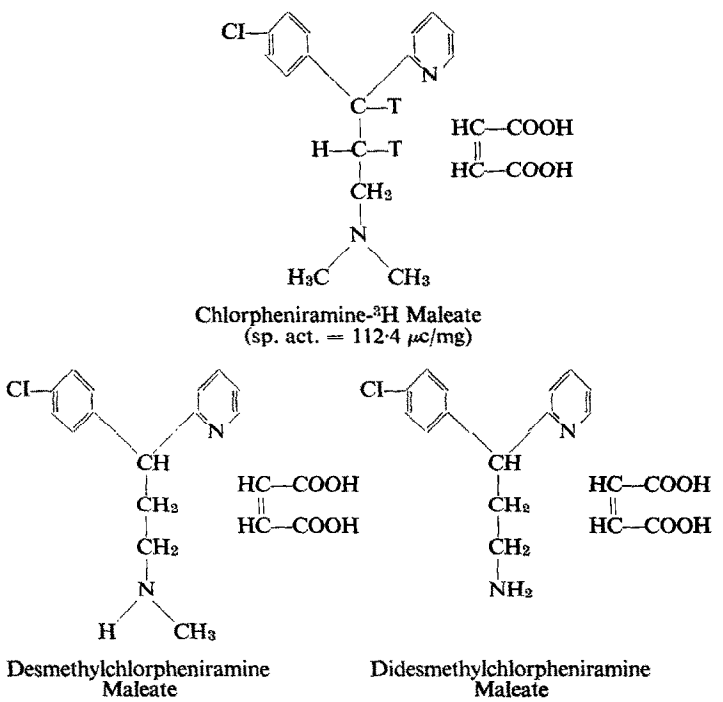

FIG. 1. Chlorpheniramine-^{3}H maleate and nonradioactive dealkylated analogs.

Animals and drug administration

Adult male Sprague–Dawley rats from the Charles River Breeding Laboratory, Wilmington, Mass., and adult female beagle dogs were used in our studies. The animals were dosed orally with saline solutions of drug (calculated as the maleate salt). For rats, a stomach tube was used for oral administration; for dogs, the drug was pipetted into a No. 000 gelatin capsule, which was forced down the animal's throat. For most rat experiments the total amount of radioactivity administered to each animal was 1·0 μc; when tissue and blood levels were determined, each animal received 81 μc tritium. Each dog received a total of 112·4 μc of radioactivity. When necessary, the solutions to be administered were appropriately diluted with non-radioactive chlorpheniramine maleate.

Collection of urine and feces

After dosing, the animals were placed in individual metabolism cages designed for the separate collection of urine and feces. Excreta were collected in 24 hr fractions for the times indicated in the tables. Standard laboratory chow and water were available *ad lib.*

Bile duct cannulation

Rats were anesthetized with ether and the common bile duct was exposed. A polyethylene cannula (Intrademic, PE-10) was inserted into the duct and secured with two silk suture ties. The incision was closed with wound clips and the animals were immobilized in restraining cages with free access to food and water. The restraining cages were designed to permit the separate collection of feces and bile. Biliary secretion experiments were performed 24 hr after surgery.

Tissue distribution studies

Rats medicated as described above were anesthetized with ether and blood was drawn by cardiac puncture with heparinized syringes. The animals were then sacrificed and the entire brain, lung, liver, kidney and epididymal fat pad were removed; in addition, samples of thigh muscle were also obtained. The tissues were rinsed in saline, blotted dry, weighed and, together with the blood, were stored frozen until they could be analyzed.

Brain, kidney, fat and liver were homogenized in 4 volumes, and lung in 9 volumes of distilled water with a glass-teflon homogenizer. Portions of each homogenate, in duplicate, were added to tetramethyl ammonium hydroxide (TMAH) and heated in a boiling water bath for 10 min. The resulting digests were cooled to room temperature and diluted with water to a fixed volume. The diluted digests were assayed for radioactivity as described below. Accurately weighed samples of muscle, in duplicate, were digested directly in TMAH without prior homogenization.

For the dog experiments, blood was obtained from a paw vein with heparinized syringes. No tissue distribution studies were done in the dog.

Estimation of radioactivity

Tritium was determined in a Packard Tri-Carb liquid scintillation counter (the tritium counting efficiency was 26·3 per cent). The observed count was corrected for quenching by the internal standard method.

The scintillation solution used for the assay of tritium in urine, feces, tissue digests and on chromatograms contained 8 g of 2,5-*bis*-[5′-tertiarybutylbenzoxazolyl (2′)] thiophene (BBOT), 80 g napthalene, 400 ml toluene and 400 ml of spectroquality dioxane diluted to 1 liter with absolute ethanol. The solution used for assay of radioactivity in bile and blood contained 7 g of 2,5-diphenyloxazole (PPO), 50 mg of 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl) benzene (dimethyl-POPOP), 50 g of thixatropic gel (Cab-O-Sil), 80 g naphthalene, 400 ml toluene and 400 ml of spectroquality dioxane diluted to 1 l. with absolute ethanol.

Suitable aliquots (0·1–0·5 ml) of urine, bile, blood or tissue digest were added directly to the appropriate phosphor for assay of their tritium content. Feces were dried in a lyophilizer, weighed and ground in a Wiley mill with a 40 mesh screen. Accurately weighed aliquots (50–100 mg) of the dried, ground feces were then burned in a Schöninger combustion apparatus. The sealed combustion flasks were placed in a dry ice–acetone bath for 15–20 min; 20 ml of phosphor was injected into the flasks and 15 ml of the phosphor was then removed to a counting vial for assay of the tritiated water produced by combustion of the ground feces.

Recovery of chlorpheniramine and metabolites from urine

Chlorpheniramine and its two dealkylated derivatives were recovered from urine by extraction with 5 vol. of *n*-hexane at pH 11. The completeness of recovery by this procedure was demonstrated as follows. Various amounts of each of the three compounds were added to separate 5-ml aliquots of urine. The urine was adjusted to pH 11 with 2·5 N NaOH and shaken with 25·0 ml *n*-hexane for 30 min. After centrifugation, exactly 20·0 ml of the hexane was transferred to a clean, dry, graduated centrifuge tube. The hexane was evaporated to dryness and the residue was dissolved in exactly 1·0 ml *n*-hexane. Exactly 5 μl of each sample was then subjected to gas chromatography as described below, and the area under each peak on the chromatogram was calculated. These areas were then converted to micrograms of compound by use of a standard curve, which was obtained by injecting known amounts of each drug, dissolved in methanol, directly into the gas chromatograph. The results indicated that chlorpheniramine and its dealkylated analogs may be recovered from urine with adequate precision (97–107 per cent).

For the identification and estimation of urinary metabolites, 10 ml urine was adjusted to pH 11 and extracted with 50 ml *n*-hexane. Exactly 40 ml of the hexane was transferred to a clean, dry container and the urine was reextracted with a fresh 50-ml aliquot of hexane; 40 ml of the second hexane extract was combined with the first 40 ml of solvent and the combined hexane aliquots were concentrated to exactly 1·0 ml in a stream of dry nitrogen. The resulting hexane concentrates were used to identify and estimate urinary metabolites as described below.

Thin-layer chromatography (TLC)

TLC was performed on Eastman Chromagram sheets (type K301R, unactivated) by the ascending technique with the solvent system: *n*-hexane:acetone:ammonia (80:20:2). A standard, comprised of chlorpheniramine and its two dealkylated analogs, was run along with the blanks and unknowns. The compounds were visualized by spraying the chromatogram with an iodochloroplatinate reagent.[8]

Gas chromatography

Gas chromatography on a stainless steel (0·25 in. i.d.) column packed with 2·5% carbowax 6000 plus 2·5% KOH coated on Gas-chrome Q (100–120 mesh) was carried out in an F & M model 400 gas chromatograph equipped with a flame ionization detector. The column, detector and flash heater temperatures were 198°, 270°, and 305° respectively. The range setting was 10 and the attenuation was 8. Helium was used as the carrier gas and was introduced into the system at a rate of 60 ml/min. The chart speed was 1/4 in./min.

Identification and estimation of urinary metaboliites

Aliquots (exactly 50 μl) of the hexane concentrates of urine extracts obtained as described above were subjected to TLC as described previously. The R_f values for chlorpheniramine and its two dealkylated derivatives, added to blank urine and carried through the procedure, were calculated. The area of the chromatogram containing the material from the experimental urine was cut into 0·5-cm segments. Each segment was then added to scintillation vials containing the appropriate phosphor for the estimation of tritium. The amount of tritium contained in each segment was then plotted against the distance from the origin, resulting in the radiohistograms shown in Fig. 4. Essentially all of the radioactivity applied to the thin-layer sheets was accounted for by this procedure. An estimate of the amount of each metabolite was made from a knowledge of: the total radioactivity in urine, the total radioactivity extracted by hexane, the total radioactivity applied to the chromatogram, and the total radioactivity under each peak on the radiohistogram. Qualitatively, the identification of each peak on the radiohistogram was made by comparing its mobility with that of the authentic compounds.

A final identification of the hexane-extractable urinary metabolites was made by comparing the gas chromatographic mobility of the material in the experimental urine with the mobilities of chorpheniramine and its dealkylated analogs, which had been added to blank urine and carried through the procedures.

RESULTS

Urinary and fecal excretion of tritium after a single oral dose of chlorpheniramine-^{3}H maleate

Table 1 summarizes the results of a study in which a single acute dose of chlorpheniramine-^{3}H maleate was administered to rats and dogs. In both species, 75–80 per cent of the administered tritium was recovered from the urine and feces in 4 days. In the rat, 85 per cent of the tritium that was recovered was excreted in the first 24 hr after the administration of the labeled drug. In the dog, excretion was somewhat slower: 44 per cent of the tritium recovered was excreted in 24 hr, 71 per cent in 48 hr and 90 per cent in 72 hr.

Urinary and fecal excretion of tritium after a single oral dose of chlorpheniramine-^{3}H maleate administered as part of a chronic dosing regimen

Since chlorpheniramine is often administered on a chronic basis, we have studied the excretion of tritium from labeled drug in animals which had been chronically

TABLE 1. URINARY AND FECAL EXCRETION OF TRITIUM AFTER THE ADMINISTRATION OF A SINGLE DOSE OF CHLORPHENIRAMINE-^{3}H MALEATE TO THE RAT AND THE DOG*

Day	Per cent of dose recovered					
	Rat			Dog		
	Urine	Feces	Urine + feces	Urine	Feces	Urine + feces
1	47·8 (41·0–58·0)	14·4 (10·1–19·2)	62·2 (52·4–73·2)	30·1 (22·0–42·4)	4·4 (2·6–8·2)	34·5 (24·6–46·8)
2	3·5 (2·9–3·9)	5·6 (4·0–8·6)	9·1 (6·9–12·3)	17·0 (12·6–20·7)	5·1 (2·9–9·5)	22·1 (17·0–32·5)
3	0·7 (0·4–0·9)	1·3 (1·0–1·7)	2·0 (1·5–2·4)	11·5 (9·0–16·2)	3·4 (2·4–4·8)	14·9 (13·3–18·6)
4	0·3 (0·2–0·4)	†	†	5·2 (3·8–6·2)	2·3 (1·8–2·9)	7·5 (5·6–8·5)
1–4	52·3 (45·5–63·1)	21·3 (16·3–27·3)	73·6 (64·7–81·8)	63·9 (50·7–84·4)	15·1 (10·7–24·3)	79·0 (63·4–98·7)

* Chlorpheniramine-^{3}H maleate at a dose of 5 mg/kg was administered orally to 6 rats and to 5 dogs. The figures are the mean recoveries for all animals; the numbers in parentheses indicate the range of recovery.

† Feces from days 3 and 4 were combined and assayed as a single sample.

treated with chlorpheniramine. The results (Table 2) in the dog are essentially the same as when a single acute dose of chlorpheniramine-^{3}H maleate was administered, i.e. about 70 per cent of the radioactivity was excreted in urine and feces in 4 days. In contrast, in the rat a chronic administration regimen resulted in the complete elimination in 4 days of the radioactivity contained in a dose of chlorpheniramine-^{3}H maleate.

TABLE 2. URINARY AND FECAL EXCRETION OF TRITIUM AFTER THE ADMINISTRATION OF A SINGLE DOSE OF CHLORPHENIRAMINE-^{3}H MALEATE ADMINISTERED AS PART OF A CHRONIC DOSING REGIMEN TO THE RAT AND THE DOG*

Day	Per cent of dose recovered					
	Rat			Dog		
	Urine	Feces	Urine + feces	Urine	Feces	Urine + feces
1	39·1 (33·6–44·2)	29·4 (20·1–38·0)	68·5 (64·3–77·6)	28·6 (24·1–31·4)	5·0 (3·0–9·2)	33·6 (28·6–40·6)
2	6·3 (5·5–7·3)	11·5 (9·2–14·1)	17·8 (15·8–19·6)	13·6 (10·6–14·8)	4·6 (2·7–6·7)	18·2 (13·3–21·0)
3	3·2 (2·8–3·5)	4·3 (3·2–5·6)	7·5 (6·6–8·4)	7·2 (4·9–8·4)	4·1 (3·2–5·1)	11·3 (8·1–13·2)
4	2·0 (1·8–2·2)	†	†	5·2 (4·7–5·6)	2·0 (1·4–2·7)	7·2 (6·7–8·3)
1–4	50·2 (43·1–56·7)	45·2 (35·9–54·6)	95·5 (92·6–97·7)	54·6 (51·5–56·5)	15·6 (13·3–18·9)	70·2 (66·4–75·4)

* Chlorpheniramine maleate, at a dose of 5 mg/kg, was administered orally to 5 rats once a day for 6 days and to 4 dogs once a day for 7 days. Twenty-four hr after the last dose of nonradioactive drug, each animal received an oral dose (5 mg/kg) of chlorpheniramine-^{3}H maleate. On each of the excreta collection days, the animals were given 5 mg/kg of nonradioactive chlorpheniramine maleate. The figures are the mean recoveries for all animals; the numbers in parentheses indicate the range of recovery.

† Feces from days 3 and 4 were combined and assayed as a single sample.

Biliary secretion of tritium after the oral administration of chlorpheniramine-^{3}H to the rat

Studies on the biliary secretion of radioactivity after the oral administration of chlorpheniramine-^{3}H maleate to the rat are summarized in Table 3. In two animals, about 46–48 per cent of the radioactivity was recovered from the bile in 24 hr. During this same period, 0·3–1·2 per cent of the dose was recovered from the feces.

TABLE 3. BILIARY SECRETION OF TRITIUM AFTER THE ADMINISTRATION OF CHLORPHENIRAMINE-^{3}H TO THE RAT*

Day	Per cent of dose recovered			
	Rat 1		Rat 2	
	Bile	Feces	Bile	Feces
1	47·8	1·2	45·8	0·3
2	1·3		2·1	
1–2	49·1	1·2	47·9	0·3

* Twenty-four hr after surgery, the animals were given 5 mg/kg of chlorpheniramine-^{3}H maleate orally.

Blood levels of apparent chlorpheniramine-^{3}H in the rat and in the dog

Whole blood levels of apparent chlorpheniramine-^{3}H after the oral administration of the labeled drug to rats and dogs are presented in Fig. 2. The corrected count rate

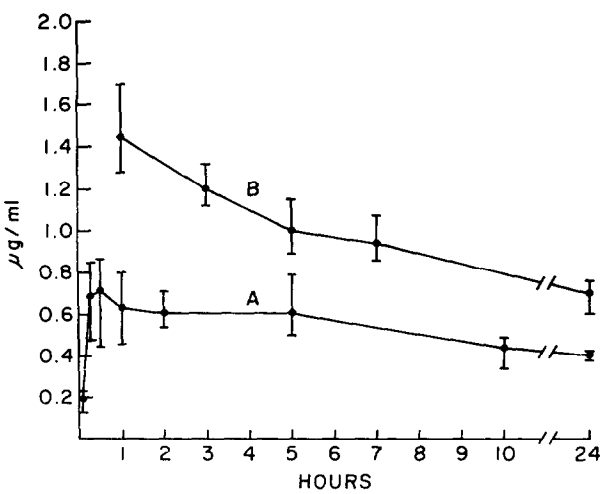

FIG. 2. Blood levels of apparent chlorpheniramine-^{3}H in the rat and in the dog. Chlorpheniramine-^{3}H maleate was administered orally to 3 rats (2·5 mg/kg) for each time interval and to 5 dogs (5·0 mg/kg). Each point is the mean value for all animals; the vertical lines show the range of the observed values. Curve A, rat; curve B, dog.

was converted to micrograms of chlorpheniramine-^{3}H on the basis of the known specific activity of the administered solutions. The data have been expressed in terms of apparent chlorpheniramine because no information was available on the chemical nature of the radioactivity observed in whole blood. This same procedure was followed

in the tissue distribution studies described below. Peak levels of apparent chlorpheniramine were observed in the rat 30 min after dosing. In the dog, radioactivity reached a maximum in 1 hr or less. In both species, a rather slow decline in blood level was noted. Thus after 24 hr, the whole blood level of apparent chlorpheniramine-^{3}H had declined to approximately one-half the maximum levels observed.

Tissue distribution of apparent chlorpheniramine-^{3}H in the rat

Table 4 presents the tissue levels of apparent chlorpheniramine-^{3}H at various times after the administration of a single dose to the rat. Chlopheniramine is rapidly absorbed and distributed to body tissues, as is evidenced by the appearance of radioactivity in the tissues investigated 15 min after dosing. Maximum levels of

TABLE 4. TISSUE LEVELS OF APPARENT CHLORPHENIRAMINE-^{3}H IN THE RAT*

Time	Tissue (μg/g, wet wt.)					
	Brain	Lung	Liver	Kidney	Fat	Muscle
15 min	1·3 (0·4–1·8)	8·3 (4·1–12·6)	16·0 (9·4–20·1)	7·6 (5·3–9·7)	1·2 (1·0–2·0)	0·8 (0·5–1·1)
30 min	1·7 (0·5–2·3)	10·2 (8·8–11·0)	12·0 (7·2–15·9)	9·2 (5·8–11·9)	1·7 (1·1–2·3)	1·1 (0·5–1·7)
1 hr	1·2 (0·6–1·6)	11·4 (8·8–13·6)	7·6 (6·3–8·4)	9·2 (7·6–10·4)	0·8 (0·2–1·5)	1·1 (0·9–1·3)
2 hr	0·8 (0·7–1·0)	11·3 (9·4–12·4)	6·1 (5·5–7·2)	7·8 (5·9–10·5)	0·3 (0·2–0·4)	1·2 (1·0–1·4)
5 hr	0·7 (0·6–0·8)	6·2 (4·8–8·1)	4·1 (3·6–4·6)	4·4 (3·5–6·1)	0·3 (0·2–0·4)	0·9 (0·8–1·1)
10 hr	0·3 (0·3–0·4)	2·2 (0·9–3·0)	2·0 (1·8–2·2)	1·5 (1·2–1·7)	0·2 (0·2–0·3)	0·5 (0·5–0·6)
16 hr	0·2 (0·2)	0·6 (0·3–1·1)	1·4 (1·0–1·7)	0·3 (0·3–0·4)	< 0·1	0·3 (0·3)
24 hr	0·2 (0·2)	0·4 (0·3–0·5)	1·4 (1·3–1·6)	0·2 (0·2)	< 0·1	0·2 (0·2)

* Chlorpheniramine-^{3}H maleate was administered orally to rats at a dose of 2·5 mg/kg. The figures are mean values for three animals at each time interval and the numbers in parenthesis are the range of observed values.

apparent chlorpheniramine-^{3}H were observed in all tissues 30–60 min after drug administration, except in the liver where the peak radioactivity was exhibited in 15 min. Liver, lung and kidney represent major sites of drug deposition. Apparent chlorpheniramine-^{3}H was detected in all tissues studied, except in fat, 24 hr after dosing.

Urinary metabolites of chlorpheniramine-^{3}H in the rat and in the dog

Chlorpheniramine-^{3}H maleate was administered orally to 4 rats (10 mg/kg) and to 3 dogs (5 mg/kg). The animals were placed in individual metabolism cages and all of the urine excreted in 24 hr was collected. The urine samples were extracted with hexane and concentrates were prepared as described under Methods.

Aliquots of the hexane concentrates of experimental, blank urine and of blank urine to which authentic samples of chlorpheniramine-^{3}H and its nonradioactive dealkylated analogs had been added were subjected to TLC. All of the experimental

urines were shown to contain material which migrated with R_f values identical with those of chlorpheniramine, desmethylchlorpheniramine and didesmethylchlorpheniramine respectively (Table 5).

TABLE 5. THIN-LAYER CHROMATOGRAPHY OF RAT AND DOG URINE EXTRACTS*

Animal	Chlorpheniramine		Desmethyl-chlorpheniramine		Didesmethyl-chlorpheniramine	
	Standard	Experimental	Standard	Experimental	Standard	Experimental
Rat 1	58	56	11	11	38	37
Rat 2	46	47	9	8	30	31
Rat 3	47	44	9	8	31	29
Rat 4	43	40	6	5	24	22
Blank urine		no spots		no spots		no spots
Dog 204	33	35	4	3	22	25
Dog 101	56	54	11	11	37	38
Dog 102	59	56	10	9	37	37
Blank urine		no spots		no spots		no spots

* Experimental and blank urines and standards which had been added to blank urine obtained from each animal prior to drug administration were carried through the method. None of the blank urines contained extractable material which reacted with the spray reagent. The numbers are the observed R_f values $\times$ 100.

Aliquots of the hexane concentrates were subjected to gas chromatography under the conditions described in the Methods. Figure 3 shows typical chromatograms resulting from the GLC of rat and dog urine hexane extracts. The hexane extracts from blank rat and dog urine contained no detectable peaks. All of the experimental rat and dog urine extracts contained 3 peaks having retention volumes corresponding to those of authentic chlorpheniramine and its two dealkylated metabolites which had been added to blank urine and carried through the same procedures (Table 6).

In these experiments, both the rat and the dog excreted 35 per cent of the administered radioactivity in urine in 24 hr. Hexane extraction of accurately measured aliquots resulted in the recovery of 70 per cent and 55 per cent of the tritium from the urine of the rat and dog respectively. None of the radioactivity was lost as a result of concentrating the hexane extracts. Carefully measured aliquots of each experimental hexane concentrate were subjected to TLC for the quantitative estimation of each component, as described in the Methods. Typical radiohistograms are presented in Fig. 4. The results (Table 7) show that didesmethylchlorpheniramine is a major metabolite, accounting for 21 per cent of the dose excreted by the rat and for almost 9 per cent of the dose excreted by the dog in the urine 24 hr after the administration of chlorpheniramine-^{3}H. The monodealkylated derivative is excreted to the extent of about 3 per cent by both species and unchanged chlorpheniramine accounts for less than 1 per cent of the dose in rats and for about 3 per cent of the dose in dogs.

DISCUSSION

Chlorpheniramine-^{3}H is well absorbed from the gastrointestinal tract of both the rat and the dog, since in both species 70–80 per cent of a single, acute 5 mg/kg dose is

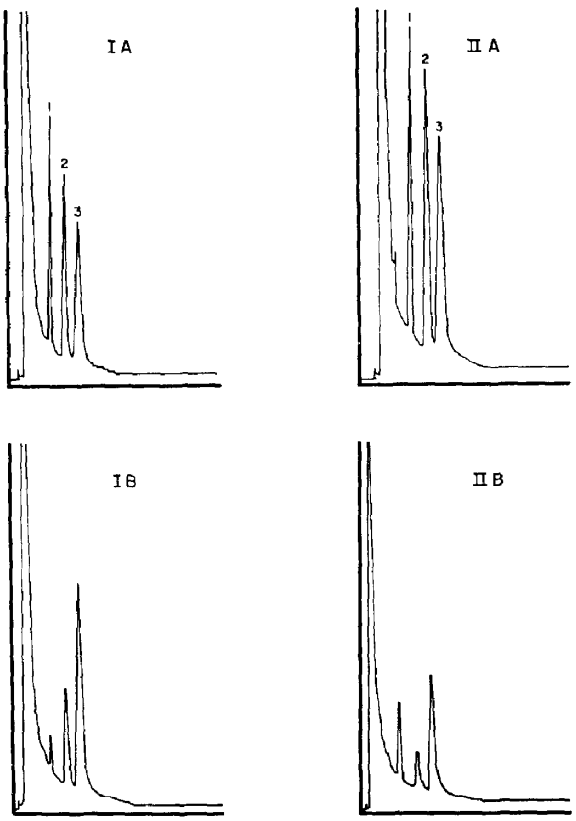

FIG. 3. Gas chromatography of rat and dog urine extracts. Chromatograms I A and II A are the hexane extracts obtained from blank rat and dog urine, respectively, to which authentic compounds had been added. Chromatograms I B and II B are the hexane concentrates from experimental rat and dog urine respectively. Peak 1 is chlorpheniramine; Peak 2 is desmethylchlorpheniramine and Peak 3 is didesmethylchlorpheniramine.

TABLE 6. GAS CHROMATOGRAPHY OF RAT AND DOG URINE EXTRACTS

Sample*	Retention volume (mm chart paper)
Chlorpheniramine	15
Desmethylchlorpheniramine	22
Didesmethylchlorpheniramine	28
Rat 1	15, 23, 29
Rat 2	16, 23, 29
Rat 3	15, 23, 29
Rat 4	15, 23, 29
Blank urine	no peaks
Chlorpheniramine	18
Desmethylchlorpheniramine	26
Didesmethylchlorpheniramine	32
Dog 204	18, 26, 32
Dog 101	18, 26, 33
Dog 102	18, 25, 33
Blank urine	no peaks

* Standards were added to blank rat or dog urine and carried through the procedures at the same time as the experimental and blank urines.

excreted in urine and feces in 4 days. For the rat, most of the radioactivity (85 per cent of the total recovered) was recovered from the excreta in the first 24 hr after drug administration; in the dog, excretion was somewhat slower and 90 per cent of the total radioactivity recovered was excreted in 72 hr.

TABLE 7. EXCRETION OF CHLORPHENIRAMINE AND DEALKYLATED METABOLITES IN URINE BY THE RAT AND THE DOG 24 hr AFTER THE ADMINISTRATION OF CHLOREPHENIRAMINE-^{3}H

Animal	Chlorpheniramine-^{3}H administered (mg)	Chlorpheniramine		Desmethylchlorpheniramine		Didesmethylchlorpheniramine		Totals	
		(mg)	(% dose)	(mg)	(% dose)	(mg)	(% dose)	(mg)	(% dose)
Rat 1	2·0	0·01	0·5	0·05	2·5	0·41	20·5	0·47	23·5
Rat 2	2·0	0·03	1·5	0·08	4·0	0·54	27·0	0·65	32·5
Rat 3	2·0	0·02	1·0	0·08	4·0	0·50	25·0	0·60	30·0
Rat 4	2·0	0·01	0·5	0·01	0·5	0·24	12·0	0·26	13·0
Mean		0·02	0·9	0·06	2·8	0·42	21·1	0·50	24·8
Dog 204	46	0·5	1·1	0·7	1·5	3·6	7·8	4·8	10·4
Dog 101	35	1·2	3·4	1·2	3·4	3·5	10·0	5·9	16·9
Dog 102	42	1·5	3·6	1·2	2·9	3·3	7·9	6·0	14·3
Mean		1·1	2·7	1·0	2·6	3·5	8·6	5·6	13·9

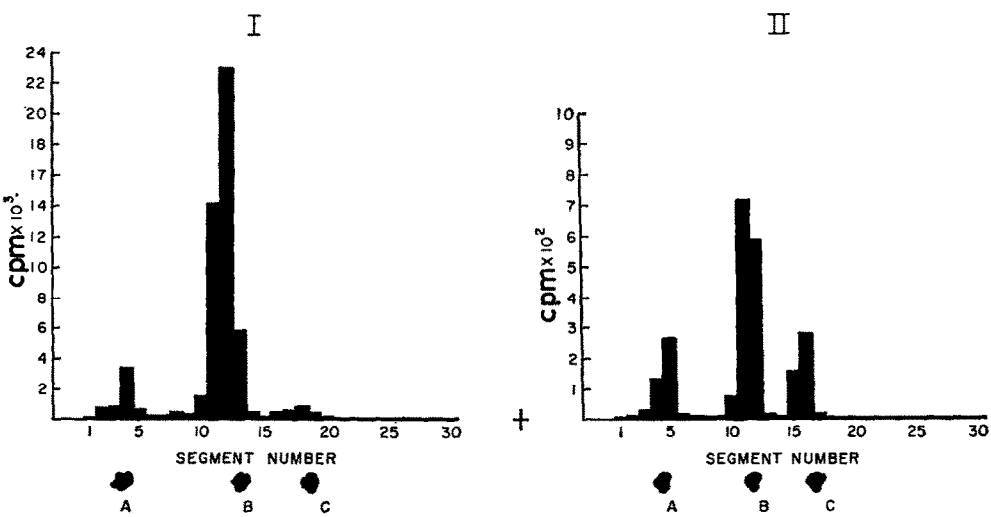

FIG. 4. Radiohistograms of rat and dog urine extracts. The spots beneath the abscissa show the location of authentic standards added to the appropriate blank urine and carried through the procedures. Spot A is desmethylchlorpheniramine; spot B is didesmethylchlorpheniramine; and spot C is chlorpheniramine. Chromatogram I is from rat urine and chromatogram II is from dog urine.

In both species, appreciable amounts of radioactivity were recovered from feces. While radioactivity in the feces after the oral administration of a drug may result from incomplete absorption, our results show that about 50 per cent of the ingested radioactivity is excreted in the bile in 24 hr. During this same time interval, about 1 per cent of the dose was recovered from the feces. In intact animals, about 15 per cent of the dose is excreted in feces in the same time interval. These data support our conclusion that chlorpheniramine-^{3}H is completely absorbed from the gastro-intestinal tract.

The biliary excretion of chlorpheniramine or its metabolites or of both offers a possible explanation for our findings that, in both the rat and the dog, small but detectable amounts of radioactivity are recovered from urine and feces 4 days after a single oral dose of chlorpheniramine-^{3}H. The biliary secretion of a drug or its metabolites may result in an enterohepatic circulation of ingested drug or metabolite which prolongs the excretion of these compounds.[9, 10] Such an explanation has been offered for the persistence of dextromethorphan,[11] morphine,[12] glutethamide,[13] digitoxin[14] and dipyridamoles[15] in man and in laboratory animals.

From our studies in dogs that were chronically treated, we observed essentially no difference in the total radioactivity excreted in urine and feces whether the animals received a single dose or chronic doses of chlorpheniramine-^{3}H. In contrast, rats chronically treated with chlorpheniramine excreted all of the radioactivity in urine and feces in 4 days, whereas they excreted only 70 per cent after receiving a single acute dose. Although we have no data which would directly support an explanation for this species difference, at least one possibility is suggested. In the rat, the total radioactivity recovered in 24 hr was the same after a single acute dose and during chronic treatment (Tables 1 and 2). We did observe, however, an increase in the fecal excretion of radioactivity in the chronic experiment in the rat, but not in the dog. Perhaps the nonradioactive drug, administered after the labeled drug, interferes in some manner with the enterohepatic circulation of chlorpheniramine-^{3}H or its metabolites or of both. Under these conditions, one might expect the radioactivity to be cleared from the body more rapidly, probably via the feces. The observed species difference may be a reflection of the relative importance of the enterohepatic circulation as a mechanism for the retention of drug or metabolite or of both in the body. In support of this argument, Smith[9] has pointed out that rodents, particularly the rat, generally show a more marked biliary excretion and enterohepatic circulation of certain steroids than does man.

The rapid absorption of chlorpheniramine-^{3}H from the gastrointestinal tract is supported by our findings that in the rat and in the dog peak blood levels of radioactivity were observed 30–60 min after oral drug administration. Furthermore, in the rat, radioactivity was detected in the tissues 15 min after oral dosing. With the exception of the liver, which had a peak level in 15 min, the tissues investigated were found to contain maximum levels of radioactivity 30–60 min after the ingestion of chlorpheniramine-^{3}H. Our studies on the blood levels of apparent chlorpheniramine-^{3}H in the rat and in the dog indicate a plasma half-life for the drug of about 24 hr. The persistence of radioactivity in whole blood may be due to the enterohepatic circulation previously discussed above. Another explanation is suggested by our observation that chlorpheniramine-^{3}H is tightly bound to plasma proteins.*

Studies on the chemical nature of the radioactivity excreted in rat and dog urine after the administration of 10 and 5 mg/kg of chlorpheniramine-^{3}H, respectively, have shown that *N*-dealkylation is a major pathway for the metabolism of chlorpheniramine-^{3}H. Mono- and di-dealkylated metabolites have been identified in urine extracts by TLC and GLC. Quantitative TLC has demonstrated that didesmethylchlorpheniramine is the major dealkylated metabolite. Chlorpheniramine-^{3}H is completely metabolized in both species, since only 1–3 per cent of the dose was excreted unchanged in the urine.

* J. J. Kamm and E. J. Van Loon, unpublished observations.

It should be emphasized that *N*-dealkylation is apparently not the only route of metabolism for chlorpheniramine-^{3}H. The extraction procedures employed herein result in the complete recovery of chlorpheniramine and its dealkylated analogs from both rat and dog urine. However, we have observed that 30 per cent of the total radioactivity in rat urine and 45 per cent of the total radioactivity in dog urine was not extracted by our technique. The extraction of radioactivity from urine is not increased by prior acid hydrolysis of urine nor by prior incubation of urine with β-glucuronidase or aryl sulfatase. The urine does not contain tritiated water, since distillation of selected samples demonstrated the absence of volatile radioactivity. At the present time, no other data concerning the nature of "nonextractable" radioactivity in urine are available.

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