Br. J. clin. Pharmac. (1979), 7, 325–331

KINETICS OF FLUPHENAZINE AFTER FLUPHENAZINE DIHYDROCHLORIDE, ENANTHATE AND DECANOATE ADMINISTRATION TO MAN

S.H.CURRY & R. WHELPTON

Department of Pharmacology and Therapeutics, The London Hospital Medical College, Turner Street, London, E1 2AD

P.J. de SCHEPPER & S. VRANCKX

Department of Pharmacology, University of Leuven, Leuven, Belgium

A.A. SCHIFF

E. R. Squibb and Sons Limited, Twickenham, Middlesex

1 Fluphenazine (1,2-ethanol ^{14}C) was administered to seven human subjects as the dihydrochloride and as the enanthate and decanoate esters.

2 The subjects had previously been treated with fluphenazine injections for at least 6 months.

3 Fluphenazine was separated from its radioactive metabolites by selective solvent extraction. Plasma concentrations were measured for up to 21 days after dosing.

4 The preparations showed differences in peak concentrations, times of the peaks and half-times of the elimination phase. The longest half-times occurred with the decanoate and the shortest with the dihydrochloride.

5 It is postulated that the differences in kinetics relate principally to the release of the compound from the site of injection.

6 There was no evidence for presence of the esters in plasma, urine or faeces.

Introduction

Long acting depot injections of fluphenazine esters (enanthate and decanoate) have been in clinical use for approximately 10 years. During this time several analytical methods for determining fluphenazine concentrations in human blood or plasma have been reported (Smulevitch, Minsker, Mazayera, Volkava & Lukanina, 1973; Larsen & Naestoft, 1973; Johansson, Borg & Gabrielsson, 1976). However, none of these methods has been applied successfully to the determination of the time-course of fluphenazine in plasma after intramuscular injections of enanthate or decanoate. Recently, radioimmunoassay has been used to describe the time-course of immuno-reactive compounds (fluphenazine and cross-reactive metabolites) in the plasma of a subject receiving fluphenazine decanoate injections (Wiles & Franklin, 1978).

The use of radioactive drug has shown greater promise. McIsaac (1971) administered up to 50 μCi of labelled fluphenazine (^{14}C) and its esters. Selective assays were applied to urine but unfortunately only total radioactivity was assessed in plasma. As fluphenazine is extensively metabolized, total radioactivity measurements provide no information on the kinetics of the unmetabolized drug. In an analogous study, up to 800 μCi of labelled flupenthixol decanoate, the thioxanthine analogue of fluphenazine decanoate, also yielded only total radioactivity data from human plasma (Jorgensen & Gottfries, 1972).

We recently reported on fractionation techniques for selective assay of fluphenazine in studies involving radioactivity (Whelpton & Curry, 1976a and b). The present study was an attempt to obtain information about the time course of fluphenazine in plasma following fluphenazine (^{14}C) dihydrochloride and its esters, employing these techniques.

Subjects

The seven subjects were patients under the care of Dr J. Haenen at the Psychiatric Hospital of the Alexian Brothers in Tienen, Belgium. The investigation was fully explained to the patients at the outset, and they gave informed consent. All were male, age range 19–51 years, weight range 64–74 kg. All had been treated with fluphenazine injections for at least six months before the study began. All received single 25 mg doses of labelled (^{14}C) compounds. With one exception these doses were substituted for routine doses of non-labelled injectable preparations, the

0306–5251/79/040325–07$01.00

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exception being one patient who received the oral dose, in whom routine treatment was stopped for two dosage periods before the test dose and for the duration of sampling after the test dose. This was without adverse results clinically. Thus treatment was not interrupted by the investigation. None of the patients needed or were given any drugs other than fluphenazine, except benzodiazepines for night sedation. In particular, no anti-parkinsonian drugs were needed or used.

Apart from the one patient receiving the oral dose, the patients received intramuscular injections (dihydrochloride, enanthate and decanoate), two subjects per preparation. Blood was collected into heparinized tubes and centrifuged to separate the plasma. Urine and faeces were 24 h collections. C.S.F. was collected at 1 and 24 h from two subjects, one receiving enanthate and one decanoate. Samples were stored, and transported to the United Kingdom, frozen, for analysis by Dr S.H. Curry and Dr R. Whelpton at The London Hospital Medical College. Fluid samples were handled as such. Weighed faeces were homogenized in methanol. The suspension was filtered, and the filtrate was evaporated to dryness. The residue was resuspended in water.

Materials

The radioactive fluphenazine (ethanol-1,2-^{14}C) dihydrochloride, fluphenazine enanthate and fluphenazine decanoate were prepared at The Radiochemical Centre, Amersham, United Kingdom, according to the method of Yale, Cohen & Sowinski (1963). The specific activities were 2.04, 2.19 and 2.36 mCi/mmol respectively. Solutions for injection (25 mg/ml) were formulated in the same manner as the non-radioactive pharmaceutical preparations. The esters were dissolved in sesame oil containing 1.2% w/v benzyl alcohol as preservative and stored at 4°C. Unlabelled standards, including 7-hydroxy-fluphenazine, were from E.R. Squibb & Sons, Limited, Twickenham, Middlesex, United Kingdom. Fluphenazine sulphoxide solutions were prepared by treating fluphenazine with 10% hydrogen peroxide solution (Whelpton & Curry, 1976a).

Reagents were analytical grade where available. Plastic backed thin layer chromatography plates consisting of 0.2 mm thick silica gel containing a fluorescent indicator were obtained from British Drug Houses, Limited, Poole, Dorset, United Kingdom. Scintillation fluids from Nuclear Enterprises, Limited, Edinburgh, Scotland, were NE250 (dioxane based) or NE260 (xylene based).

Total radioactivity

This was determined in all samples by assessment of 0.1 ml aliquots of fluid samples or of faecal extracts in 10 ml dioxane based scintillation fluid.

Extraction procedures

The biological samples (plasma, urine and the faecal extracts) were examined by a fractionation scheme described previously. In this scheme ether and toluene extract fluphenazine, its esters and its sulphoxide, and also 7-hydroxyfluphenazine. Heptane extracts only fluphenazine and its esters. Specificity for any of these compounds is achieved by manipulation of solvents and pH. Conjugates are assayed as the aglycones after hydrolysis with β-glucuronidase (Whelpton & Curry, 1976a and b).

The first extract in studies of fluphenazine dihydrochloride was with heptane. The initial step in determination of plasma concentrations of fluphenazine after the fluphenazine esters was ether extraction. Non-radioactive fluphenazine dihydrochloride (100 µg in 1 ml water) as cold carrier and internal standard was added to plasma (up to 20 ml). The samples were mixed and the volumes measured before being transferred to 50 ml polypropylene centrifuge tubes. Diethyl ether (20 ml) and 10 N NaOH (1 ml) were added and the tubes were shaken mechanically for 30 min. After centrifugation the ether layers were transferred to 30 ml glass centrifuge tubes and the ether evaporated under a gentle stream of nitrogen. The ether extraction was repeated twice and the residues collected in the same glass centrifuge tubes. Heptane (5 ml) and 1 N HCl (5 ml) were added and the tubes shaken for 15 min and centrifuged. The acid layer (4.5 ml) was transferred to a clean tube and heptane (5 ml) and 10 N HaOH (1 ml) added. After shaking and centrifugation the heptane layer (4.5 ml) was removed for spectrophotometric analysis of the internal standard. The heptane and cuvette washings (2×0.5 ml) were transferred to counting vials containing xylene based scintillation fluid.

Total radioactivity was also determined in 1 ml of C.S.F. and the remainder ($\sim$3.5 ml) fractionated as for urine (Whelpton & Curry, 1976b).

Radioactivity measurements

A Tracerlab Corumatic Series 2000 liquid scintillation spectrometer was used to measure the radioactivity. After solvent extraction, the solvent solutions were counted in xylene based fluid. Quenching was determined by the channels ratio method. A total counting efficiency of 90% was obtained with the Tracerlab standard. Experimental samples generally had counting efficiencies between 85 and 90%.

Thin layer chromatography

Plasma extracts were pooled and evaporated to a small volume under a stream of nitrogen. Samples were applied to plastic backed thin layer plates which were developed in chloroform : ethanol :

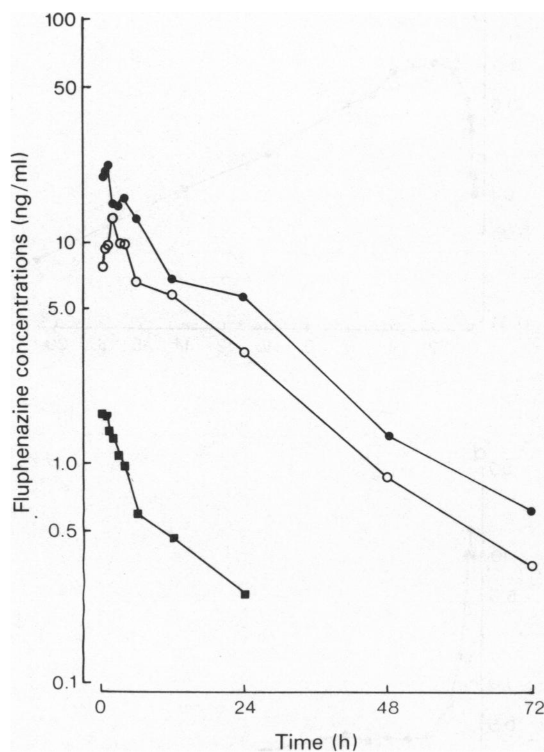

Figure 1 Concentrations of fluphenazine radioactivity in plasma following 25 mg fluphenazine dihydrochloride. Subjects 1 (O) and 2 (●) received intramuscular injections, subject 3 (■) received the solution orally.

ammonia (80:10:1). The plates were viewed under short wavelength ultraviolet light (250 nm) to locate the non-radioactive carrier. The plates were cut into 0.5 cm segments and each segment counted in dioxane based scintillation fluid.

Gas-liquid chromatography

A Perkin-Elmer model F33 gas chromatograph equipped with a thermionic (nitrogen sensitive) detector was used. A glass column (1 m x 3 mm i.d.) packed with 3% OV-17 on Chromosorb W HP (80–100 mesh) was maintained at 265°C. The injector/detector block temperature was 300°C. The helium carrier gas flow was 80 ml/min.

Analysis of data

Plasma fluphenazine concentrations, Cp_t, were plotted against time on semilogarithmic paper (Figures 1 and 2). The apparent first order rate constants of the decline of the terminal phases were obtained from least squares linear regression of $\ln Cp_t$ against time using a Compucorp 327 calculator. Regressions were performed with the last three data points and then the last four, the last five, etc., until a maximum value of correlation coefficient was estimated. The 95% confidence limits were obtained from $\pm ts_b$ where s_b is the standard deviation of the regression coefficient and the value of t is obtained from $\alpha=0.05$ for N-2 degrees of freedom.

Data after fluphenazine enanthate were analysed graphically by a method of residuals (Riggs, 1970; Curry, 1977).

Table 1. Half-times of the apparent first order decline of fluphenazine concentrations in plasma after 25 mg doses

Subject	*Compound*	*Route*	N*	*Half-time*†	*Correlation coefficient*
1	Dihydrochloride	i.m.	5	14.9 h (16.4–13.4)	0.997
2	Dihydrochloride	i.m.	5	15.3 h (18.4–12.2)	0.989
3	Dihydrochloride	oral	3	14.7 h (18.9–11.5)	0.999
4	Enanthate	i.m.	6	3.6 days (3.8–3.4)	0.998
5	Decanoate	i.m.	5	9.6 days (11.7–7.5)	0.986
6	Enanthate	i.m.	5	3.7 days (4.0–3.4)	0.998
7	Decanoate	i.m.	4	6.8 days (8.3–5.3)	0.997

*N is the number of terms in the regression equation
†numbers in parentheses are 95% confidence limits

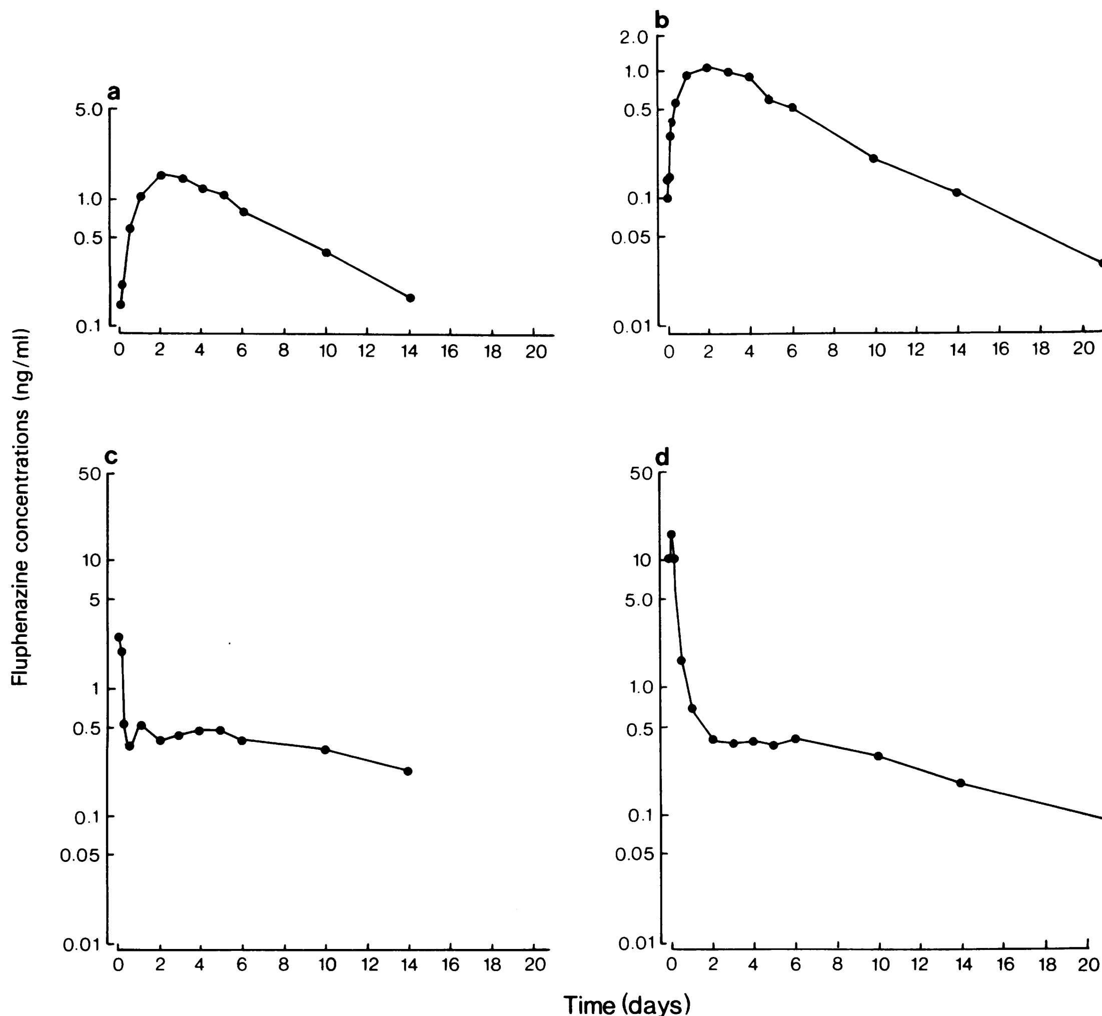

Figure 2 Plasma concentrations of fluphenazine following 25 mg i.m. of the esters. Subjects 4 and 6 received fluphenazine enanthate (2a and 2b respectively). Subjects 5 and 7 received fluphenazine decanoate (2c and 2d respectively).

Results

Spare ampoules containing fluphenazine (^{14}C) enanthate and decanoate, as well as commercial samples of the esters, were opened at the end of the study and samples were diluted with heptane. Aliquots of the dilutions were injected into the chromatograph without further treatment. The ratio of fluphenazine to fluphenazine esters was obtained by comparing the peaks obtained from the injection solutions with calibration curves obtained from pure solutions of the reference compounds. The ampoules of labelled material contained less than 1% of their phenothiazine content as non-esterified fluphenazine.

The possibility of hydrolysis of esters causing artifactual results was tested in control experiments in which fluphenazine enanthate and decanoate were added to blank plasma. The resulting solutions were subjected to the standard work-up procedure. Fluphenazine detected in the final solutions indicated at most 3% hydrolysis.

Plasma concentrations of radioactive fluphenazine following dihydrochloride administration were considerably less than the concentrations of total radioactivity. The times of the peak and the peak concentrations in subjects 1 and 2 were 2 h, 13.0 ng/ml, and 1.5 h, 22.6 ng/ml respectively. After oral administration the peak concentration of 1.65 ng/ml

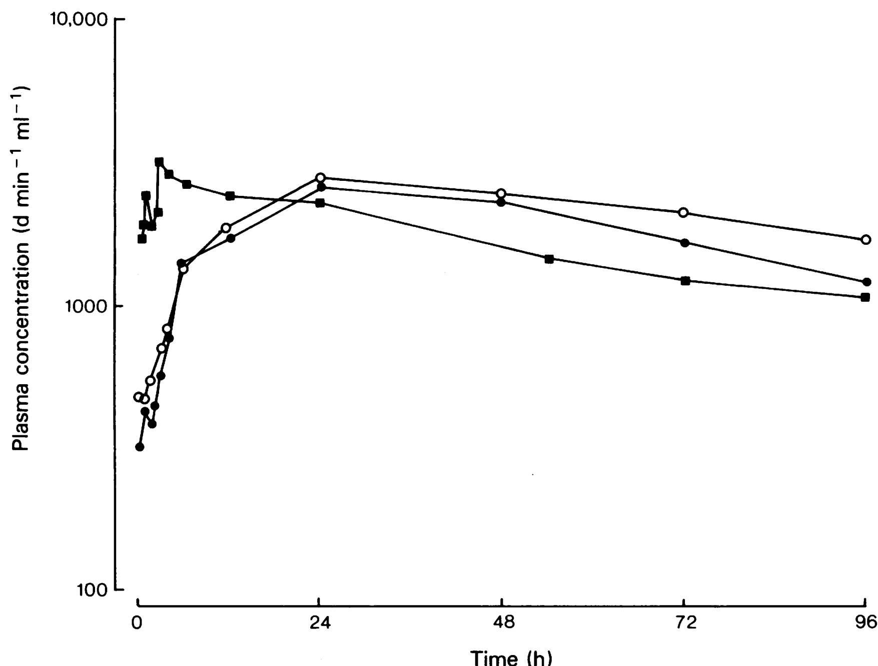

Figure 3 Total radioactivity concentrations after fluphenazine dihydrochloride, in subjects 1 (○). 2 (●) and 3 (■). 1 ng fluphenazine ≡ 10.4 d min^{-1}.

was measured at 0.5 h, the time of the first blood sample. Concentrations at 96 h after i.m., and 48 h after oral doses were below the lower limit of analytical detection. The terminal phases declined with half-times of approximately 15 h in the three subjects (Table 1). Total radioactivity in plasma peaked at 24 h following intramuscular fluphenazine and 3 h after oral administration. The peak concentrations and half-times of the apparent first order decline of this material were similar for the three treatments (Figure 3).

Plasma concentrations of fluphenazine after intramuscular fluphenazine enanthate were less than those obtained from intramuscular dihydrochloride. The data show a rising and a declining phase. The peak concentrations of fluphenazine, 1.51 and 1.06 ng/ml in subjects 4 and 6 respectively, were measured at 48 h. Linear regression gave half-times of 3.6 and 3.7 days. Using the method of residuals, the data were resolved into two exponential processes with half-times of 16.8 h and 3.5 days for subject 4 and 14.4 h and 3.5 days for subject 6. The times of the peaks were calculated to be 2.02 and 1.83 days, respectively.

Plasma concentrations of fluphenazine after fluphenazine decanoate were characterized by an initial high concentration, declining rapidly at first, followed by either a rise or a plateau from which the decline was more gradual. The half-times were 9.6 and 6.8 days.

Examination of urine and faeces from subject 2 for identifiable metabolites by selective solvent extraction, thin-layer chromatography and gas-liquid chromatography, showed the presence of fluphenazine, fluphenazine sulphoxide and 7-hydroxyfluphenazine in faeces. These compounds, and also conjugates of them, were in urine (Tables 2 and 3). Similar examination of plasma failed to demonstrate the presence of any identifiable radioactive compound other than fluphenazine.

Radioactivity was detectable in C.S.F. at 1 h and 24 h after enanthate and decanoate (subjects 6 and 7). The concentrations were 4 and 14 d min^{-1} ml respectively, for enanthate, and 5 and 39 d min^{-1} ml^{-1} after decanoate. If fluphenazine, 7-hydroxyfluphenazine and fluphenazine sulphoxide, were present, their concentrations were below the sensitivity limits of the investigation. It can be assumed that fluphenazine was present, but in concentrations similar to those in plasma water (approximately 1% of the plasma concentration).

Discussion

At the commencement of this study, in 1974, non-radioactive methods had failed to yield data on the

Table 2 Cumulative excretion in the urine of subject 2

Time (days)	*Total*	*Cumulative excretion (µCi)* Fluphenazine		*Fluphenazine sulphoxide*		*7-Hydroxyfluphenazine*	
		Free	*Conjugated*	*Free*	*Conjugated*	*Free*	*Conjugated*
0–1	4.18	0.022	0.478	0.247	0.006	0.021	0.134
0–2	11.60	0.093	1.649	1.039	0.391	0.082	0.484
0–3	13.07	0.106	2.361	1.180	1.285	0.095	0.870
0–4	13.84	0.113	2.446	1.240	1.348	0.103	0.925
0–5	14.27	0.117	2.483	1.269	1.368	0.105	0.958

Table 3 Cumulative excretion in the faeces of subject 2.

Time (day)	*Total*	*Fluphenazine*	*Cumulative excretion (µCi)* Fluphenazine sulphoxide	*7-Hydroxy-fluphenazine*
0–2	2.05	0.53	0.22	1.50
0–3	9.68	1.84	0.82	4.73
0–5	13.36	2.18	1.04	5.94

time-course of fluphenazine, free of metabolites, in man, and prior to the present report, the time-course has remained undefined. Using double the quantity of radioactivity used by McIsaac (1971) up to 20 ml plasma and a high efficiency counting system with a relatively low background count rate, it was possible in our study to measure unmetabolized fluphenazine after oral and intramuscular fluphenazine dihydrochloride, and after intramuscular fluphenazine enanthate and decanoate. This is a significant improvement over total radioactivity measurements. A combination of factors, principally large plasma volumes and high counting efficiencies, negated the need for the much higher doses of radioactivity used for flupenthixol by Jorgensen & Gottfries (1972).

The position of the radioactive label (ethanol-1,2-^{14}C) reduced the number of radioactive metabolites containing the trifluoromethyl-phenothiazine residue, hence simplifying the separation procedures. The extraction of fluphenazine into heptane from alkaline aqueous solutions was essentially 100% but the recovery of fluphenazine added to plasma was reduced to ~50% (Whelpton & Curry, 1976a). Concentrations of fluphenazine after the dihydrochloride preparation were such that a single heptane extraction was sufficient to quantify fluphenazine. Diethyl ether extracted more than 98% of added fluphenazine and so ether extractions were used to improve the yield of material from plasma after intramuscular injections of the esters. The specificity of the method was maintained by including an alkali/heptane extraction stage after interfering endogenous ultraviolet absorbing materials had been removed by an acid/heptane wash. In this method, the recovery of non-radioactive fluphenazine from ten samples was $76.2 \pm 1.5\%$ (mean $\pm$ s.e. mean) which, allowing for the aliquots taken at the various stages accounts for 94% of the added material. All experimental results were individually corrected for losses. The recovery of added non-radioactive fluphenazine was used to correct individual experimental results. When 10 ml samples of plasma containing between 2.5 and 0.025 ng/ml of fluphenazine were taken through the method a linear calibration was obtained, $r = 0.9998$, $n = 5$. The errors of radioactivity counting are a function of the total number of counts collected and the background count rate (Kobayshi & Maudsley, 1974). Ten thousand counts per sample were collected. Samples with less than 1.1 x the background count rate (> 15% error) were rejected. Of the 84 plasma samples with measurable concentrations of fluphenazine 92% were determined to better than 10% and 85% better than 5% confidence.

The kinetics of fluphenazine after the three preparations, dihydrochloride, enanthate and decanoate are different. Elimination half-times of intramuscular and oral fluphenazine dihydrochloride were however very similar. Plasma concentrations after oral fluphenazine were less than those after intramuscular injections. Total radioactivity however showed a higher peak concentration at an earlier time after oral administration indicating that the lower fluphenazine plasma concentrations are due to 'first pass' metabolism rather than poor or slow absorption, as long-known for chlorpromazine (Curry, D'Mello & Mould, 1971). The advantages of fluphenazine injections over oral chlorpromazine have been demonstrated (Curry & Adamson, 1972; Adamson,

Curry, Bridges, Firestone, Lavin, Lewis, Watson, Xavier & Anderson, 1973). It would appear that the same is true with regard to intramuscular and oral fluphenazine preparations.

Fluphenazine plasma concentrations declined with half-times of approximately 3.5 days after the injections of fluphenazine enanthate. Thin layer chromatography of pooled heptane extracts showed only one spot, corresponding to fluphenazine. It is reasonable to deduce that this indicates absence of the ester from plasma. In the absence of detectable amounts of enanthate ester it is difficult to explain why the elimination rate constant of fluphenazine after fluphenazine enanthate should be different from that after fluphenazine dihydrochloride. On the other hand, the concentration-time curve can be explained in terms of a one compartment pharmacokinetic model where the absorption process is much slower than the elimination processes and, consequently, is rate limiting. In such a situation the terminal phase gives an estimate of the half-time of absorption and the rising phase the elimination half-time (Gibaldi & Perrier, 1975). The elimination half-times estimated by the method of residuals (16.8 h and 14.4 h) are in very good agreement with those obtained from the dihydrochloride data. Animal studies confirm the hypothesis that the rate of release of enanthate controls the plasma concentration of fluphenazine. For example, Dreyfuss, Ross & Schreiber (1971) showed that 12% and 19% of a dose was still at the site of injection 21 days after injection of fluphenazine enanthate in two dogs.

Applying the same reasoning to the decanoate data, the absorption rate half-times were 9.6 and 6.8 days in subjects 5 and 7, respectively. The initially high plasma concentrations complicated analysis of the data and it was considered inappropriate to attempt to obtain fluphenazine elimination half-times in these cases. The reason for the high plasma concentrations is not understood. These early high plasma concentrations are too great to be ascribed to the presence of non-esterified fluphenazine in the injection solutions. They may have considerable significance for the use of fluphenazine in acute conditions and in the generation of extrapyramidal effects on the first day of each dosage period.

We are grateful to Dr J. Haenen for assistance with patients, and to the patients themselves. Squibb, Benelux kindly assisted with transfer of samples between the two countries.

References

ADAMSON, L., CURRY, S.H., BRIDGES, P.K., FIRESTONE, A.F., LAVIN, N.I., LEWIS, D.M., WATSON, R.D., XAVIER, C.M. & ANDERSON, J.A. (1973). Fluphenazine decanoate trial in chronic in-patient schizophrenics failing to absorb oral chlorpromazine. *Dis. Nerv. Syst.*, **34**, 181—191.

CURRY, S.H. (1977). *Drug Disposition and Pharmacokinetics*. 2nd edn. London: Blackwell Scientific Publications.

CURRY, S.H. & ADAMSON, L. (1972). Double-blind trial of fluphenazine decanoate. *Lancet*, **ii**, 543–544.

CURRY, S.H., D'MELLO, A. & MOULD, G.P. (1971). Destruction of chlorpromazine during absorption in the rat *in vivo* and *in vitro*. *Br. J. Pharmac.*, **42**, 403–411.

DREYFUSS, J., ROSS, J.J. & SCHREIBER, E.C. (1971). Excretion and biotransformation of the enanthate ester of fluphenazine-^{14}C by the dog. *J. pharm. Sci.*, **60**, 829–833.

GIBALDI, M. & PERRIER, D. (1975). *Pharmacokinetics*. New York: Marcel Dekker, Inc.

JOHANSSON, R., BORG, K.D. & GABRIELSSON, M. (1976). Determination of fluphenazine in plasma by ion-pair partition chromatography. *Acta Pharm. Suecica*, **13**, 193–200.

JORGENSEN, A. & GOTTFRIES, C.G. (1972). Pharmacokinetic studies on flupenthixol and flupenthixol decanoate in man using tritium labelled compounds. *Psychopharmacologia*, **27**, 1–10.

KOBAYASHI, Y. & MAUDSLEY, D.V. (1974). *Biological Applications of liquid Scintillation Counting*. New York: Academic Press.

LARSEN, N-E. & NAESTOFT, J. (1973). Determination of perphenazine and FPZ in whole blood by gas chromatography. *Med. Lab. Tech.*, **30**, 129–132.

McISAAC, C. (1971). The human metabolism of long-acting fluphenazine drugs. *Unpublished data, quoted by Schreiber, E., and Grozier, M.L., in Therapie, 1973*, **28**, 441–449.

RIGGS, D.S. (1970). *The mathematical approach to physiological problems*. Cambridge, Massachusetts: M.I.T. Press.

SMULEVITCH, A.B., MINSKER, E.L., MAZAYERA, N.A., VOLKAVA, R.P. & LUKANINA, S.K. (1973). The problem of clinical activity of long-acting neuroleptics. *Comprehensive Psych.*, **14**, 227–233.

WHELPTON, R. & CURRY, S.H. (1976a). Methods for study of fluphenazine kinetics in man. *J. Pharm. Pharmac.*, **28**, 869–873.

WHELPTON, R. & CURRY, S.H. (1976b). Solvent extraction as applied to fluphenazine and its metabolites. *Methodological developments in biochemistry*, **5**, 115–120.

WILES, D.H. & FRANKLIN, M. (1978). Radioimmunoassay for fluphenazine in human plasma. *Br. J. clin. Pharmac.*, **5**, 265–268.

YALE, H.L., COHEN, A.I. & SOWINSKI, F. (1963). Esters of 4{3-[2-(trifluoromethyl)phenothiazin-10-yl]propyl}-1 piperazineethanol as long-acting tranquillizing agents. Synthesis of 4-{3-[2-(trifluoromethyl)phenothiazin-10-yl]propyl}-1-piperazineethanol ethyl-C^{14} and its ester with heptanoic acid. *J. med. Chem.*, **6**, 347–350.

(Received May 15, 1978)