including the intact drug itself but that conditions during the second period of dosing were not such as to precipitate the reaction.

The present findings, however, do not exclude the possibility that toxic metabolite formation is responsible for the adverse effects of practolol, since these might be produced only in very small amounts, be highly reactive and covalently bound (Orton & Lowery, 1977) and not readily detectable by the methods used here.

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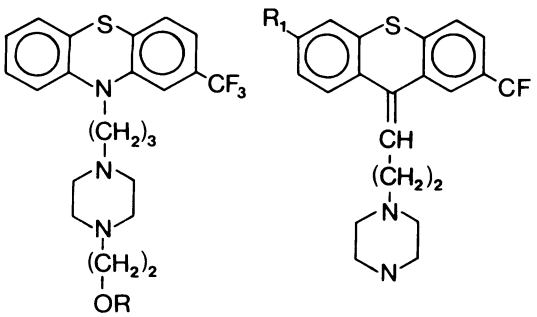
RADIOIMMUNOASSAY FOR FLUPHENAZINE IN HUMAN PLASMA

Fluphenazine (FPZ, Figure 1a) is a phenothiazine derivative. It is widely used in the maintenance treatment of schizophrenia as an intramuscular slow release preparation containing its decanoic acid ester (Figure 1b). Although treatment is generally effective (Ayd, 1975) there are individual differences in both clinical response and in side effects. Measurement of plasma fluphenazine levels would allow greater control of dosage and may improve therapeutic control. However, fluphenazine decanoate is given in low doses (typically 25 mg every 1–4 weeks) and plasma drug levels attained are below the range of current assay techniques (Larsen & Naestoft, 1973: Whelpton & Curry, 1976). The following account describes the development of a highly sensitive radioimmunoassay for fluphenazine in plasma which does not require extraction and preliminary data from the analysis of clinical samples.

Antiserum was raised in an adult female sheep. The immunogen (Figure 1d) was prepared by covalent linkage of bovine serum albumin (BSA) to 7-carboxy flupenthixol (Figure 1c) employing the carbodiimide condensation reaction (Sheehan & Hlavka, 1956) followed by dialysis first against a citrate buffer (pH 3) and then saline (0.9%). The sheep was inoculated at 4-weekly intervals with 1 ml conjugate in saline (250 µg flupenthixol) suspended in 4 ml of complete Freund's adjuvant, the dose being divided between four intramuscular sites. Venous blood samples taken on the same occasion were allowed to clot overnight at 4°C and serum, separated by centrifugation, was stored at −20°C.



a R=-H

b R=-C(=O)-$(CH_2)_8$-CH_3

c R_1=-COOH

d R_1=-CONH-BSA

Figure 1 Structural formulae of (a) fluphenazine (FPZ); (b) FPZ decanoate; (c) 7-COOH-Fluphethixol; (d) flupenthixol-BSA.

Serum from the immunized sheep was tested by radioimmunoassay. Reaction mixtures were perpared in plastic tubes in an ice bath. Drug solutions were protected from light as far as was practicable. Dilutions of serum made in gelatine buffer pH 7.6 (phosphate buffered saline containing: sodium phosphate, 0.1 M; saline, 0.9%; gelatine, 0.1% and thiomersal, 0.01%) were incubated overnight at 4°C with [^{3}H]-fluphenazine (1500 counts/min, 34 pg; specific activity 199/mmol, purity > 99%, purchased from Nuclear Research Centre, Negev, Israel). Duplicate tubes contained in addition non-radioactive fluphenazine. Anti-body-bound fluphenazine was separated from the free fraction by the dextran-coated charcoal technique (Herbert, Lau, Gottlieb & Bleicher, 1965). Bound radioactivity was measured with a liquid scintillation spectrometer. A suitable antiserum which bound 50% of the [^{3}-H]-fluphenazine and allowed 80% displacement by 500 pg of the cold drug at 1:300,000 final dilution, was obtained after seven injections.

Table 1 Intra-assay precision and accuracy. Immuno-reactive FPZ levels obtained by analysis of known amounts of FPZ (ranging from 20–2000 pg) added to human plasma. The mean and coefficient of variation (CV) are shown using one 200 µl plasma sample from nine individuals at each concentration of FPZ.

FPZ (pg)	*Mean (pg)*	*FPZ CV (%)*	*n*
20	19.92	7.78	9
50	50.70	9.76	9
100	98.33	7.45	9
200	204.60	6.19	9
500	508.80	9.39	9
1000	990.00	19.60	9
2000	2033.00	15.30	9

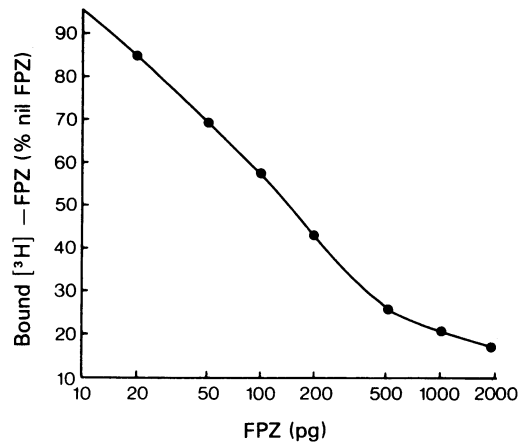


Figure 2 Standard calibration curve. Inhibition of the binding of [^{3}H]-FPZ to anti-FPT-BSA (1 : 300,000 final dilution) by various amounts of unlabelled FPZ in the presence of 200 µl of human plasma. Antibody bound radioactivity is expressed as a percentage of that obtained in the absence of unlabelled FPZ. Each point represents the mean of 9 determinations ± s.d.

Using the antiserum, calibration standards were prepared in triplicate containing fluphenazine (10–2000 pg), antiserum (1 :300,000, finally), [^{3}H]-fluphenazine (1500 counts/min) human control plasma (200 µl) and gelatine buffer (to a final volume of 600 µl). These were processed as described above and the bound radioactivity, expressed as a percentage of that bound in the absence of unlabelled fluphenazine, was used to construct a standard curve (Figure 2). The least amount of fluphenazine, which could be distinguished from zero was 10 pg (equivalent to a concentration of 50 pg/ml when a 200 µl plasma sample is assayed). A measure of intra-assay precision and accuracy was obtained by assaying known concentrations of fluphenazine in 200 µl plasma samples from each of nine subjects. The mean drug levels and coefficients of variation (C.V.) were then calculated (Table 1). Over the range 20–500 pg, fluphenazine was estimated with a coefficient of variation of less than 10%. Precision was not improved when the plasmas were pooled and the experiment repeated, showing that individual plasmas did not differ in their effect on the assay.

The ability of other drugs used in psychiatry to compete with [^{3}-H-fluphenazine for binding sites on the antibody was examined. Test compounds

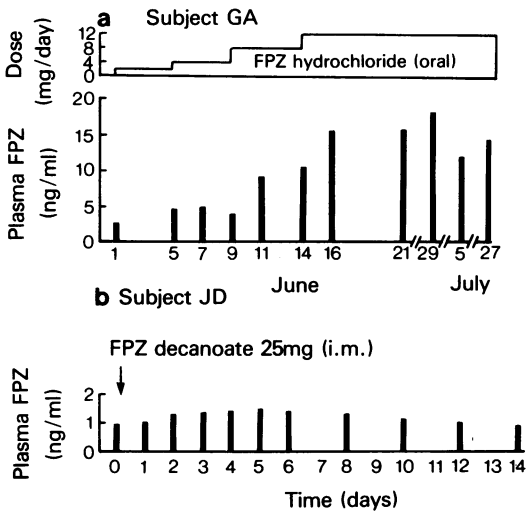


Figure 3 Plasma FPZ levels in two subjects receiving the usual therapeutic doses of the drug. a: Plasma FPZ in plasma samples taken at 2 h after the morning dose of FPZ hydrochloride given orally in subject GA who received between 2 and 12 mg/day in two doses. b: Plasma FPZ levels in morning samples from subject JD who received chronic treatment with 25 mg FPZ decanoate given intramuscularly at intervals of 2 weeks.

(100 pg–100 ng were incubated with [^{3}H]-fluphenazine and antibody in the system described earlier. The percentage cross reaction of each compound was calculated (Exley, Johnson & Dean, 1971) by reference to the quantity of fluphenazine which displace 50% of antibody-bound [^{3}-H]-fluphenazine (Table 2). Among the metabolites of fluphenazine available for testing, the hydroxy compounds showed greatest cross-reactivity (98 and 57 % for 8- and 7-OH–fluphenazine respectively), the sulphoxide gave only 0.6% cross-reaction.

Venous blood samples from patients receiving treatment with fluphenazine were taken into heparin, centrifuged and the plasma stored at −20°C. Up to 200 µl of plasma were assayed in triplicate. Smaller volumes of unknown plasma were made up to 200 µl with human control plasma. Measurable levels of fluphenazine were found in all plasma samples from nine patients receiving fluphenazine, of whom one received oral dosage. Figure 3 shows examples of plasma fluphenazine concentrations in two subjects: Subject GA, who received the hydrochloride orally and Subject JD who was treated with intramuscular injections of the decanoate.

The antigen used here was designed so that the point of attachment of BSA was remote from important functional groups on the hapten molecule. Consequently, the resulting antiserum favoured tricyclic compounds having piperazine in the side chain.

The metabolic transformations of fluphenazine in man have not been studied extensively. Whelpton & Curry (1976) found only unmetabolized fluphenazine by thin-layer chromatography of a plasma extract from a patient receiving intramuscular doses of radiolabelled fluphenazine, although in addition the sulphoxide and 7-hydroxy-fluphenazine, mainly as glucuronic acid conjugates were found in the urine. Animal studies in the dog (Dreyfuss, Ross & Schreiber, 1971) and rhesus monkey (Dreyfuss & Cohen, 1971) confirm these as the major metabolites. From these findings and from the cross-reaction studies reported here it is clear that 'immunoreactive fluphenazine' as determined by this assay will include contributions from it hydroxylated metabolites should they occur in significant amounts in plasma. However, it is likely that these metabolites will possess some of the pharmacological properties of the parent drug, as is the case with chlorpromazine (Manian, Efron & Goldberg, 1965). The level of immuno-reactive fluphenazine may therefore be a truer estimate of the biologically effective dose of the drug, although this matter needs further investigation.

Drugs closely related to fluphenazine in structure, possessing piperazine in the side-chain (trifluoperazine, perphenazine and flupenthixol) cross-reacted sufficiently strongly to be assayed using the same antiserum. Such assays are being developed. Of other neuroleptics examined, chlorpromazine cross-reacted

Table 2 Cross-reaction of phenothiazine derivatives and other psychiatric drugs with anti–FPT–BSA.

Test Compound	*Cross-reaction %*
Fluphenazine (FPZ)	100.0
8 – OH – FPZ	98.0
7 – OH –FPZ	57.0
FPZ – SO	0.6
Cis–Flupenthixol (FPT)	22.0
FPT–NH	45.6
FPT–SO	4.8
Chlorpromazine (CPZ)	3.6
NOR CPZ	0.5
CPZ²SO	0.08
Trifluoperazine	82.0
Perphenazine	63.0
Thioridazine	0.2
Amitriptyline	0.08
Imipramine	0.08
Desipramine	0.08
Haloperidol	0 at 100 ng
Benzhexol	0 at 100 ng
Diazepam	0 at 100 ng
Nitrazepam	0 at 100 ng
Chlordiazepoxide	0 at 100 ng

weakly but significantly. Representative tricyclic antidepressants cross-reacted very weakly and the benzodiazepines and benzhexol were not recognized at all.

The preliminary clinical data presented here (Figure 3) show that plasma fluphenazine levels in a subject (JD) receiving a typical dose of the long-acting depot preparation of fluphenazine are low (1–2 ng/ml) but measurable by radioimmunoassay.

The stepwise increase in plasma fluphenazine concentration produced by raising the dosage in Subject GA confirms that the assay can pick up changes in the plasma drug level.

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RELATIONSHIP BETWEEN PLASMA AND SALIVARY CONCENTRATIONS OF AMITRIPTYLINE

There is increasing evidence (Lader, 1974) that the relationship between therapeutic response to antidepressive drugs and their steady state plasma levels is a complex one. For some drugs subtherapeutic levels appear to be ineffective, and levels above the therapeutic range are also less effective, as well as associated with an increased incidence of adverse effects. This reduced efficacy at higher levels may be due to other pharmacological effects of these drugs which assume greater importance at higher concentrations and to problems of patient compliance.

It is desirable, therefore, that the plasma levels of patients receiving antidepressive drug treatment should be measured, particularly if they are not demonstrating the expected response to treatment. However, such patients are often unwilling to be subjected to venesection, especially on repeated occasions, and the fear of such venesection may positively discourage patients from regularly attending their psychiatrist. We have, therefore, carried out a small pilot study to investigate the possibility of using salivary drug levels instead of plasma levels in order to provide a non-invasive alternative to venesection. The patients studied were receiving amitriptyline for a depressive illness, but no other tricyclic antidepressive compound.

Unfortunately, due to the anticholinergic properties of many tricyclic antidepressants (such as amitriptyline), salivary flow is greatly diminished, creating difficulty in obtaining sufficient sample size for accurate analysis of drug concentration. In order to increase flow, patients sucked 'acid drop sweets' and all saliva thus produced was collected in a small container. In general, it was possible to obtain the required amount although some patients experienced difficulty (particularly those patients admitted with drug overdose). Mixed saliva was used as it was found difficult to obtain a 4 ml sample by more sophisticated methods such as the parotid cup (Stephen & Speirs, 1976). As this was a pilot study no parameters were controlled apart from the method of stimulation and all samples were taken, together with simultaneous blood samples, at random intervals after drug administration. Whenever possible, at least 2 h had elapsed since the last dose of amitriptyline. Preliminary studies had shown that acid drops