Biological Disposition and Metabolic Fate of Fluphenazine-^{14}C in the Dog and Rhesus Monkey

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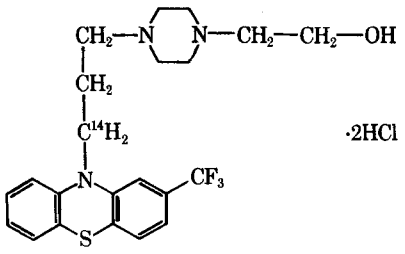
Abstract □ Fluphenazine-^{14}C, 4-[1-^{14}C-3-[2-(trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol dihydrochloride, was administered orally to dogs at 10 and 40 mg./kg. and to monkeys at 10 mg./kg. After an oral dose of 10 mg./kg., dogs excreted 2–4% and 75–89% of the dose in the urine and feces, respectively. Monkeys that received 10 mg./kg. orally excreted 12–19% and 56–69% of the dose in the urine and feces, respectively. After an oral dose of 40 mg./kg., dogs excreted 5–18% and 85–110% of the dose in the urine and feces, respectively. After 10 mg./kg. i.v., a dog excreted 1.2, 62.0, and 0.03% of the dose in the urine, bile, and expired air, respectively, in 7 hr. After an oral dose of 40 mg./kg., dogs had the highest concentrations of radioactivity in the liver; lungs; combined retina, choroid, and sclera; and kidneys. Localization of radioactivity to gross areas of the brain was not demonstrable. In addition to fluphenazine sulfoxide, several metabolites were present in the urine of dogs and monkeys. In the feces, in addition to fluphenazine-^{14}C, one major and two minor metabolites were found in both species. As the oral dose of fluphenazine-^{14}C administered to dogs was increased from 10 to 40 mg./kg., the amount of unchanged fluphenazine-^{14}C increased relative to the major metabolite. The major metabolite was unconjugated in the feces but was present as the glucuronide conjugate in the bile.

Keyphrases □ Fluphenazine-^{14}C dihydrochloride—metabolic fate, dog, monkey □ Biliary, urinary, fecal excretion—fluphenazine-^{14}C and metabolites □ Metabolites, fluphenazine-^{14}C—isolation, identification □ Scintillometry, liquid—analysis

Fluphenazine[1] is a phenothiazine derivative bearing a trifluoromethyl substituent and a piperazine-containing side chain (Structure I). The compound was labeled with



I

Structure of fluphenazine-^{14}C

^{14}C in the 1-propyl position and administered to dogs, rhesus monkeys, and baboons in an attempt to determine the kinds of metabolites formed. It was hoped to synthesize subsequently some of these metabolites and examine their pharmacological activities, thus adding to the understanding of the structure–activity relationships within this class of potent drugs. The results obtained with rhesus monkeys and baboons did not differ appreciably. Therefore, for the most part, only the results found with dogs and rhesus monkeys, the subhuman primate species more commonly employed in experimental studies, are presented here.

[1] Prolixin.

Table I—Chromatographic Systems Employed to Separate Fluphenazine-^{14}C and Its Metabolites

System Number	Solvent System[a]	R_f Fluphenazine	R_f Fluphenazine Sulfoxide	R_f Metabolite C
1	Chloroform–95% ethanol (9:1)	0.37	0.11	—
2	Chloroform–95% ethanol–ammonia (80:20:1)	0.91	0.83	—
3	Benzene–ammonia–dioxane (10:10:80)	0.70	0.42	0.62
4	Benzene–ammonia–dioxane (60:5:35)	0.41	0.06	0.05
5	Chloroform–95% ethanol–ammonia (10:85:2.5)	0.82	0.58	0.60
6	Chloroform–100% ethanol–ammonia (80:10:1)	0.69	0.47	0.18
7	Chloroform–95% ethanol–ammonia (20:75:1)	0.82	0.37	—

[a] Activated thin-layer plates coated with silica gel PF (Brinkmann) were used.

METHODS AND MATERIALS

Purity and Specific Activity of Fluphenazine-^{14}C—Fluphenazine-^{14}C dihydrochloride was synthesized[2] and had a specific activity of 5.5 μc./mg. and a radiochemical purity of 98.4%, as determined by chromatography in Solvent Systems 3 and 4 (Table I).

Animals—Rhesus monkeys[3] (2.0 kg.) were maintained on a diet of Purina Monkey Chow. Primates and pure-bred beagles (7.8–14.9 kg.) were housed in cages that allowed for the separate collection of urine and feces. All animals received about 50 μc. of fluphenazine-^{14}C. Samples of blood were collected at frequent intervals from the jugular veins of the dogs or from the femoral veins of the primates. Samples of urine or feces were collected from all animals every 24 hr. for 14 days. All samples were stored in the frozen state while awaiting analysis.

Animal Surgery—A pure-bred male beagle was anesthetized with pentobarbital (30 mg./kg. i.v.). The radial vein of the dog was infused with a buffered solution of mannitol and pentobarbital at the rate of about 3 ml./min. during the entire 7-hr. experiment. The solution contained: mannitol, 100 g.; KH_2PO_4, 0.2 g.; K_2HPO_4, 0.9 g.; pentobarbital, 25.5 mg./kg. of body weight; and sufficient water to make a final volume of 2 l. An endotracheal tube was inserted in the trachea and a catheter in the urinary bladder. After a midline incision was made, the entrance to the gall bladder from the bile duct was clamped, and the common bile duct was cannulated with polyethylene tubing (No. 100) near its entrance into the duodenum.

Measurement of $^{14}CO_2$ Evolution—To remove respiratory water from the air expired by the dog through the endotracheal tube, it was led through a trap kept cold with dry ice. The air stream was then bubbled through a trap containing 250 ml. of a mixture of 2-methoxyethanol and 2-aminoethanol (3:1). The system was connected to a vacuum line with an appropriate bleeder valve so that the dogs were able to obtain enough air through a twoway valve located just past the endotracheal tube and still force the expired air through the CO_2-trap. The contents of the CO_2-trap were renewed every 105

[2] By Mr. A. Restivo.
[3] Obtained from Primate Imports, Inc.

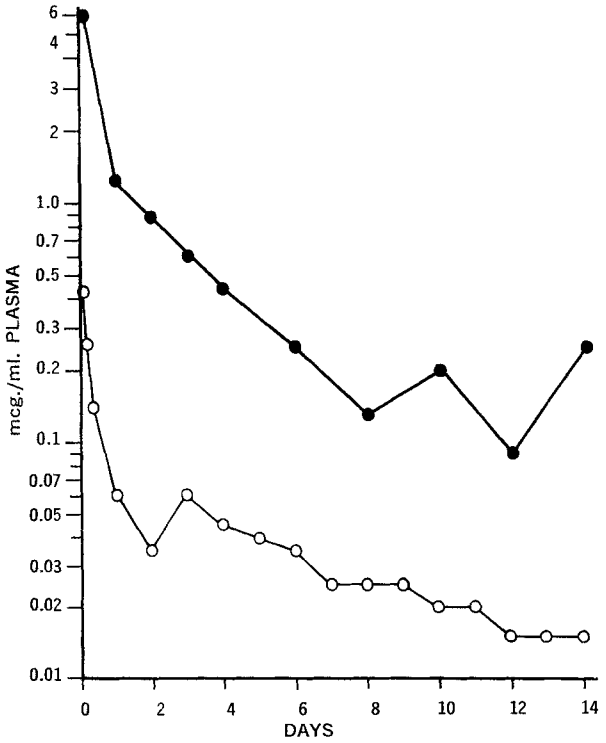


Figure 1—*Levels of fluphenazine-^{14}C equivalents in the plasma of dogs after 10 or 40 mg./kg. p.o. Key: ●, 40 mg./kg.; and ○, 10 mg./kg.*

min. The trapping solution was analyzed for the presence of radioactivity by placing 0.5 ml. into 1.0 ml. of solubilizer[4], followed by 15 ml. of Bray's scintillation fluid (1).

Analysis of Blood and Plasma—A 0.2-ml. sample of heparinized blood was digested in 0.5 ml. of 1.0 *N* NaOH by being heated overnight at 80°. The sample was then bleached with 30% hydrogen peroxide, neutralized with 0.2 ml. of 2-ethylhexanoic acid, and counted in 15 ml. of Bray's scintillation fluid.

A 0.4-ml. sample of plasma was dissolved in 2 ml. of solubilizer[4] and counted in 15 ml. of toluene scintillation fluid. This scintillation fluid contained, per liter of toluene, 5 g. of 2,5-diphenyloxazole and 300 mg. of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

Analysis of Urine and Bile—Samples of urine and bile were counted directly in 15 ml. of Bray's scintillation fluid.

Analysis of Feces—Fecal samples were homogenized in 2–3 volumes of methanol. A 1-g. sample of the homogenate was transferred to a vial, and 5 ml. of 0.5 *N* sodium hydroxide was added. The sample was digested for 16 hr. at 80°. One milliliter of the digested material was transferred to another vial, bleached with 30% hydrogen peroxide, neutralized with 0.2 ml. of 2-ethylhexanoic acid, and counted in 15 ml. of Bray's scintillation fluid. Certain samples of feces that contained the major portion of the dose were also assayed by the combustion method of Kelly *et al.* (2), as described by Ebert and Hess (3).

Extraction Procedure for Feces—A sample of feces was homogenized with 2–3 volumes of methanol and then centrifuged; the supernatant fluid was recovered and saved. Chloroform–methanol (1:1), equal in volume to the supernatant fluid recovered, was added to the fecal residue, and the mixture was shaken for 30 min. and centrifuged; the supernatant fluid was saved. The supernatants were combined and spotted directly for chromatography or were first concentrated to a suitable volume at 40–50° under reduced pressure. This extraction procedure removed an average of 71% of the radioactivity present in the feces.

Analysis of Tissues—The brain was grossly dissected into the cortex and subcortex, cerebellum, brain stem, and dorsal and hypothalamus (4). All samples of brain and other tissues taken at autopsy were homogenized with 2–3 volumes of methanol. About

[4] NCS, Amersham/Searle.

Table II—Summary of the Excretion of Fluphenazine-^{14}C and/or Its Metabolites by Dogs and Rhesus Monkeys

Animal	Dose, mg./kg.	Sex	Percent of Dose		
			Urine	Feces	Total
Dog 143[a]	10	M	1.86	88.99	90.85
Dog 144[a]	10	F	3.88	75.09	78.97
Monkey 231[a]	10	M	19.35	56.43	75.78
Monkey 232[a]	10	F	12.38	68.54	80.92
Dog Q7-5[a]	40	M	10.15	84.69	94.84
Dog Q8-137[a]	40	F	5.11	89.19	94.29
Dog 131[b]	40	M	5.77	109.86	115.63
Dog 107[b]	40	F	18.04	95.97	114.01

[a] Acute drug administration. [b] Nonradioactive fluphenazine was administered orally at 40 mg./kg./day prior to and following the administration of fluphenazine-^{14}C.

0.2 g. of homogenate was solubilized in 2 ml. of solubilizer[4] and counted in 15 ml. of toluene scintillation fluid.

Instrumentation—All samples were counted in a Packard Tri-Carb liquid scintillation spectrometer, model 3375. Counting efficiency was determined by the use of external standardization. Chromatograms were examined for the presence of radioactivity with an Actigraph III scanner[5].

Enzymatic Hydrolysis—Samples of bile (0.5 ml.) were mixed with 0.4 ml. of 0.1 *M* acetate buffer, pH 4.5, and incubated at 37° with either 0.2 ml. of β-glucuronidase[6] or about 1 mg. of aryl sulfatase (Sigma, type III) for 18 or 3 hr., respectively.

Chromatography—With the exception of feces, all samples were spotted on thin-layer plates without prior extraction. Several solvent systems were employed to separate fluphenazine-^{14}C from its metabolites. These different solvent systems and the R_f values of the reference compounds are listed in Table I. Solvent Systems 3 and 4 were routinely employed to detect the presence of any unchanged fluphenazine-^{14}C. In Solvent System 4, metabolites were retained at the origin. Solvent Systems 5 and 6 were routinely employed to separate the polar metabolites. The other solvent systems were employed occasionally, as described under *Results*.

RESULTS

Plasma Levels—Figure 1 depicts the levels of fluphenazine-^{14}C equivalents present in the plasma of dogs that received 10 or 40 mg./kg. p.o. The kinetics of the elimination of radioactivity during the 24 hr. following drug administration (data not shown) indicate the occurrence of two first-order rate processes, with half-lives of 3–5 and 12–13 hr., respectively. Thereafter, the plasma levels decline with a half-life of 5–6 days in the dogs that received 10

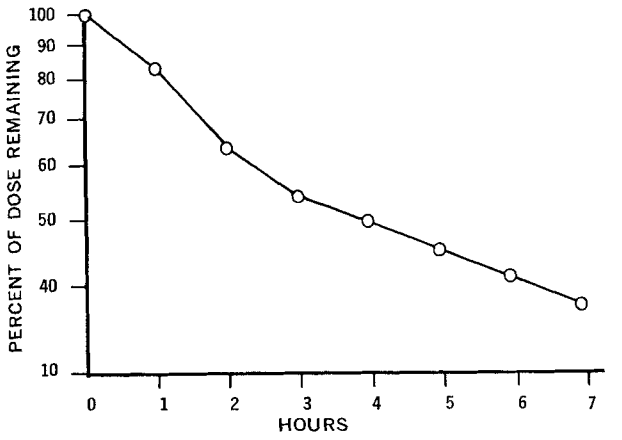


Figure 2—*Excretion of fluphenazine-^{14}C and/or its metabolites in the urine, bile, and expired air. A dog with an externalized bile duct received 10 mg./kg. i.v. of drug.*

[5] Nuclear Chicago.
[6] Ketodase, Warner Chilcott.

Table III—Excretion of Fluphenazine-^{14}C and/or Its Metabolites in the Urine, Bile, and Expired Air of Dog Q8-262[a]

Hours	Percent of Dose		
	Urine	Bile	$^{14}CO_2$
1.0	0.29	16.86	—
1.75	—	—	0.007
2.0	0.22	19.27	—
3.0	0.18	9.52	—
3.5	—	—	0.006
4.0	0.14	4.47	—
5.0	0.13	4.40	—
5.25	—	—	0.006
6.0	0.11	3.83	—
7.0	0.11	3.64	0.006
	1.18	61.99	0.025

[a] A female beagle received 10 mg./kg. i.v. of fluphenazine-^{14}C.

mg./kg. of drug; a good linear fit of the data was obtained (Fig. 1). Monkeys exhibited elimination kinetics similar in their magnitude to those found for the dogs, except that the phase having the shortest half-life in the dogs (3–5 hr.) was not present for the monkeys. In general, the complexity of the elimination kinetics points to the formation of a broad spectrum of metabolites.

The levels of radioactivity found in the plasma of the dogs that received 40 mg./kg. were much higher than those in dogs given 10 mg./kg. of fluphenazine-^{14}C, even when the fourfold difference in the level of drug administered is taken into account. These data suggest that dogs given the higher dose of drug saturated those sites that bind fluphenazine-^{14}C, its metabolites, or both more completely than did the dogs given the lower dose. In dogs that received 40 mg./kg., a larger proportion of the drug was available to the circulation, thus accounting for the higher plasma levels.

Excretion—Table II summarizes the elimination of radioactivity in the urine and feces of dogs and rhesus monkeys. Since the administered drug may tranquilize the animals during the early part of the 14-day test, the intake of food and water may be greatly reduced, resulting in a reduced and delayed excretion of feces. Thus, the data are useful for determining the routes of elimination of the radioactivity but are difficult to interpret from a kinetic standpoint. The primary route for the excretion of fluphenazine-^{14}C, its metabolites, or both was *via* the feces for both species (10 mg./kg.). Compared with the monkeys, the dogs excreted a greater percentage of the dose in the feces. Monkeys, as opposed to dogs, are unable to tolerate oral doses of 40 mg./kg. of fluphenazine-^{14}C; thus, data are available only for dogs given this dose level. Dogs Q7-5 and Q8-137 received single oral doses of 40 mg./kg. of fluphenazine-^{14}C. Dogs 131 and 107 received oral doses of 40 mg./kg. per day of nonradioactive fluphenazine before and after a single oral dose of 40 mg./kg. of fluphenazine-^{14}C. In all four animals, the major portion of the radioactivity was again excreted in the feces and a smaller portion in the urine. However, the amount excreted in the urine by dogs given 40 mg./kg. was several times higher than that excreted by dogs given 10 mg./kg. In each case, the major portion of the radioactivity was

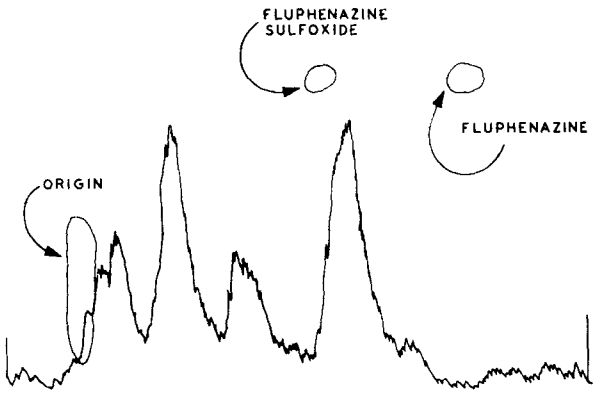


Figure 3—*Chromatogram of urine from the dog in Solvent System 3. The dog received 40 mg./kg. p.o. of fluphenazine-^{14}C.*

Table IV—Levels of Fluphenazine-^{14}C and/or Its Metabolites in Selected Tissues of Dogs

Tissue	Dog 107[a] (mcg./g.)	Dog 113[b] (mcg./g.)
Cerebellum	27.1	22.7
Brain cortex and subcortex	33.2	28.4
Dorsal and hypothalamus	33.6	25.1
Brain stem	31.8	27.9
Combined retina, choroid, and sclera	95.7	61.5
Aqueous and vitreous humors	0.31	0.19
Lens	0.32	0.23
Cornea	4.5	4.5
Heart	14.8	34.3
Kidneys	41.9	47.2
Liver	136.8	109.4
Lungs	61.3	134.3
Omental fat	8.1	7.0
Skeletal muscle	9.8	11.7
Skin with hair	17.0	12.1
Blood, mcg./ml.	1.5	1.4
Plasma, mcg./ml.	2.4	2.0

[a] A female beagle received 40 mg./kg. of fluphenazine-^{14}C by gavage. [b] A male beagle received 40 mg./kg. of fluphenazine-^{14}C by gavage.

excreted during the first 4 days, depending upon the physiological state of the particular animal. Thereafter, small amounts of radioactivity continued to be excreted in the urine and the feces for the duration of the test.

Biliary Excretion—A female beagle with an externalized bile duct received fluphenazine-^{14}C as an intravenous infusion lasting 5 min. The excretion of radioactivity by this dog in the urine and bile and as respiratory $^{14}CO_2$ is shown in Table III. The excretion of fluphenazine-^{14}C, its metabolites, or both takes place primarily in the bile; only 1.2% of the dose was eliminated in the urine. Small

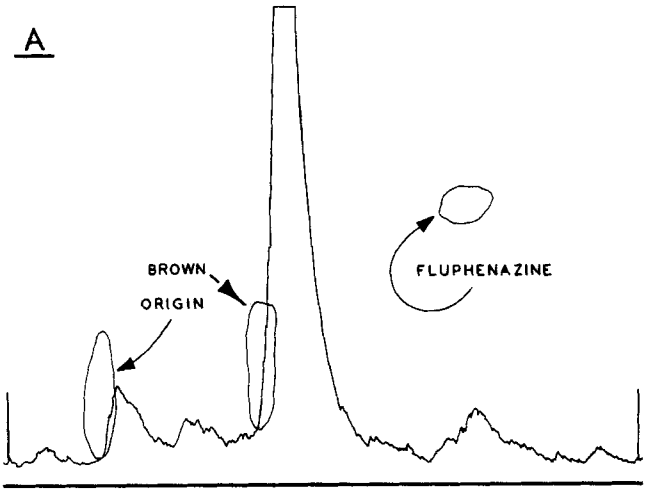


Figure 4—*Chromatograms of extracts of feces from dogs in Solvent System 6. Dogs received: (A) 10 mg./kg. p.o. of fluphenazine-^{14}C, or (B) 40 mg./kg. p.o. of fluphenazine-^{14}C.*

Table V—Summary of the Metabolism of Fluphenazine-^{14}C in the Urine of Dogs and Rhesus Monkeys

Animal	Time of Sample, hr.	Percent of Dose: Fluphenazine Sulfoxide	Percent of Dose: Fluphenazine-^{14}C
Dog 144[a]	0–4	0.95	1.14
Dog 144[a]	24–48	0.68	0.16
Monkey 231[a]	0–4	3.96	0.00
Monkey 232[a]	0–4	1.89	0.00
Dog Q7-5[b]	0–8	2.68	0.92
Dog Q8-137[b]	0–8	—	—
Dog 131[c]	8–24	1.85	0.13
Dog 107[c]	0–8	6.59	0.38

[a] Acute drug administration (10 mg./kg. p.o.). [b] Acute drug administration (40 mg./kg. p.o.). [c] Nonradioactive fluphenazine was administered orally at 40 mg./kg./day prior to and following a single dose of fluphenazine-^{14}C (40 mg./kg. p.o.).

amounts of radioactivity were eliminated in the expired air as $^{14}CO_2$. During the 7 hr. of the test, a total of 63% of the dose was excreted. The excretion of fluphenazine-^{14}C, its metabolites, or both is plotted in Fig. 2. The results show clearly that the radioactivity is eliminated according to a biphasic pattern.

Tissue Distribution—Two dogs, one male and one female, used in a chronic toxicity test, received *nonradioactive* fluphenazine dihydrochloride orally at a level of 40 mg./kg. per day for 52 weeks. Both dogs received a single dose of 40 mg./kg. of fluphenazine-^{14}C by gavage. The animals were sacrificed 19 hr. later, and various tissues were removed and analyzed for the presence of fluphenazine-^{14}C, its metabolites, or both (Table IV). Radioactivity was present in the four areas of the brain examined in approximately equal concentrations. Other organs with concentrations of radioactivity equal to or greater than those in the brain were the kidneys, liver, lungs, and the combined retinal, choroid, and scleral layers of the eye. The concentrations of radioactivity present in the brains of Dogs 107 and 113 were 18–21 times higher than those found in the blood at the times the animals were sacrificed. The dog with the externalized bile duct, which had received 10 mg./kg. i.v. of fluphenazine-^{14}C, had 20 times more radioactivity in the brain at autopsy than had been present in the blood at the time of sacrifice (7 hr. after drug administration). Thus, it is apparent that fluphenazine-^{14}C, its metabolites, or both are highly localized in the various tissues of the body including the target organ, the brain.

Biotransformation—The amounts of unchanged fluphenazine-^{14}C and fluphenazine sulfoxide present in the urine of dogs and monkeys are summarized in Table V. In the urine of the two species, about 1% of the dose or less is excreted as unchanged fluphenazine-^{14}C. Fluphenazine sulfoxide appears to be present in the urine to the extent of 4% of the dose or less. Fluphenazine sulfoxide was identified in the urine of Dog 144 by chromatography in Solvent Systems 3 and 4, with authentic fluphenazine sulfoxide as a reference. Each chromatogram yielded three radioactive peaks, one of which, in each case, corresponded in its R_f value to that of fluphenazine sulfoxide.

Table VI—Summary of the Metabolism of Fluphenazine-^{14}C in the Feces of Dogs and Rhesus Monkeys

Animal	Time of Sample, hr.	Percent of Dose: A	B	C	Fluphenazine-^{14}C
Dog 143[a]	72–96	2.04	8.45	72.5	2.84
Dog 144[a]	48–72	2.55	9.54	53.8	5.32
Dog 144[a]	72–96	1.43	5.03	57.9	4.06
Monkey 231[a]	24–48	3.49	5.70	30.8	9.70
Monkey 232[a]	0–24	3.42	6.51	39.4	13.4
Dog Q7-5[b]	24–48	2.94	3.44	20.2	17.6
Dog Q8-137[b]	24–48	13.0	8.29	20.6	3.12
Dog 131[c]	24–48	0.98	6.76	18.5	35.5
Dog 107[c]	24–48	0.58	7.12	29.0	26.6

[a] Acute drug administration (10 mg./kg. p.o.). [b] Acute drug administration (40 mg./kg. p.o.). [c] Nonradioactive fluphenazine was administered orally at 40 mg./kg./day prior to and following a single dose of fluphenazine-^{14}C (40 mg./kg. p.o.).

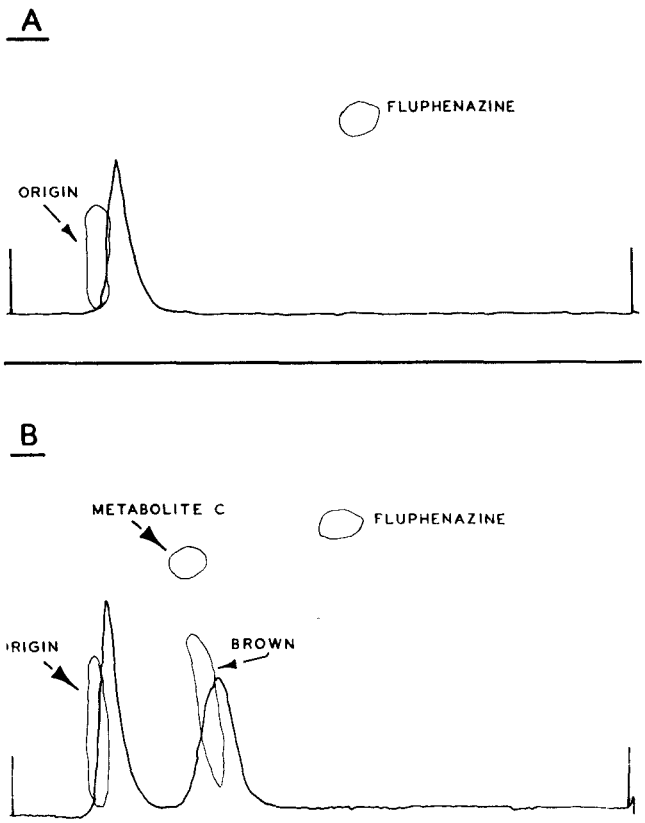


Figure 5—*Chromatograms of bile (0–1 hr.) from the dog (A) before and (B) after treatment with β-glucuronidase. Solvent System 6 was employed. The dog received 10 mg./kg. i.v. of fluphenazine-^{14}C.*

The amounts of fluphenazine and its sulfoxide present in the urine of dogs do not vary significantly as the dose administered is raised from 10 to 40 mg./kg. Figure 3 illustrates a chromatogram of urine from Dog 131 collected during the interval of 0–8 hr. A prominent peak corresponds in R_f value to that of fluphenazine sulfoxide; three, possibly four, other radioactive components are also observed.

Table VI summarizes the disposition of fluphenazine-^{14}C in the feces of dogs and monkeys. The distribution of metabolites in the feces of baboons was essentially similar to that for the rhesus monkey. Fluphenazine-^{14}C was identified in the feces of a baboon by chromatography in Solvent Systems 1, 2, and 3. All three chromatograms showed a minor peak that corresponded, in each case, with the R_f value of authentic fluphenazine. In the feces of animals that received oral doses of 10 mg./kg. of fluphenazine-^{14}C, the unaltered drug was excreted in the amounts of 3–5% of the dose by the dogs and 10–13% of the dose by the monkeys. One major and two minor components are present for each animal species. The results for the dog appear to represent the simplest case, since most of the radioactivity is present in a single peak, as shown in Fig. 4A. This large peak corresponds neither to fluphenazine nor to fluphenazine sulfoxide. In fact, no radioactivity present in the fecal extracts corresponds to fluphenazine sulfoxide. Since the rhesus monkeys and baboons showed a sizable component remaining at the origin of the chromatograms, an attempt was made to separate further the possible components of this peak. The peak that remains at the origin in Solvent System 6 separated into two peaks which moved with R_f values of 0.08 and 0.32 in Solvent System 5; these metabolites are designated in Table VI as A and B, respectively. The major peak shown in the chromatogram of a fecal extract from the dog (Fig. 4A) is designated as Metabolite C, which characteristically forms a brownish zone if the thin-layer plate is allowed to stand overnight. The presence of Metabolites A, B, and C could be detected in the fecal extracts of all three species. The most notable difference in distribution of these components was that Metabolite C was more abundant in the dog than in the primates.

Evidence was obtained that, at least in the baboon, urinary metabolites were distinctly different from those present in the feces. In a chromatogram of a urine sample, essentially all of the radio-

activity moves with an R_f value of 0.1. In a chromatogram of a fecal extract, the major portion of the radioactivity moves with an R_f value of 0.68. Since both of these chromatograms were developed in Solvent System 7, the results indicate that most of the radioactivity present in the urine is not of the same nature as that found in the feces.

As the dose of fluphenazine-^{14}C administered to dogs is raised from 10 to 40 mg./kg., a shift is observed in the relative amounts of unchanged fluphenazine-^{14}C and Metabolite C (Fig. 4B and Table VI). The amount of unchanged fluphenazine-^{14}C generally ranged from 18 to 36% of the dose. The fecal extracts from Dog Q8-137 contained only 3% unchanged fluphenazine-^{14}C, but this dog excreted a correspondingly higher amount of Metabolite A than did the other three animals. Although the fecal samples analyzed were not all excreted during the same interval, in each case they represented the first samples that contained appreciable amounts or the major portion of the dose. With the exception of Dog Q8-137, the other three animals again showed that Metabolite A was the component least present in the extracts of feces. Metabolite B was the next most prominent, and Metabolite C was the most abundant biotransformation product. It is obvious that as the dose of fluphenazine-^{14}C is raised from 10 to 40 mg./kg., the amount of Metabolite C decreases and, correspondingly, the amount of fluphenazine-^{14}C increases. No obvious differences are seen between the two groups of dogs that received 40 mg./kg. of fluphenazine-^{14}C with or without pretreatment with *nonradioactive* fluphenazine.

To determine whether the metabolites present in the feces of dogs had undergone any alteration in the intestines, a sample of bile collected during the 1st hr. after an intravenous dose of 10 mg./kg. of fluphenazine-^{14}C was examined. Chromatography in Solvent System 6 showed that none of the radioactivity present corresponded to unchanged fluphenazine-^{14}C; all of the radioactivity remained at the origin (Fig. 5A). This result is in sharp contrast to the chromatogram of feces shown in Fig. 4A, where most of the radioactivity moved as a single peak with an R_f value of about 0.4. Thus, the radioactive material present in the bile of dogs is not the same as the material extracted from their feces.

To determine whether the bile contained glucuronide conjugates that could have been hydrolyzed in the intestine, a sample of bile was hydrolyzed with a β-glucuronidase preparation. Chromatography now yielded two peaks in both Solvent Systems 5 and 6 instead of the single peaks found with the unhydrolyzed samples of bile (Solvent System 6 is shown in Fig. 5B). This peak, which only appears after hydrolysis, corresponds well to the R_f value of Metabolite C. One can conclude that, at least in the dog, the major metabolite of fluphenazine-^{14}C is excreted in the bile as the glucuronide conjugate. This conjugate is subsequently hydrolyzed in the gastrointestinal tract.

DISCUSSION

There appear to be no appreciable differences between the modes of excretion of fluphenazine-^{14}C in dogs that received 10 or 40 mg./kg. orally, whether as a single dose or chronically; however, at the higher dose, more radioactivity in the feces is unchanged fluphenazine-^{14}C. Also, at the higher dose, the levels of fluphenazine-^{14}C, its metabolites, or both present in the blood and plasma were much higher than would be expected from the fourfold difference in dose level, suggesting that saturable tissue-binding sites are present.

The localization of phenothiazine derivatives and their metabolites in body tissues has been well documented (5). In fact, the pharmacological effects of this class of drugs appear to be closely related to their distribution in certain tissues, like the brain. In the present studies, the authors were unable to demonstrate that fluphenazine-^{14}C or its metabolites localized preferentially in any of the gross anatomic areas of the brain.

Metabolite C is excreted in the bile of the dog as the glucuronide conjugate but appears in the feces as the phenolic compound. The hydrolysis of glucuronide conjugates by intestinal microorganisms has been well documented (6). Sulfoxidation, a metabolic pathway for the conversion of chlorpromazine and related compounds (7), also occurs with fluphenazine in rats (8), in man (9), and, as shown in the present study, in dogs and monkeys, although the excretion of fluphenazine sulfoxide in the urine accounts for only a small percentage of the dose.

As discussed in a companion article (10), Metabolite C was identified as 7-hydroxyfluphenazine.

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