Fluphenazine Enanthate and Fluphenazine Decanoate: Intramuscular Injection and Esterification as Requirements for Slow-Release Characteristics in Dogs

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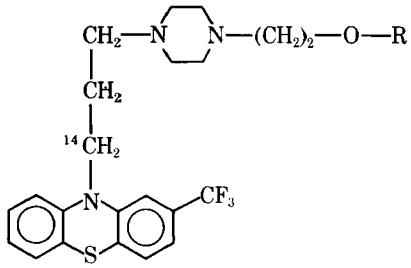
Abstract □ ^{14}C-Fluphenazine base was administered intramuscularly in sesame oil to five male beagles (2 mg/kg). The concentration of radioactivity in plasma and the excretion of radioactivity in urine and feces were measured for 14 days. Maximum concentrations of radioactivity were found in plasma 2 hr after administration. These levels declined with elimination half-lives of 3.20 hr during the 2–12-hr interval after dosing and of 4.02 days during the 2–14-day interval. Most administered radioactivity was excreted during the first 2 days after dosing, predominantly in the feces. An average of 0.43% of the dose was present at the injection site 14 days after dosing; some residual radioactivity was found in the liver and in the ocular portion consisting of the combined retina, choroid, and sclera. ^{14}C-Fluphenazine and its enanthate and decanoate esters were each administered intravenously to three different groups of intact dogs at doses of 1 mg/kg. Regardless of which compound was administered, concentrations of radioactivity in the plasma of these dogs were comparable. Thirty minutes after these dogs had been dosed with ^{14}C-fluphenazine enanthate or ^{14}C-fluphenazine decanoate, most radioactivity in the plasma was present as ^{14}C-fluphenazine base and other unidentified metabolites; at this time, at least 79% of either of the two ^{14}C-fluphenazine esters had been biotransformed. The excretion of radioactivity by these same three groups of dogs was very similar, regardless of which of the three compounds was given. In 7 days, an average of only 3–4% of the dose was excreted in urine; the remainder was excreted in feces. ^{14}C-Fluphenazine and its enanthate and decanoate esters (1 mg/kg) were administered intravenously to dogs whose bile ducts had been cannulated. The amounts of radioactivity excreted in the urine and bile in 8 hr were very similar, as were the residual amounts of radioactivity present in selected tissues. Comparison of the data obtained from dogs given these three compounds intravenously (unformulated) or intramuscularly in sesame oil points to the following conclusions: (*a*) fluphenazine base *per se* does not provide slow-release characteristics unless it has been esterified, for example, with heptanoic or decanoic acid, and (*b*) intramuscular rather than intravenous administration of these two esters is responsible for producing their slow-release characteristics.

Keyphrases □ Fluphenazine—base, enanthate, and decanoate esters, comparison of slow-release characteristics, effect of route of administration, dogs □ Pharmacokinetics—fluphenazine base, enanthate, and decanoate, comparison of slow-release characteristics, effect of route of administration, dogs □ Excretion—fluphenazine base, enanthate, and decanoate, comparison of slow-release characteristics, effect of route of administration, dogs □ Tranquilizers—fluphenazine base, enanthate, and decanoate esters, comparison of slow-release characteristics, effect of route of administration, dogs

The administration to dogs of the long-acting esters of fluphenazine base (I), the enanthate (II) and the decanoate (III), was studied to reveal the conditions necessary for producing slow-release characteristics. An earlier study (1) compared the relative rates of release of ^{14}C-fluphenazine enanthate and ^{14}C-fluphenazine decanoate (1) when each compound was administered intramuscularly in sesame oil to dogs. After that study, it became apparent that the data, which had been based only on measurements of radioactivity, could not be fully interpreted without additional supporting studies in which ^{14}C-fluphenazine and the enanthate and decanoate esters were administered intravenously in the unformulated state. Thus, it was recognized that some



I: R = H

II: R = $C(CH_2)_5CH_3$ (=O)

III: R = $C(CH_2)_8CH_3$ (=O)

information regarding the nature of the radioactivity present in the circulation after the administration of each compound was needed. An additional objective was to determine to what extent ^{14}C-fluphenazine base, administered intramuscularly in sesame oil in the unesterified state, produced slow-release characteristics.

EXPERIMENTAL

Purity and Specific Activity—^{14}C-Fluphenazine hydrochloride and ^{14}C-fluphenazine base had specific activities of 5.8 and 5.5 μCi/mg, respectively, in their undiluted states. The compounds were not less than 98% chemically and radiochemically pure.

^{14}C-Fluphenazine enanthate had a specific activity of 4.6 μCi/mg in the undiluted state. The compound was not less than 97% chemically and radiochemically pure.

^{14}C-Fluphenazine decanoate had a specific activity of 4.4 μCi/mg. The compound was not less than 98% chemically and radiochemically pure.

Design of Intramuscular Studies with ^{14}C-Fluphenazine Base—Unesterified ^{14}C-fluphenazine base was dissolved in sesame oil at a concentration of 40.7 mg/ml. The sesame oil also contained 1.5% benzyl alcohol. Five purebred male beagles each received an intramuscular injection into the biceps femoris of formulated ^{14}C-fluphenazine base (0.5 ml of sesame oil preparation/10 kg of body weight). The dogs weighed 9.8 ± 0.4 kg (±*SE*); each was given a dose of 2.04 ± 0.02 mg/kg, containing 110 ± 5 μCi of total radioactivity.

The dogs were housed in metabolism cages that permitted the separate collection of urine and feces. Blood samples were drawn into heparinized syringes periodically for 14 days. The total urinary and fecal excretions were collected daily for 14 days; then the dogs were anesthetized with pentobarbital (30 mg/kg iv) and sacrificed by the intravenous injection of 5 ml of a saturated solution of potassium chloride.

The dogs were necropsied, and selected tissues were analyzed for residual radioactivity. In addition, the entire musculature of the left and right thighs was removed and analyzed so that the amount of radioactivity remaining in the injection site could be determined.

Analysis of Plasma—A 0.8-ml plasma sample was dissolved in 4 ml of solubilizer[1]. The sample was counted with 15 ml of toluene

[1] NCS solubilizer, Amersham/Searle Corp., Des Plaines, Ill.

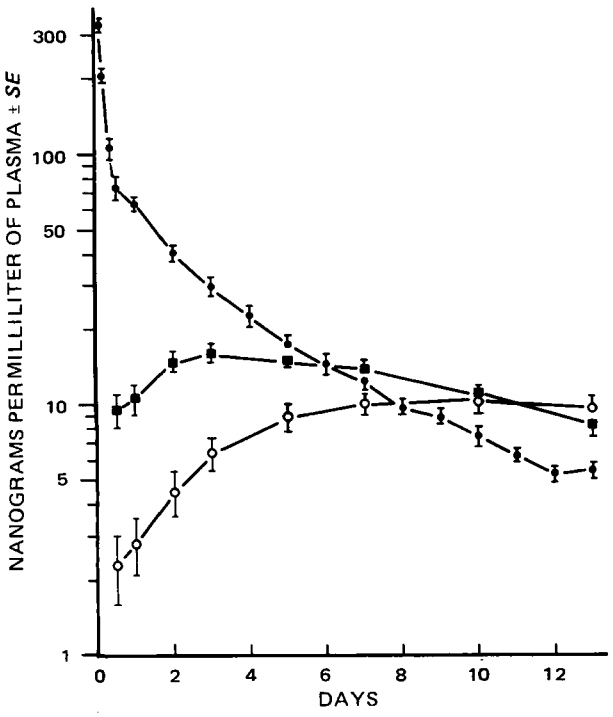


Figure 1—*Average concentrations of radioactivity in the plasma of male dogs after the intramuscular administration of 2-mg/kg doses of ^{14}C-fluphenazine base (●), ^{14}C-fluphenazine enanthate (■), or ^{14}C-fluphenazine decanoate (○) (n = 5/group). All three compounds were administered in sesame oil. The dogs ranged in weight from 9.2 to 11.6 kg. To facilitate comparison among the three compounds, the data were truncated at 13 days.*

scintillation fluid containing 5 g of 2,5-diphenyloxazole and 300 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene/liter of toluene.

Extraction Procedure for Plasma—Plasma (9–12 ml) from dogs was mixed with four volumes of methanol (40 ml) and shaken for 20 min. The samples were centrifuged at 200×*g* (10°), and the supernatant fluid was recovered and saved. The pellet that remained was extracted by shaking it with the same volume of methanol (40 ml) originally added to the plasma and centrifuging. The combined supernatant fluids were concentrated almost to dryness under reduced pressure at 40–50°. This extraction procedure removed the following average amounts of radioactivity from the plasma of dogs after intravenous treatment with the indicated drug: 77%, ^{14}C-fluphenazine; 68%, ^{14}C-fluphenazine enanthate; and 84%, ^{14}C-fluphenazine decanoate.

Analysis of Urine, Bile, and Feces—Samples of feces (200 g) were homogenized with 400 ml of methanol; 790–825 mg of homogenate was combusted[2]. Samples of urine (0.2 ml) and bile (50 μl) were counted directly in 15 ml of scintillation fluid (2).

Analysis of Tissues—Brain samples (80 g) were homogenized in water (100 ml), using an all-glass homogenizer[3]; 790–810 mg of homogenate was combusted. Certain portions of the eye (lens, cornea, aqueous and vitreous humors, and combined retina, choroid, and sclera) and blood were combusted. Similarly, representative portions of omental fat (180–220 mg) and skin without hair (280–320 mg) were combusted. Samples of kidney, liver, and thigh muscle were ground in a meat grinder; then 780–820 mg of the well-mixed sample was combusted.

Bile Collection—Purebred male beagles (8.8–15.8 kg) were anesthetized with pentobarbital (30 mg/kg iv). The radial vein of each dog was cannulated, and a buffered solution of mannitol and pentobarbital was infused throughout the 8-hr experiments at a rate of about 3 ml/min. The solution contained: mannitol, 100 g; monobasic potassium phosphate, 0.2 g; dibasic potassium phosphate, 0.9 g; pentobarbital, 25.5 mg/kg; and sufficient water to make a final volume of 2 liters.

A catheter was inserted into the urinary bladder of each dog. Then a midline incision was made, the entrance to the gallbladder from the bile duct was clamped, and the common bile duct was cannulated with polyethylene tubing (No. 100) near its point of entry into the duodenum. The midline incision was closed with wound clips. At the conclusion of the experiment, each dog was sacrificed by the intravenous administration of 5 ml of a saturated solution of potassium chloride.

Chromatography—Samples of bile (25–50 μl) and extracts of plasma (50–100 μl) were applied onto thin-layer plates coated with silica gel[4] and developed in benzene–28% ammonia–dioxane (60:5:35). This solvent system resolved fluphenazine base from its enanthate and decanoate esters and also from some of the unidentified metabolites (R_f values of 0.00, 0.06, and 0.23) of these three compounds.

The utility of this chromatographic system for separating fluphenazine base from its enanthate and decanoate esters was demonstrated by the following experiment. Solutions (5 ml, 80 μg/ml) of the dihydrochloride salts of the enanthate and decanoate esters of ^{14}C-fluphenazine were prepared in dog plasma, which had been denatured by heating for 30 min in a boiling water bath. These solutions were made strongly alkaline (pH 12–13) by the addition of 1 drop of 50% sodium hydroxide and incubated in a boiling water bath for 90 min. After incubation, the pH was adjusted to 6.5–7.5 and the samples were chromatographed. None of the radioactivity was now present at the R_f value of either ester; instead, all of it had been transformed to unesterified ^{14}C-fluphenazine base, as judged by the coincidence of the radioactive peak and the authentic reference compound.

Counting of Samples—Radioactivity in each sample was measured by a liquid scintillation spectrometer[5]. Counting efficiency was determined with automatic external standardization and the use of previously prepared quench curves. Chromatograms were scanned[6] for the presence of radioactivity, and the area under each curve was integrated in duplicate by using a planimeter. The average areas were accepted if the duplicate measurements agreed within 1.5%.

RESULTS

Intramuscular Studies with Unesterified ^{14}C-Fluphenazine Base—Average concentrations of radioactivity in the plasma of dogs dosed with 2 mg/kg im of unesterified ^{14}C-fluphenazine base in sesame oil are shown in Fig. 1. For comparison, plasma concentrations from an earlier study (1) are shown; the enanthate and decanoate esters had been given to dogs at 2 mg/kg under identical conditions. Even though ^{14}C-fluphenazine base was administered intramuscularly in sesame oil, no sustained-release effect was found for any of the five dogs, as was the case for the enanthate and decanoate esters.

Concentrations of radioactivity, already at a maximum 2 hr after the dogs were dosed, declined rapidly at first (2–12 hr) and then more slowly during the remainder of the test. Half-lives of elimination of radioactivity from the plasma were calculated for each dog based on a linear regression analysis. The regression lines for the concentrations of radioactivity in the plasma of each dog were calculated for the 2–12-hr and 2–14-day intervals. The pooled average values, considered to represent the most accurate analysis of regression, indicate an elimination half-life of radioactivity from plasma of 3.20 hr for the 2–12-hr interval after dosing (by the method of residuals). This portion of the plasma curve may represent a combination of distribution of radioactivity into tissues and excretion, but this point has not been verified experimentally. The elimination half-life of radioactivity from plasma during the 2–14-day interval is a pooled average of 4.02 days.

The average daily excretion of radioactivity in the urine and feces by the five dogs is shown in Table I. No more than 6% of the dose was excreted in urine by any individual dog, with the remainder appearing in the feces. Most of the radioactivity (102.3% of the dose) was excreted during the first 4 days, particularly during the first 2 days after dosing (90.1% of the dose). Four days after the dogs were dosed, they excreted only an additional 1.7–5.8% of the dose. This rapid excretion during the first 2 days after dosing also indicates a general lack of slow-release characteristics in dogs dosed with ^{14}C-fluphenazine base.

Fourteen days after the dogs were dosed, they were necropsied;

[2] Biological material oxidizer, R. J. Harvey Instrument Corp., Hillsdale, N.J.

[3] Potter-Elvehjem, Fisher Scientific Co., Springfield, N.J.

[4] QIF, Quantum Industries, Fairfield, N.J.

[5] Packard Tri-Carb model 3380, Packard Instrument Co., Downers Grove, Ill.

[6] Nuclear-Chicago Actigraph III, Amersham/Searle Corp., Des Plaines, Ill.

Table I—Average Daily Excretion of Radioactivity by Five Male Dogs after Intramuscular Administration of ^{14}C-Fluphenazine Base in Sesame Oil (2 mg/kg)

Days	Average[a] Percent of Dose ± *SE*		
	Urine	Feces	Total
1	2.30 ± 0.24	25.53 ± 10.63	27.84 ± 10.79
2	1.19 ± 0.26	61.09 ± 9.98	62.27 ± 10.07
3	0.40 ± 0.07	2.36 ± 0.23	2.76 ± 0.25
4	0.21 ± 0.04	9.18 ± 1.84	9.39 ± 1.85
5	0.13 ± 0.02	1.30 ± 0.35	1.44 ± 0.35
6	0.080 ± 0.014	0.70 ± 0.18	0.78 ± 0.18
7	0.047 ± 0.009	0.33 ± 0.07	0.38 ± 0.07
8	0.044 ± 0.006	0.26 ± 0.03	0.30 ± 0.03
9	0.028 ± 0.004	0.20 ± 0.01	0.23 ± 0.01
10	0.018 ± 0.001	0.15 ± 0.01	0.17 ± 0.01
11	0.014 ± 0.001	0.094 ± 0.009	0.11 ± 0.01
12	0.017 ± 0.003	0.10 ± 0.01	0.12 ± 0.01
13	0.019 ± 0.002	0.093 ± 0.004	0.11 ± 0.01
14	0.013 ± 0.002	0.067 ± 0.018	0.080 ± 0.019
Pan rinse	0.11 ± 0.02	—	0.11 ± 0.02
	4.64 ± 0.48	101.47 ± 0.92	106.08 ± 0.92

[a] All average values were rounded to two significant figures.

Table II—Average Residual Radioactivity in Selected Tissues of Five Male Dogs 14 Days after Intramuscular Administration of ^{14}C-Fluphenazine Base in Sesame Oil (2 mg/kg)

Tissue	Average ± *SE*	
	μg/g	% of Dose
Brain	0.000	0.000
Combined retina, choroid, and sclera	0.83 ± 0.13	—
Cornea	0.021 ± 0.003	—
Aqueous humor	0.013 ± 0.006	—
Vitreous humor	0.015 ± 0.006	—
Lens	0.006 ± 0.003	—
Kidneys	0.043 ± 0.004	0.010 ± 0.001
Liver	0.37 ± 0.03	0.54 ± 0.04
Omental fat	0.006 ± 0.003	—
Skin	0.024 ± 0.003	—
Left thigh muscle (contains injection site)	0.18 ± 0.02	0.44[a] ± 0.06
Right thigh muscle	0.003 ± 0.001	0.006 ± 0.001
Blood, μg/ml	0.004 ± 0.001	—

[a] The radioactivity remaining at each injection site, as presented in the text, was determined by subtracting the total amount of radioactivity present in the right thigh muscle from that present in the left thigh muscle.

selected tissues were removed for the analysis of residual radioactivity, including the thigh muscle containing the site of injection (Table II). The small amounts of radioactivity remaining at the sites of injection, an average of 0.43% (range of 0.29–0.55% of the dose), were consistent with the finding of essentially complete excretion of radioactivity by each dog. Of the other tissues analyzed, only the liver and the portion of the eye consisting of the combined retina, choroid, and sclera contained any appreciable quantities of radioactivity. The binding of phenothiazines to melanin is a well-documented phenomenon (3).

The excretion of ^{14}C-fluphenazine, its metabolites, or both in bile probably accounts for the relatively greater amounts of residual radioactivity found in liver than in the other tissues. Interestingly, no radioactivity could be detected in a homogenate of whole brain from any of the dogs, even though small amounts of radioactivity were still detectable in plasma 14 days after dosing. This finding is in contrast to the intramuscular injection of the enanthate and decanoate esters. In those cases, small amounts of radioactivity were present in the brain and blood 35 days after the dogs were dosed (1).

Intravenous Studies in Intact Dogs—^{14}C-Fluphenazine and the dihydrochloride salts of the enanthate and decanoate esters were administered to intact dogs at doses of 1 mg/kg iv. Regardless of which compound was administered, concentrations of radioactivity in the plasma of these dogs were quite comparable, except during the first 30 min after drug administration (Fig. 2). During this early time, radioactivity from each compound was being distributed to the various tissues of the body. Thereafter, the rates of elimination of total radioactivity from plasma were similar for each of the three compounds. Therefore, no slow-release characteristics were found when the enanthate and decanoate esters of fluphenazine were administered intravenously; such an effect was found previously when the esters were administered intramuscularly in sesame oil to dogs (1).

A large sample of blood (25–30 ml) was taken from each dog 30 min after it was dosed intravenously with either ^{14}C-fluphenazine or its enanthate or decanoate ester. The plasma (9–12 ml) from each blood sample was recovered, extracted, and chromatographed (Fig. 3). For two of the three dogs given ^{14}C-fluphenazine (upper portion of Fig. 3), there were detectable amounts of unchanged ^{14}C-fluphenazine in plasma, amounting to 28 and 72% of the radioactivity applied to the chromatograms. For both of these dogs, an unidentified metabolite with an R_f value of about 0.20 was also present, and there were additional unidentified metabolites present at (R_f value of 0.00) or just

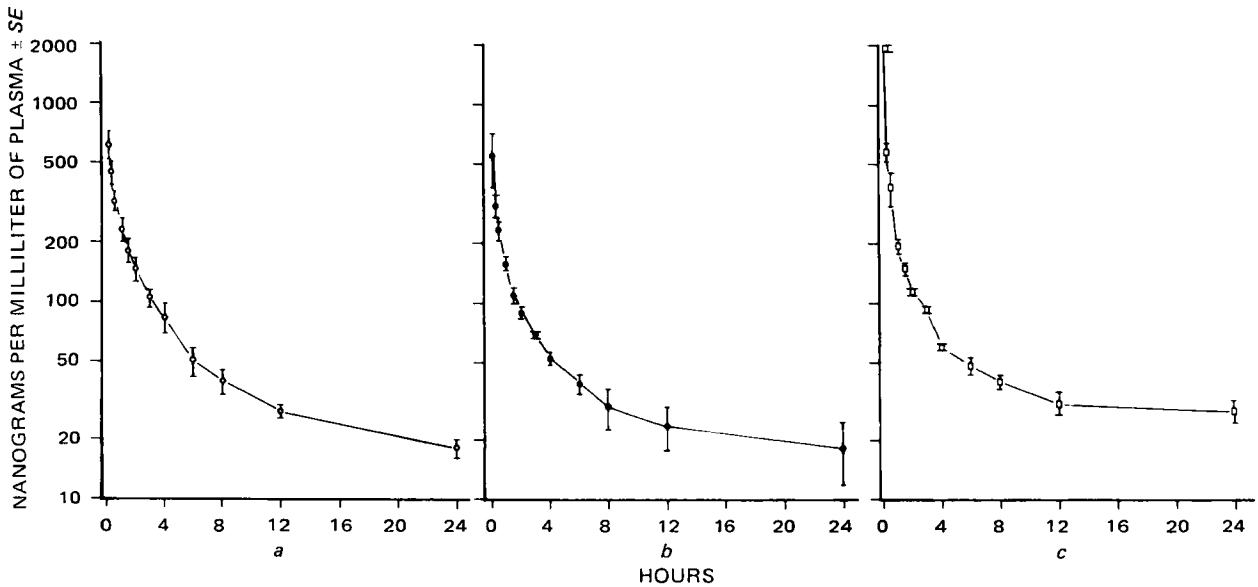


Figure 2—*Average concentrations of radioactivity in the plasma of intact male dogs after the intravenous administration of 1-mg/kg doses of ^{14}C-fluphenazine* (n = 3) (a), *^{14}C-fluphenazine enanthate dihydrochloride* (n = 2) (b), *or ^{14}C-fluphenazine decanoate dihydrochloride* (n = 2) (c). *All three compounds were dissolved in 1.5 ml of water containing either 25 or 50% ethanol and injected during 2-min periods.*

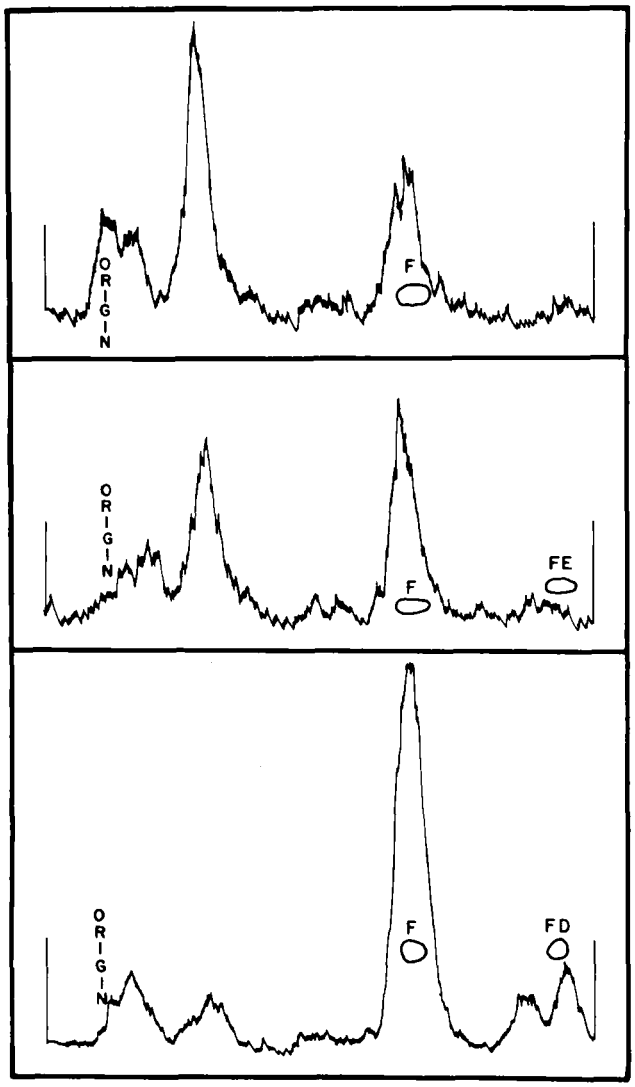


Figure 3—*Typical thin-layer chromatograms of extracts obtained from the plasma of dogs after the intravenous administration of 1-mg/kg doses of ^{14}C-fluphenazine (top), ^{14}C-fluphenazine enanthate dihydrochloride (middle), or ^{14}C-fluphenazine decanoate dihydrochloride (bottom). The circled areas represent material visible under UV light (254 nm). F is ^{14}C-fluphenazine base, FE is ^{14}C-fluphenazine enanthate, and FD is ^{14}C-fluphenazine decanoate.*

ahead of (R_f values of 0.06 and 0.23) the origin of this chromatogram.

Chromatograms of the extracts of plasma prepared 30 min after the dogs had been dosed intravenously with the enanthate ester are shown in the middle portion of Fig. 3. Virtually no unchanged ^{14}C-fluphenazine enanthate was present in these plasma samples (about 6%); however, significant amounts of ^{14}C-fluphenazine base (15 and 44%) were found, as well as the other metabolites that had been found previously in the plasma of dogs dosed with ^{14}C-fluphenazine. Chromatograms of plasma extracts of the dogs dosed intravenously with the decanoate ester (lower portion of Fig. 3) generally resembled those for dogs dosed with the enanthate ester: 9 and 21% of the radioactivity were unchanged ^{14}C-fluphenazine decanoate, 11 and 57% were unchanged ^{14}C-fluphenazine base, and the same additional metabolites were present as had been found in the plasma of dogs dosed with ^{14}C-fluphenazine.

The excretion of radioactivity in the urine and feces of each intact dog dosed intravenously with either ^{14}C-fluphenazine or its enanthate or decanoate ester is shown in the appropriate columns of Tables III and IV, respectively. Regardless of whether ^{14}C-fluphenazine or its enanthate or decanoate ester was administered intravenously, the excretion of radioactivity in urine and feces was similar as a function of time, and the total amounts of radioactivity excreted in the urine and feces in 7 days were essentially the same. Significantly, based on data for excretion, no slow-release characteristics were produced after the intravenous administration of either of the two esters.

Intravenous Studies in Bile-Cannulated Dogs—Since most of the radioactivity after an intravenous dose of ^{14}C-fluphenazine or its enanthate or decanoate ester was excreted in feces, it was difficult to follow the rates of excretion and biotransformation of any of these three compounds unless the bile was collected continuously. Accordingly, a comparison was made of excretion and biotransformation after administration of each of the three compounds to dogs whose bile ducts had been cannulated for the continuous collection of bile.

Single dogs prepared surgically in this manner were given 1-mg/kg doses of either ^{14}C-fluphenazine or the dihydrochloride salts of the enanthate or decanoate esters. As shown in Table V, approximately the same amounts of total radioactivity were excreted in urine and bile during an 8-hr test (58–75% of the dose). In addition, the approximate rates at which these total amounts of radioactivity were excreted as a function of time were comparable, and no unchanged ^{14}C-fluphenazine base or either of its esters was detectable in the earliest bile samples (0–1 hr) collected from the dogs given any of the three compounds.

An earlier study (4) showed that the nature of the radioactivity excreted in the bile of dogs dosed with ^{14}C-fluphenazine enanthate consisted primarily of the glucuronide conjugate of 7-hydroxyfluphenazine.

Eight hours after they were dosed, these three bile-cannulated dogs had similar amounts of radioactivity remaining in selected tissues (Table VI). No matter which of the three compounds was administered, radioactivity was most concentrated in the liver and lungs and less concentrated in the blood and other tissues examined.

DISCUSSION

Additional data summarizing the comparative rates of excretion of radioactivity in the urine and feces of intact dogs after intramus-

Table III—Average Excretion of Radioactivity in the Urine of Dogs after Intravenous (1 mg/kg) or Intramuscular (2 mg/kg in Sesame Oil) Administration of ^{14}C-Fluphenazine, ^{14}C-Fluphenazine Enanthate, or ^{14}C-Fluphenazine Decanoate

	Average Percent of Dose ± *SE*					
	^{14}C-Fluphenazine		^{14}C-Fluphenazine Enanthate		^{14}C-Fluphenazine Decanoate	
Days	Intravenously[a]	Intramuscularly[b]	Intravenously[a]	Intramuscularly[b]	Intravenously[a]	Intramuscularly[b]
1	2.22 ± 0.29	2.30 ± 0.24	1.86 ± 0.04	0.20 ± 0.04	—[c]	0.04 ± 0.00
2	0.80 ± 0.10	1.19 ± 0.26	0.76 ± 0.31	0.29 ± 0.03	2.98 ± 0.09	0.07 ± 0.02
3	0.43 ± 0.06	0.40 ± 0.07	0.26 ± 0.01	0.36 ± 0.07	0.41 ± 0.23	0.10 ± 0.02
4	0.36 ± 0.24	0.21 ± 0.04	0.18 ± 0.05	0.43 ± 0.03	0.22 ± 0.06	0.14 ± 0.03
5	0.16 ± 0.09	0.13 ± 0.02	0.07 ± 0.01	0.35 ± 0.05	0.12 ± 0.02	0.13 ± 0.03
6	0.06 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	0.33 ± 0.01	0.11 ± 0.06	0.16 ± 0.02
7	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.27 ± 0.02	0.03 ± 0.01	0.17 ± 0.03
	4.08[d] ± 0.06	4.35[d] ± 0.45	3.23[d] ± 0.41	2.18[d] ± 0.10	3.87[d] ± 0.28	0.81[d] ± 0.07

[a] Dihydrochloride salt. [b] Base. [c] None excreted. [d] The average weekly totals (±*SE*) for each treatment group were calculated based on the total amount of radioactivity excreted during the first 7 days by *each* dog. The average amounts (±*SE*) excreted per day for each treatment group were calculated based only on those dogs that actually excreted a sample on that particular day.

Table IV—Average Excretion of Radioactivity in the Feces of Dogs after Intravenous (1 mg/kg) or Intramuscular (2 mg/kg in Sesame Oil) Administration of ^{14}C-Fluphenazine, ^{14}C-Fluphenazine Enanthate, or ^{14}C-Fluphenazine Decanoate

	Average Percent of Dose ± *SE*					
	^{14}C-Fluphenazine		^{14}C-Fluphenazine Enanthate		^{14}C-Fluphenazine Decanoate	
Days	Intravenously[a]	Intramuscularly[b]	Intravenously[a]	Intramuscularly[b]	Intravenously[a]	Intramuscularly[b]
1	48.67 ± 15.51	31.92 ± 10.97	42.31 ± 36.35	1.23 ± 0.98	—[c]	0.50 ± 0.15
2	29.73 ± 14.43	61.09 ± 9.98	51.70 ± 30.77	10.57 ± 1.88	60.99 ± 21.30	1.29 ± 0.20
3	18.50 ± 9.54	2.36 ± 0.23	5.73 ± 2.30	7.17 ± 1.66	22.77 ± 17.50	2.98 ± 0.62
4	17.27 ± 15.12	9.18 ± 1.84	1.57 ± 0.01	8.16 ± 0.60	4.56 ± 1.39	2.63 ± 0.41
5	4.14 ± 3.24	1.30 ± 0.35	0.59 ± 0.05	9.20 ± 0.90	2.32 ± 1.33	3.22 ± 0.53
6	1.00 ± 0.60	0.70 ± 0.18	0.24 ± 0.05	5.18 ± 0.96	0.65 ± 0.09	3.60 ± 0.61
7	0.32 ± 0.15	0.33 ± 0.07	0.32 ± 0.02	5.90 ± 1.46	0.58 ± 0.46	2.84 ± 0.43
	103.40[d] ± 2.81	100.49[d] ± 0.92	102.46[d] ± 3.36	44.80[d] ± 2.58	91.87[d] ± 0.55	16.26[d] ± 2.34

[a] Dihydrochloride salt. [b] Base. [c] None excreted. [d] The average weekly totals (±*SE*) for each treatment group were calculated based on the total amount of radioactivity excreted during the first 7 days by *each* dog. The average amounts (±*SE*) excreted per day for each treatment group were calculated based only on those dogs that actually excreted a sample on that particular day.

Table V—Excretion of Radioactivity in the Urine and Bile of Dogs after Intravenous Administration of ^{14}C-Fluphenazine, ^{14}C-Fluphenazine Enanthate, or ^{14}C-Fluphenazine Decanoate (1 mg/kg)

	Percent of Dose		
Hours	^{14}C-Fluphenazine[a]	^{14}C-Fluphenazine Enanthate[a]	^{14}C-Fluphenazine Decanoate[a]
0–1	17.60	18.37	23.46
1–2	19.93	19.33	21.36
2–3	10.21	6.81	6.83
3–4	7.34	4.08	4.32
4–5	5.44	3.16	3.77
5–6	4.98	2.29	2.69
6–7	4.57	2.00	2.42
7–8	4.55	2.31	1.81
	74.62	58.35	66.66

[a] Dihydrochloride salt.

cular doses of ^{14}C-fluphenazine base or of its enanthate or decanoate ester (2 mg/kg) are shown in Tables III and IV. Although the intramuscular studies were for longer periods (14–35 days) than the intravenous studies (7 days), the data for both routes of administration were truncated at 7 days to permit direct comparison.

After intact dogs were dosed either intravenously or intramuscularly with ^{14}C-fluphenazine, similar amounts of radioactivity (intravenously, 107.5% of the dose; intramuscularly, 104.8% of the dose) were excreted in 7 days in urine and feces. After the administration of ^{14}C-fluphenazine by either route, most of the dose was excreted during the first 2 days (intravenously, 65.2% of the dose; intramuscularly, 90.1% of the dose). Thus, the administration of unesterified ^{14}C-fluphenazine base, either intravenously or intramuscularly, does not produce slow-release characteristics.

After the dosing of intact dogs with ^{14}C-fluphenazine enanthate, the excretion of radioactivity within 7 days was almost complete for dogs dosed intravenously but was only about half completed for dogs dosed intramuscularly. The results for intact dogs that were dosed either intravenously or intramuscularly with ^{14}C-fluphenazine decanoate were similar to those after dosing of such animals with the enanthate ester; however, for the decanoate ester, the rate of excretion of radioactivity was still slower than it was for the enanthate ester after the latter was administered intramuscularly. Thus, the intravenous administration of ^{14}C-fluphenazine or of its enanthate or decanoate ester to intact or bile-cannulated dogs produced no slow-release characteristics. In addition, the intramuscular administration of ^{14}C-fluphenazine base in sesame oil produced no slow-release characteristics, unless the parent molecule was esterified with heptanoic or decanoic acid.

An earlier study (1) compared the relative rates of release of ^{14}C-fluphenazine enanthate and ^{14}C-fluphenazine decanoate when these drugs were given intramuscularly (2 mg/kg) in sesame oil to dogs under the same conditions used in the present study with ^{14}C-fluphenazine base. ^{14}C-Fluphenazine decanoate was released from the site of injection more slowly than was the enanthate ester. By contrast, the administration of ^{14}C-fluphenazine base did not produce any slow-release characteristics; a large fraction of the dose was released from the site of injection during the first 12 hr after dosing.

Since, after the intramuscular administration of ^{14}C-fluphenazine base, most of the radioactivity was released from the site of injection

Table VI—Residual Radioactivity in Selected Tissues of Dogs 8 hr after Intravenous Administration of ^{14}C-Fluphenazine, ^{14}C-Fluphenazine Enanthate, or ^{14}C-Fluphenazine Decanoate (1 mg/kg)

	^{14}C-Fluphenazine[a,b]		^{14}C-Fluphenazine Enanthate[a,c]		^{14}C-Fluphenazine Decanoate[a,d]	
Tissue	Amount in Tissue, μg/g	Amount in Entire Organ, % of Dose	Amount in Tissue, μg/g	Amount in Entire Organ, % of Dose	Amount in Tissue, μg/g	Amount in Entire Organ, % of Dose
Brain cortex and subcortex	0.56	—	0.43	—	0.37	—
Brain stem	0.67	—	0.40	—	0.34	—
Cerebellum	0.27	—	0.52	—	0.26	—
Dorsal and hypothalamus	0.58	—	0.75	—	0.35	—
Heart	0.38	0.30	0.42	0.38	0.26	0.25
Kidneys	0.55	0.37	0.69	0.39	0.44	0.30
Liver	3.31	9.62	3.69	8.00	2.79	7.02
Lungs	2.91	2.52	2.73	1.71	2.67	2.07
Skeletal muscle	0.19	—	0.63	—	0.18	—
Omental fat	0.15	—	0.24	—	0.15	—
Skin	0.27	—	0.15	—	0.096	—
Blood, μg/ml	0.050	—	0.031	—	0.050	—

[a] Dihydrochloride salt. [b] A male dog was given 1 mg/kg of ^{14}C-fluphenazine dissolved in 3.4 ml of water as an intravenous injection lasting 2 min. [c] A male dog was given 1 mg/kg of ^{14}C-fluphenazine enanthate dissolved in 6.2 ml of water containing 28% ethanol as an intravenous injection lasting 2 min. [d] A male dog was given 1 mg/kg of ^{14}C-fluphenazine decanoate dissolved in 4.6 ml of water as an intravenous injection lasting 2 min.

and excreted from the body during the first 2–4 days after dosing, the concentrations of radioactivity remaining in the plasma during the 2–14-day interval after dosing are assumed to represent largely metabolites of fluphenazine rather than the parent compound. However, this point could not be established experimentally. On the other hand, in the case of the esters of fluphenazine base, because of their slow-release characteristics, the esters themselves, or fluphenazine base, the product of their hydrolysis, may be released continuously into the circulation for a much longer period.

For all intact dogs given intravenous doses (1 mg/kg) of ^{14}C-fluphenazine or of its enanthate or decanoate ester, virtually identical concentrations of radioactivity were present in the circulation. In addition, the enanthate and decanoate esters of ^{14}C-fluphenazine were rapidly converted to ^{14}C-fluphenazine base and other unidentified metabolites. Thus, slow-release characteristics were not produced by the intravenous administration of the esters of ^{14}C-fluphenazine base.

A number of other lines of evidence point to the fact that the slow-release characteristics produced by the esterified fluphenazine base are a function both of esterification *per se* and of intramuscular administration. In an earlier study (1), a comparison was made, during a 35-day period, of the relative rates of release of ^{14}C-fluphenazine enanthate and ^{14}C-fluphenazine decanoate. The rates of excretion of total radioactivity in urine and feces after administration of either of these two esters intramuscularly in sesame oil were dissimilar; however, the radioactivity was excreted at the same rate in *both* urine and feces for each ester but more slowly for the decanoate ester than for the enanthate ester. This finding is consistent with the idea that the rate-limiting step for the release of either ester is its diffusion into the circulation from the injection site.

Yet another experimental situation points to the necessity of administering ^{14}C-fluphenazine enanthate intramuscularly, although not necessarily in the presence of sesame oil, to produce slow-release characteristics. In that situation, a dog whose bile duct had been cannulated was given a 1-mg/kg im dose of ^{14}C-fluphenazine enanthate in absolute ethanol (4). During the 8-hr test, concentrations of radioactivity in the plasma increased consistently. Only about 0.55% of the dose was excreted during the 8 hr, the major portion of the dose (88%) remaining at the site of injection. Thus, the administration of ^{14}C-fluphenazine enanthate intramuscularly, but not necessarily in sesame oil, was capable of producing slow-release characteristics.

Studies *in vitro* have shown that ^{14}C-fluphenazine enanthate (4) and ^{14}C-fluphenazine decanoate[7] can be hydrolyzed slowly by plasma esterases of the dog. The rates of hydrolysis of these two esters in plasma, however, appeared to be much slower than those occurring in intact dogs when the compounds were injected intravenously. This observation suggests that other organs, such as the liver, may have a much greater enzymatic capacity than does the plasma to cleave these esters. The observation that most of an intravenous dose of ^{14}C-fluphenazine enanthate or ^{14}C-fluphenazine decanoate (1 mg/kg) can be hydrolyzed within 30 min suggests that the hydrolytic capabilities of the dog are much greater than the rates at which these two esters, formulated in sesame oil, are usually released from their intramuscular injection sites.

All of these observations point to the conclusions that:

1. The slow-release characteristics produced by the enanthate and decanoate esters of fluphenazine base are a consequence of their relative rates of diffusion into the circulation from an injection site.
2. Cleavage of these esters in the circulation occurs rapidly and plays little, if any, role in the observed differences between their relative rates of release.

Once either of these two esters is released into the circulation of dogs, it is rapidly hydrolyzed to fluphenazine base and further metabolized according to the metabolic pathways previously described for ^{14}C-fluphenazine (5).

[7] J. Dreyfuss and J. M. Shaw, unpublished data.

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dc Polarographic Assay of Tetracyclines

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Abstract □ The optimum conditions for the polarographic reduction of some tetracycline antibiotics were studied. A boric acid–sodium borate buffer provided the best conditions for the electroreduction of tetracycline, oxytetracycline, chlortetracycline, and demeclocycline. Conditions are described for the quantitative determination of these tetracyclines in various dosage forms.

Keyphrases □ Tetracycline—polarographic analysis, pharmaceutical formulations □ Oxytetracycline—polarographic analysis, pharmaceutical formulations □ Chlortetracycline—polarographic analysis, pharmaceutical formulations □ Demeclocycline—polarographic analysis, pharmaceutical formulations □ Polarography—analysis, tetracycline, oxytetracycline, chlortetracycline, and demeclocycline in pharmaceutical formulations □ Antibiotics—tetracycline, oxytetracycline, chlortetracycline, demeclocycline, polarographic analysis in pharmaceutical formulations

Despite their well-known disadvantages, microbiological assays remain as the official methods of analysis for the tetracyclines (1, 2). However, the search for more accurate alternatives continues. Recently, two reviews (3, 4) dealt with the many methods reported for this important class of antibiotics.

Polarographic analyses of tetracyclines were first reported in the early 1950's (5, 6), and numerous studies