Excretion and Biotransformation of the Enanthate Ester of Fluphenazine-^{14}C by the Dog

JACQUES DREYFUSS, JOHN J. ROSS, Jr., and ERIC C. SCHREIBER

Abstract □ Fluphenazine-^{14}C enanthate {4-[1-^{14}C-3-[2-(trifluoromethyl)phenothiazin-10-yl]propyl]-1-piperazineethanol ester with heptanoic acid} in a sesame oil formulation was administered intramuscularly to dogs. Only traces of radioactivity were found in the circulation. Excretion was predominantly in the feces; 2–3% of the dose was excreted in the urine. Radioactivity was still present at the sites of injection 21 days after the dogs had received the dose. Unformulated fluphenazine-^{14}C enanthate was excreted according to a biphasic, exponential decay curve after intravenous administration to a dog with an externalized bile duct. The half-life of the slower portion of this decay curve was considerably *faster* than that observed in dogs that had received formulated fluphenazine-^{14}C enanthate intravenously. Thus, the rate-limiting step in the biological disposition of formulated fluphenazine-^{14}C enanthate after intramuscular administration is probably the release from an oily depot rather than excretion. Unformulated fluphenazine-^{14}C enanthate, administered intramuscularly to a dog with an externalized bile duct, also represents a slow-release situation. Therefore, the sesame oil employed in the formulation serves as a convenient vehicle for the administration of the drug, although its presence is not an absolute requirement for slow release. Fluphenazine-^{14}C enanthate is hydrolyzed to fluphenazine-^{14}C by plasma esterases. Radioactivity present in the bile of a dog that had received fluphenazine-^{14}C enanthate intravenously was identical with the glucuronide conjugate of 7-hydroxyfluphenazine. Thus, fluphenazine-^{14}C enanthate appears to be metabolized by the dog according to the metabolic pathway established for fluphenazine-^{14}C.

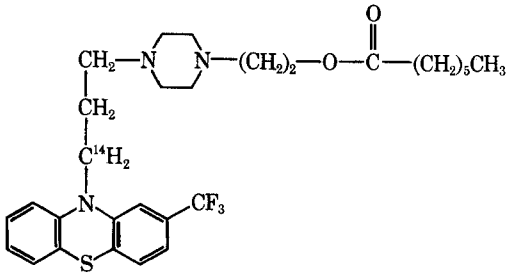
Keyphrases □ Fluphenazine-^{14}C enanthate—metabolism, excretion □ Biliary, urinary, fecal excretion—fluphenazine-^{14}C enanthate □ Tissue levels—fluphenazine-^{14}C, metabolites □ Metabolite—7-hydroxyfluphenazine glucuronide □ TLC—analysis □ Scintillometry—analysis

Fluphenazine enanthate[1] is a long-acting ester of fluphenazine. This ester (Structure I) is administered intramuscularly in a sesame oil-based formulation which permits a convenient mode of treatment for outpatients and obviates uncertainties attendant on administration of drugs to psychically disturbed individuals.

The present study with dogs was designed to answer these questions: Is the metabolism of the ester of fluphenazine similar to that of the unesterified compound, the metabolism of which was discussed in a companion publication (1)? Is the slow-release property of the formulated preparation a consequence of the physical characteristics of the drug molecule, the release characteristics of a drug molecule present in an oily depot, or both?

METHODS AND MATERIALS

Fluphenazine-^{14}C enanthate (Structure I) was synthesized[2] and had a specific activity of 2.47 μc./mg. and a radiochemical purity of 96.8%. Fluphenazine-^{14}C enanthate was injected into the thigh muscle of pure-bred beagles at a dose of 1 mg./kg. The standard formulation contained 25 mg./ml. of fluphenazine-^{14}C enanthate



I

Structure of fluphenazine-^{14}C enanthate

(free base), 1.5% benzyl alcohol, and sesame oil. In some experiments, the ester was administered intravenously as the dihydrochloride salt to facilitate dissolution of the compound in mixtures of water and ethanol. In other experiments, when unformulated ester was administered intramuscularly, the free base was dissolved in absolute ethanol. Methods pertaining to the collection and analysis of excreta, blood and plasma, and tissues, as well as surgical procedures, have been described (1).

Chromatography—Three solvent systems were employed to develop activated thin-layer plates of silica gel PF (Brinkmann): (*a*) 1, benzene–ammonia–dioxane (60:5:35); (*b*) 2, chloroform–95% ethanol–ammonia (10:85:2.5); and (*c*) 3, chloroform–100% ethanol–ammonia (80:10:1). The R_f values of fluphenazine-^{14}C enanthate and some of its potential metabolites are indicated on the individual chromatograms.

Hydrolysis by Plasma Esterases—Blood was obtained from several dogs of each sex and pooled. The plasma was recovered, and one portion was placed in a boiling water bath for 30 min.; the sample was centrifuged to recover the supernatant fluid. Fluphenazine-^{14}C enanthate was dissolved in 95% ethanol, and sufficient 0.1 *N* HCl was added to convert the compound to the dihydrochloride salt. The solution thus prepared contained 88 mcg. of drug per milliliter of plasma. Both undenatured and denatured plasma samples were incubated at 37° for 20 hr.; then the undenatured plasma sample was denatured with an equal volume of methanol and centrifuged. The supernatants from each tube were chromatographed in Solvent System 3.

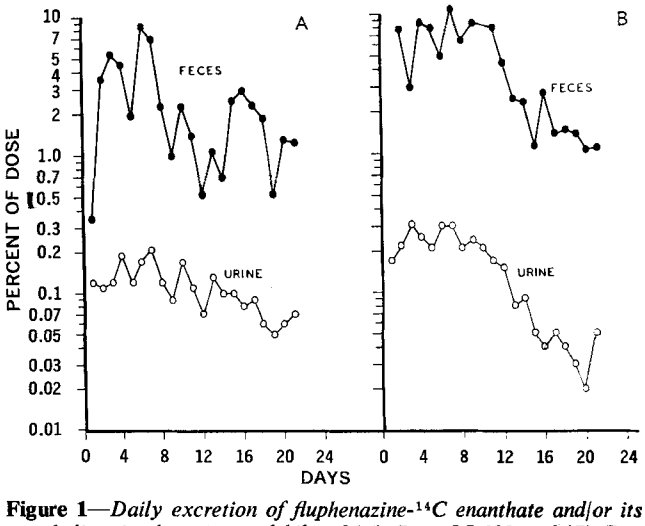


Figure 1—*Daily excretion of fluphenazine-^{14}C enanthate and/or its metabolites in the urine and bile of (A) Dog Q7-132 and (B) Dog Q7-222. The dogs received 1 mg./kg. i.m. of formulated fluphenazine-^{14}C enanthate.*

[1] Prolixin Enanthate, E. R. Squibb & Sons, Inc., New York, N. Y.
[2] By Mr. F. Dondzila.

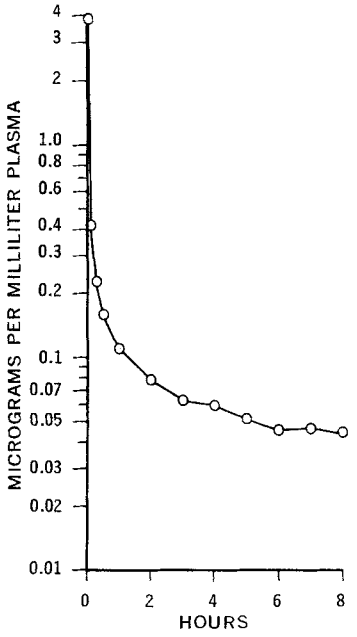


Figure 2—*Levels of fluphenazine-^{14}C enanthate equivalents in the plasma of Dog Q7-147. The dog received 1 mg./kg. i.v. of fluphenazine-^{14}C enanthate dihydrochloride in water made 28% in ethanol.*

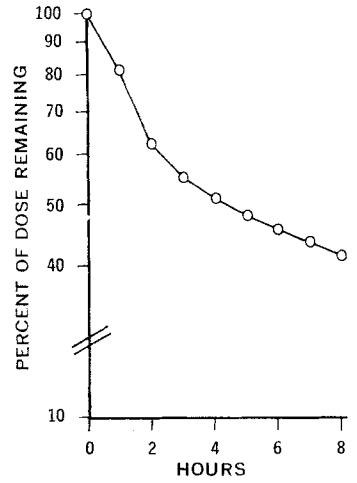


Figure 3—*Excretion of fluphenazine-^{14}C and/or its metabolites in the urine and bile of Dog Q7-147. The dog received 1 mg./kg. i.v. of fluphenazine-^{14}C enanthate dihydrochloride in water made 28% in ethanol.*

Enzymatic and Alkaline Hydrolysis of Bile—Bile (0.5 ml.) from Dog Q7-147 was placed into each of two tubes. One tube was made strongly alkaline with a small drop of 50% NaOH. To the other tube was added 0.4 ml. of 0.1 *M* acetate buffer, pH 4.5, and 0.2 ml. of β-glucuronidase (Ketodase). Alkaline hydrolysis was carried out at 90–100° for 1.5 hr. Enzymatic hydrolysis was carried out at 35–40° for 20 hr. The pH of both samples was adjusted to about 7 by the addition of acid or base. These samples were then chromatographed in Solvent Systems 2 and 3.

The reverse treatments were also conducted. The tube that had previously been made strongly alkaline had added to it 0.4 ml. of acetate buffer, pH 4.5, followed by 0.2 ml. of β-glucuronidase. The tube that had previously received β-glucuronidase was made strongly alkaline with NaOH. The conditions for incubation were the same as described previously. After incubation, the pH of the samples was again adjusted to about 7 and they were chromatographed in Solvent Systems 2 and 3.

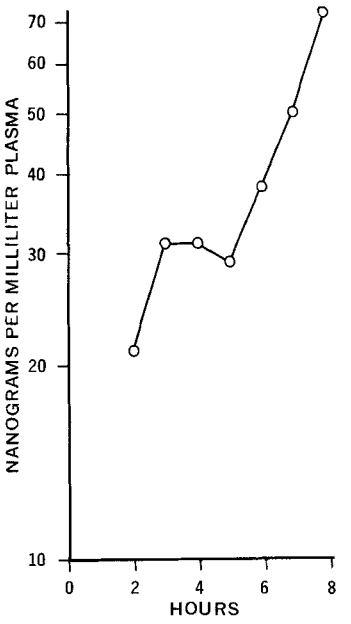


Figure 4—*Levels of fluphenazine-^{14}C enanthate equivalents in the plasma of Dog Q7-111. The dog received 1 mg./kg. i.m. of fluphenazine-^{14}C enanthate in absolute ethanol.*

RESULTS AND DISCUSSION

Disposition of Formulated Drug Intramuscularly—Figure 1 shows the values obtained for the daily excretion of fluphenazine-^{14}C enanthate, its metabolites, or both in the urine and feces of Dogs Q7-132 and Q7-222 after intramuscular injection of 1 mg./kg. of the standard formulation. During the 21 days, about 55–87% of the dose was excreted, chiefly in the feces; only 2–3% of the radioactivity was excreted in the urine. The rate of excretion was highest from the 4th through the 10th days. The half-lives for the excretion of radioactivity by these two animals (estimated graphically) ranged from 4 to 7 days in the urine and about 4 days in the feces. The total amounts of radioactivity excreted by Dogs Q7-132 and Q7-222, as well as the portions of the dose still present at the injection site, are summarized in Table I. A total of 74–99% of the dose was accounted for during the 21 days of the experiment. The very low levels of radioactivity found in the plasma of Dogs Q7-132 and Q7-222 ranged from 3 to 11 ng./ml. during the entire test. Two to three days after drug administration, levels of radioactivity in the plasma were found to be about one-half of the maximal levels.

Disposition of Unformulated Drug Intravenously—A dog with an externalized bile duct received a 1-mg./kg. i.v. dose of unformulated fluphenazine-^{14}C enanthate dihydrochloride. The levels of radioactivity in the plasma of this dog (Q7-147) are shown in Fig. 2. During the first several minutes following drug administration, the levels of radioactivity in the plasma declined precipitously. Elimination of radioactivity from the circulation then proceeded ever more slowly, with a half-life of about 9 hr. during the interval of 3–8 hr. During the 8-hr. experiment, only 1.40% of the dose was excreted in the urine; 56.95% was excreted in the bile. The excretion of fluphenazine-^{14}C enanthate, its metabolites, or both in the urine and bile of Dog Q7-147 is shown in Fig. 3. The decay curve fits a biphasic, exponential pattern. The portion of the curve for the first 3 hr. has a half-life of about 0.5 hr. (by the "method of residuals"), while the portion of the curve for the

Table I—Excretion of Fluphenazine-^{14}C Enanthate and/or Its Metabolites by Dogs

Dog[a]	Sex	Route	Formulated	Duration of Experiment	Percent of Dose			
					Urine	Feces or (Bile)	Injection Site	Total
Q7-132	M	i.m.	Yes	21 days	2.34	52.56	19.55	74.45
Q7-222	F	i.m.	Yes	21 days	3.19	83.88	12.12	99.19
Q7-111	M	i.m.	No	8 hr.	0.06	(0.49)	87.82	88.37
Q7-147	M	i.v.	No	8 hr.	1.40	(56.95)	—	58.35

[a] Dogs received 1 mg./kg.

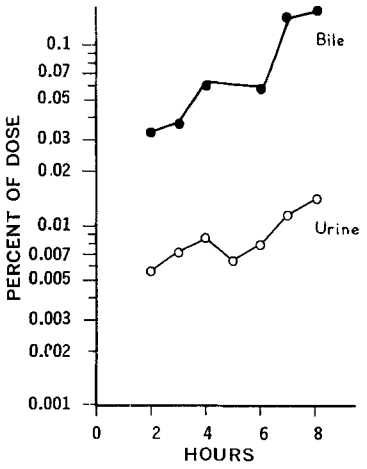


Figure 5—*Excretion of fluphenazine-^{14}C enanthate and/or its metabolites in the urine and bile of Dog Q7-111. The dog received 1 mg./kg. i.m. of fluphenazine-^{14}C enanthate in absolute ethanol.*

3rd to 8th hr. has a half-life of 12–13 hr. Even the longer of these half-lives (12–13 hr.) is much shorter than the value of 4–7 days cited earlier for the dogs that received formulated fluphenazine-^{14}C enanthate intramuscularly. Thus, when the formulated drug is administered intramuscularly, the rate-limiting step for the elimination of radioactivity from the body is not a function of the rate of excretion but probably of the rate of drug release from the site of injection.

Disposition of Unformulated Drug Intramuscularly—Unformulated fluphenazine-^{14}C enanthate was administered intramuscularly to a dog with an externalized bile duct. During the 8-hr. experiment, the levels of fluphenazine-^{14}C enanthate equivalents in the plasma of this dog (Q7-111) rose consistently (Fig. 4). The excretion of fluphenazine-^{14}C enanthate, its metabolites, or both in the urine and bile of Dog Q7-111 is shown in Fig. 5. Only about 0.55% of the dose was excreted (Table I), most of it, again, in the bile. The major portion of the dose (88%) remained at the injection site of Dog Q7-111. Thus, fluphenazine-^{14}C enanthate, which is itself an oily compound, is slowly released from the site of intramuscular injection after the unformulated drug has been administered.

Distribution—Dogs Q7-132 and Q7-222 were sacrificed at the end of 21 days. Only trace amounts of radioactivity were present in the brain, kidneys, fat, skin, and left thigh muscle (Table II). Of the tissues examined, the livers had the highest concentrations of radioactivity.

Dog Q7-111 was sacrificed after 8 hr. The lungs and liver contained the highest concentrations of radioactivity. The three areas of the brain examined showed approximately equal levels of radioactivity.

Dog Q7-147 was also sacrificed after 8 hr. All the tissues examined contained measurable amounts of radioactivity (Table

Table II—Residual Fluphenazine-^{14}C Enanthate and/or Its Metabolites in Selected Tissues of Dogs

Tissue	—After 21 Days— Dog Q7-132[a] (mcg./g.)	Dog Q7-222[a] (mcg./g.)	—After 8 Hr.— Dog Q7-111[b] (mcg./g.)	Dog Q7-147[c] (mcg./g.)
Lungs	—	—	0.20	2.73
Liver	0.35	0.25	0.20	3.69
Kidneys	0.05	0.06	0.05	0.69
Omental fat	0.03	0.03	0.03	0.24
Skin	0.03	0.01	0.02	0.15
Heart	—	—	0.06	0.42
Brain	0.04	0.03	—	—
Dorsal and hypothalamus	—	—	0.13	0.75
Brain cortex and subcortex	—	—	0.11	0.43
Brain stem	—	—	0.13	0.40
Cerebellum	—	—	—	0.52
Right thigh muscle (contains injection site)	4.78	3.61	21.77	—
Left thigh muscle	0.02	0.03	0.05	0.63

[a] Dogs Q7-132 and Q7-222 received 1 mg./kg. of formulated fluphenazine-^{14}C enanthate intramuscularly. [b] Dog Q7-111 received 1 mg./kg. of fluphenazine-^{14}C enanthate in absolute ethanol intramuscularly. [c] Dog Q7-147 received 1 mg./kg. of fluphenazine-^{14}C enanthate dihydrochloride in water made 28% with respect to ethanol, intravenously.

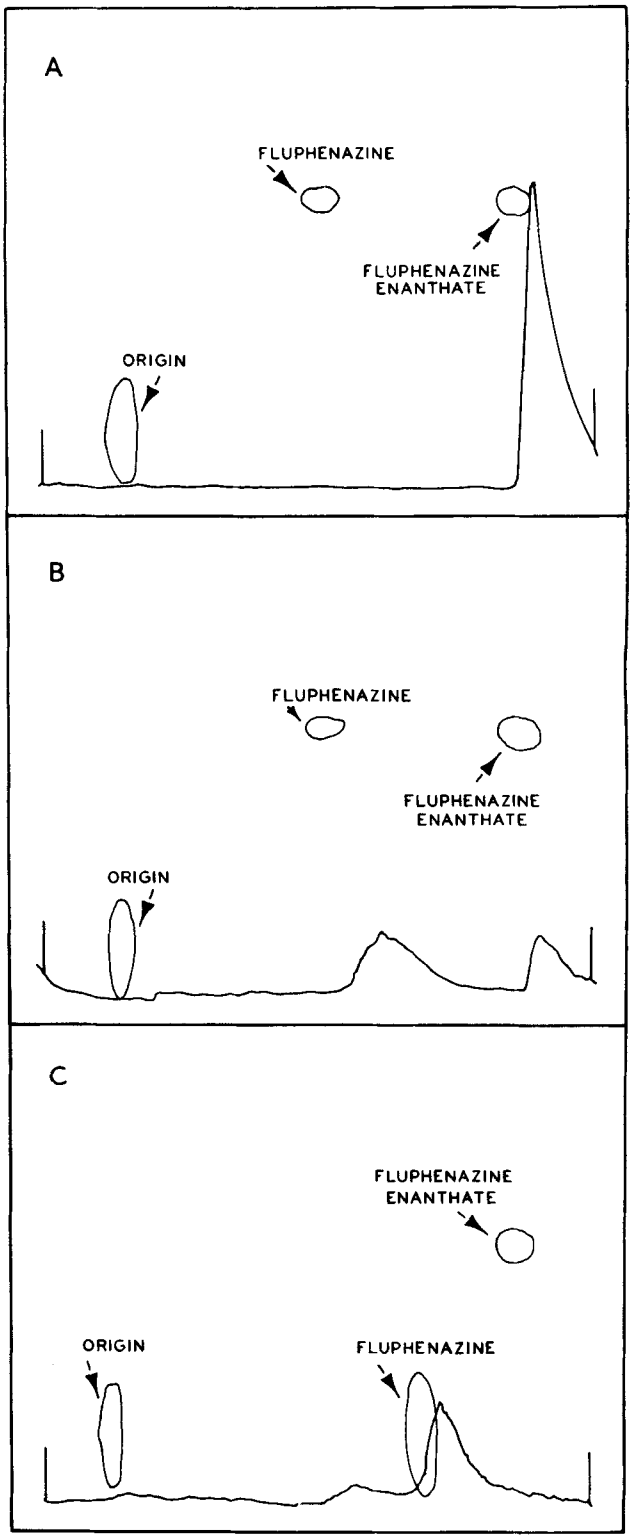


Figure 6—*Chromatograms of fluphenazine-^{14}C enanthate incubated with dog plasma: (A) denatured plasma, (B) intact plasma, and (C) intact plasma after alkaline hydrolysis. Solvent System 3 was employed.*

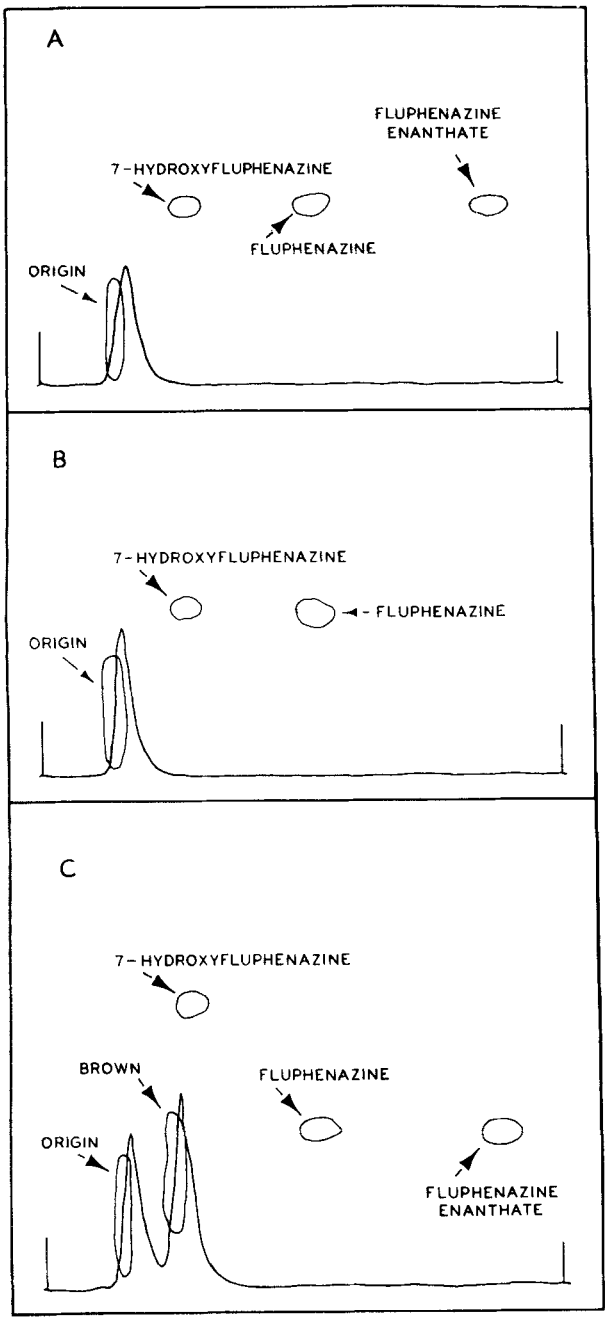


Figure 7—*Chromatograms of bile (0–1 hr.) developed in Solvent System 3: (A) bile from Dog Q7-147 was subjected to alkaline hydrolysis; (B) bile from Dog Q8-262 was chromatographed without any prior treatment; and (C) bile from Dog Q7-147 was subjected to hydrolysis with β-glucuronidase.*

II). No striking localization was noted in the areas of the brain examined. The lungs and liver, again, contained the greatest concentrations of radioactivity; the liver contained 8% of the dose at the time of sacrifice. The approximately 12% of the dose present in the various tissues of Dog Q7-147, plus the amounts excreted, accounted for about 70% of the dose.

Biotransformation—The biological disposition of fluphenazine-^{14}C dihydrochloride in dogs and subhuman primates was presented in a companion paper (1). There, evidence was presented to support the notion that the major metabolite of fluphenazine-^{14}C was unconjugated in the feces of dogs and was present as the glucuronide conjugate in the bile. To determine whether fluphenazine-^{14}C enanthate could be metabolized *via* the pathway previously established for fluphenazine-^{14}C, fluphenazine-^{14}C enanthate was incubated with either intact or denatured dog plasma. The samples were then chromatographed to determine the amount of fluphenazine-^{14}C enanthate remaining. Incubation of fluphenazine-^{14}C enanthate with denatured dog plasma led to the complete recovery of a peak corresponding in R_f value to fluphenazine enanthate (Fig. 6A). Incubation of fluphenazine-^{14}C enanthate with intact dog plasma led to two peaks on the chromatogram (Fig. 6B), one corresponding in R_f value to that of fluphenazine enanthate, the other to that of fluphenazine.

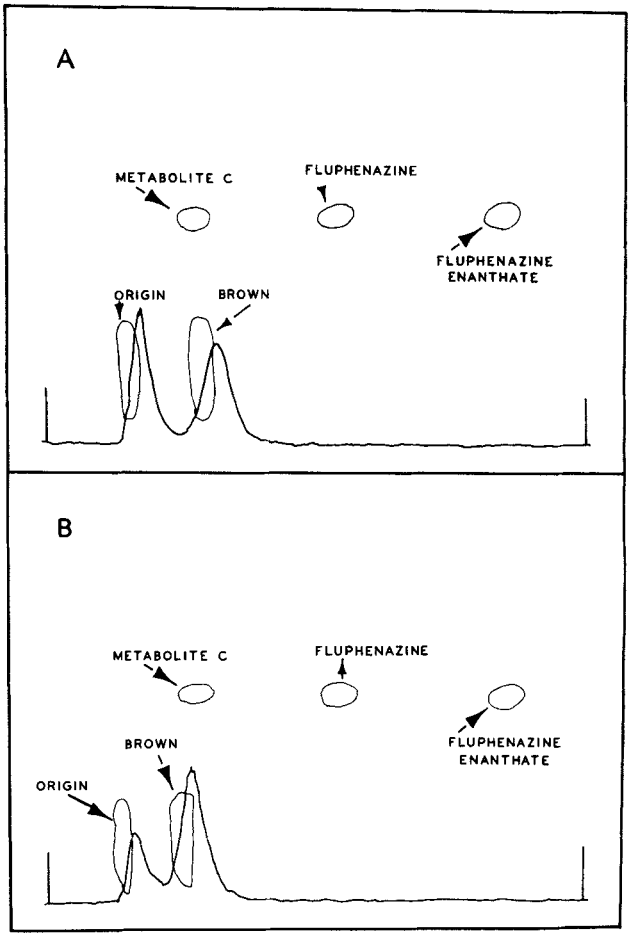


Figure 8—*Chromatograms of bile from Dog Q7-147 (0–1 hr.) developed in Solvent System 3: (A) bile was subjected to hydrolysis with β-glucuronidase followed by alkaline hydrolysis; and (B) bile was subjected to alkaline hydrolysis followed by hydrolysis with β-glucuronidase.*

To provide better evidence that the peak corresponding in R_f value to that of fluphenazine in Fig. 6B was, in fact, attributable to fluphenazine, the incubated sample of dog plasma was made alkaline, hydrolyzing the ester bond. The sample was then chromatographed again in Solvent System 3, as shown in Fig. 6C. Most of the radioactivity now appeared under a single peak that corresponds to the R_f value of fluphenazine; another minor, broad peak, with a slightly slower R_f value, probably represents products resulting from the alkaline hydrolysis of fluphenazine-^{14}C enanthate at elevated temperatures. Thus, the authors concluded that dog plasma contains esterases capable of hydrolyzing fluphenazine-^{14}C enanthate to fluphenazine-^{14}C. These results are comparable to those reported by Ebert and Hess (2). In their study of the metabolism of fluphenazine-^{14}C enanthate in the rat, fluphenazine-^{14}C was recovered after the direct injection of fluphenazine-^{14}C enanthate dissolved in sesame oil into the brains of rats.

Bile collected from Dog Q7-111 between the 7th and 8th hr. and from Dog Q7-147 during the 1st hr. and the 7th to 8th hr. was chromatographed in Solvent Systems 1 and 3. No unchanged fluphenazine-^{14}C enanthate was observed in any of these bile samples in either solvent system. Thus, no fluphenazine-^{14}C enanthate was recoverable after the administration of unformulated fluphenazine-^{14}C enanthate to dogs, whether intramuscularly or intravenously.

To determine whether conjugates were present in the bile, samples obtained from Dog Q7-147, which had received fluphenazine-^{14}C enanthate intravenously, were analyzed. Bile from this animal was treated with alkali in order to remove the heptanoic acid group. A chromatogram of this sample (Fig. 7A) shows that all of the radioactivity remained at the origin. Figure 7B shows the same result for a sample of bile from Dog Q8-262, which had received fluphenazine-^{14}C (10 mg./kg. i.v.) (1). Similar results are also obtained with these two samples of bile if the chromatograms are developed in Solvent System 2. Figure 7C reveals that a second peak, which corresponds in R_f value to Metabolite C of fluphenazine-^{14}C, is formed after bile from Dog Q7-147 has been treated with β-glucuronidase. Metabolite C was shown in a companion paper (3) to be identical with 7-hydroxyfluphenazine.

Confirmatory evidence that Metabolite C can be derived from the bile of a dog that had received fluphenazine-^{14}C enanthate was obtained by the following series of experiments. Bile obtained from Dog Q7-147 was given one of two sequential treatments. Either the bile was treated first with β-glucuronidase, followed by alkaline hydrolysis, or the bile was subjected first to alkaline hydrolysis and then treated with β-glucuronidase. In both cases, the samples were chromatographed after the second treatment in Solvent Systems 1, 2, and 3. The results obtained with Solvent System 3 are shown in Fig. 8. Regardless of the sequence of treatments, the same results are obtained in each solvent system; two peaks are observed, one of which corresponds to a brownish zone on the chromatogram and also to the R_f value of Metabolite C. These results are explainable only by the conversion of fluphenazine-^{14}C enanthate to the glucuronide of 7-hydroxyfluphenazine. Although the formation of the glucuronide of 7-hydroxyfluphenazine enanthate is a theoretical possibility for the metabolism of this compound, no 7-hydroxyfluphenazine enanthate was available as a chromatographic reference standard. Such a material would be expected to have an R_f value in Solvent System 3 intermediate between that of fluphenazine and fluphenazine enanthate. In fact, none of the radioactivity seen in Fig. 7C, for example, was observed to move with such an R_f value.

In conclusion, fluphenazine-^{14}C enanthate was shown to be hydrolyzed to fluphenazine-^{14}C by plasma esterases of the dog *in vitro*. Radioactivity present in the bile of a dog that had received fluphenazine-^{14}C enanthate was excreted as the glucuronide conjugate of 7-hydroxyfluphenazine. From the present studies, the authors are unable to determine whether fluphenazine-^{14}C enanthate is first hydroxylated and then has the ester bond cleaved, or whether the ester bond is first cleaved and the compound is then metabolized in a manner essentially like that previously described for fluphenazine-^{14}C (1).

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Separation and Spectrofluorometric Assay of the β-Adrenergic Blocker Sotalol from Blood and Urine

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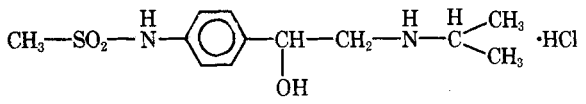
Abstract □ A sensitive spectrofluorometric assay of sotalol·HCl (*d,l*-4-(2-isopropylamino-1-hydroxyethyl)methanesulfonanilide hydrochloride; MJ 1999), a potent and specific β-adrenergic blocking agent, was developed. The compound fluoresces in alkali at 250/350 nm. and in acid at 235/309 nm. The maximum extractability was found at a pH of 9, where the substance is primarily the zwitterion with potentiometric pKa′ values of 8.30 and 9.80, where the former has been spectrophotometrically assigned to the dissociation of the conjugated sulfanilino group. A mixture of *n*-amyl alcohol and chloroform (1 : 3, v/v) at a volume ratio of organic solvent and pH 9 buffer solution of 8 : 1 extracted 85% of the compound, which was completely reextracted into 5.2 *N* HCl. The fluorescence of the monocharged molecule was measured at 235/309 nm. in the acid solution. The sensitivity of the assay was 0.1 mcg./ml. in plasma and 2.5 mcg./ml. in urine. Statistical analyses of assays of sotalol-spiked plasmas, urines, and acidic extraction blanks showed no dose × day interactions and no significant variations of the slopes of calibration curves within 3–4 days of assay; no significant assay differences when the drug was stored under refrigeration in water, acidic extraction blanks, whole blood, plasma, serum, and urine for 2–6 days; and no significant effects from blood and urine obtained from different dogs. The recovery of the drug was 69.4% from plasma and 81% from urine. The sensitivity of 0.1 mcg./ml. in plasma and 2.5 mcg./ml. in urine and the reproducibility of 2% at a midrange concentration of 0.5 mcg./ml. in plasma and of 2.2% at a midrange concentration of 10 mcg./ml. in urine allow the assay to be applicable for monitoring blood levels and urinary excretion of the drug when administered in therapeutic amounts.

Keyphrases □ Sotalol determination—blood, urine □ Partition coefficients—sotalol·HCl □ pKa determination—sotalol·HCl □ Variability parameters—sotalol·HCl fluorometric analysis □ Fluorometry—analysis, biological fluids

Sotalol·HCl, *d,l*-4-(2-isopropylamino-1-hydroxyethyl)methanesulfonanilide hydrochloride (MJ 1999), is a specific and potent β-adrenergic blocking agent in animals (1, 2) and man (2–4), with a structure (I) similar to isoproterenol.

The plasma levels of tritium-labeled drug were shown to be proportional to its pharmacological activity in dogs (2). However, the study of distribution and excretion of drugs in animals and man to establish opti-



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