

DETERMINATION OF HALOPERIDOL IN HUMAN SERUM BY RADIOIMMUNOASSAY

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A radioimmunoassay has been developed which enables accurate determination of haloperidol in human serum. Antiserum was prepared by immunizing guinea pigs with haloperidol (O-carboxymethyl)oxime derivative (III) coupled with bovine serum albumin. With the antiserum, ^3H -haloperidol and dextran-coated charcoal, the assay of haloperidol in serum was possible over a concentration range of 1 to 50 ng/ml, using 0.1 ml of human serum without the need of an extraction procedure. Data obtained by radioimmunoassay are in good agreement with those obtained by gas chromatography. No appreciable cross-reactivity was observed neither with haloperidol metabolites nor with other butyrophenone neuroleptics. Serum levels of haloperidol in schizophrenic patients receiving single oral dosing (6 mg/subject) have also been determined.

Keywords—radioimmunoassay; haloperidol; cross-reactivity of haloperidol antiserum; haloperidol level in patient serum; haloperidol-(O-carboxymethyl)oxime-BSA conjugate

Haloperidol, 1-[3-(4-fluorobenzoyl)propyl]-4-hydroxy-4-(4-chlorophenyl)piperidine (I, Fig. 1), is a butyrophenone neuroleptic drug widely used in the treatment of psychoses. For the rational clinical use of the drug, it is particularly helpful to monitor the blood levels of the unchanged drug in patients.^{1,2)}

The gas chromatographic method developed by Forsman *et al.*³⁾ permits the specific determination of haloperidol in plasma but is not highly sensitive, and in addition the method involves tedious extraction procedures. Recently, Clark *et al.*⁴⁾ determined haloperidol in serum by a radioimmunoassay developed by use of conjugate II-BSA (Fig. 1) as an antigen. Shostak *et al.*⁵⁾ also developed a similar radioimmunoassay method by use of another conjugate III-BSA as an antigen, but details of the method have not been reported.

In this paper, we describe a radioimmunoassay developed by use of III-BSA as an antigen with a

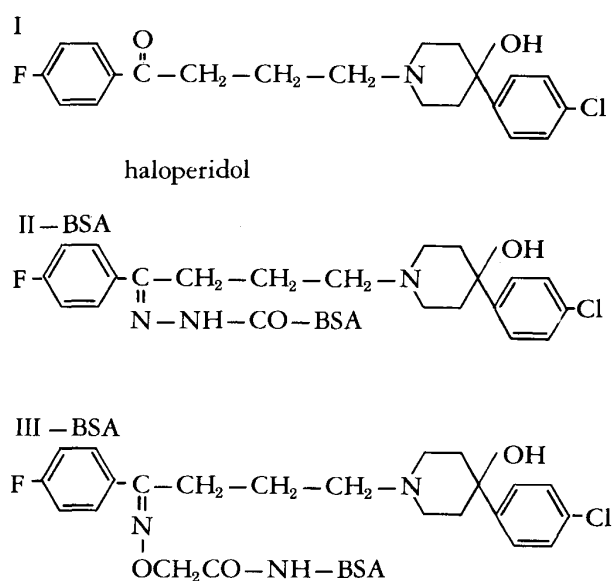


FIG. 1. Structures of Haloperidol and Antigens
[III]-BSA conjugate was used as an antigen.
BSA: Bovine serum albumin.

high sensitivity and specificity for haloperidol in unextracted human serum, and also describe the serum levels of haloperidol in patients.

EXPERIMENTAL

Chemicals and Reagents

³H-Haloperidol (labeled at the 3-position of the 4-chlorophenyl moiety) with a specific activity of 10.5 Ci/mmol was supplied by I.R.E. (Belgium) and the radiochemical purity was checked by thin-layer chromatography prior to use. 1-[3-(4-Chlorobenzoyl)propyl]-4-hydroxy-4-(4-chlorophenyl)piperidine hydrochloride (an internal standard in gas chromatography) and 4-fluorophenylacetic acid were synthesized in this laboratory. Floropipamide, trifluoperidol, droperidol, promethazine and chlorpromazine were extracted with solvent from their commercial preparations and were purified. 4-Hydroxy-4-(4-chlorophenyl)piperidine was kindly supplied by Janssen Pharmaceutica (Beerse, Belgium), and clofluperol, biperiden and haloperidol (I) were obtained from Dainippon Pharmaceutical Co. (Osaka). 4-Fluorobenzoylpropionic acid and 4-fluorophenylacetic acid were purchased from Aldrich Chemical Co. Inc. (San Leandro, Calif.), bovine serum albumin (BSA, crystallized and lyophilized) from Sigma Chemical Co. (St. Louis, Mo.), Sephadex G-25 and dextran T-70 from Pharmacia Fine Chemicals (Uppsala, Sweden), Freund's complete adjuvant from Difco Laboratories (Detroit, Mich.), γ -Venin from Hoechst Aktiengesellschaft (Germany), activated carbon (Norit A) from Nutritional Biochemical Corp. (Cleveland, Ohio) and human control serum from Flow Laboratories (Rockville, Md.).

All other chemicals used were of analytical-reagent grade.

Synthesis of 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone-1-(O-carboxymethyl)oxime (III)

III was prepared according to the procedure of Sasajima *et al.*⁶⁾ To a solution of 1.45 g of carboxymethoxylamine hemihydrochloride in 4 ml of water were added 7.6 ml of 2 N KOH and 2.0 g of I in 40 ml of ethanol. The mixture was refluxed

for 3 hr with stirring and evaporated *in vacuo* to remove ethanol. The residue was cooled with ice-water and alkalized with 4 ml of 2 N KOH. The solution was washed with three 100 ml portions of ether and brought to pH 5–6 with conc. HCl. The precipitate formed was collected by filtration, washed with water and recrystallized from ethanol-ether (1:4) to give 1.89 g of III, mp 180–185° (dec.). *Anal.* Calcd for C₂₃H₂₆N₂ClFO₄: C, 61.54; H, 5.84; N, 6.24; Cl, 7.90; F, 4.23. Found: C, 61.32; H, 5.97; N, 5.99; Cl, 7.72; F, 4.15.

Preparation of Antigen

The hapten III was covalently coupled to BSA by the mixed anhydride procedure⁷⁾ as follows: 136.9 mg (0.305 mmol) of III and 0.2 ml (0.945 mmol) of tri-*n*-butylamine were dissolved in 3 ml of absolute dioxane. The solution was cooled and treated with 0.63 ml (6.0 mmol) of isobutyl chlorocarbonate. The reaction was allowed to proceed at 5–10° for 20 min, and then the mixture was added dropwise to a stirred, cooled solution of 420 mg (6.1 μ mol) of BSA in 11 ml of water, 11 ml of dioxane and 0.42 ml of 1 N NaOH. Stirring and cooling were continued for 3 hr. The solution was dialyzed against running water for about 20 hr and brought to pH 4.5 with 1 N HCl. The resultant precipitate was allowed to stand at 4° overnight and then centrifuged. After removal of the supernatant, the residual solution was neutralized with NaHCO₃ to dissolve the precipitate and then passed through a column of Sephadex G-25 and lyophilized to give a fluffy powder.

Spectrophotometric analysis⁷⁾ of the conjugate against control BSA and III indicated that about 38 mol of the hapten had been covalently coupled to 1 mol of BSA.

Immunization and Measurement of Antibody Titer

Six male Hartley guinea pigs weighing 300–350 g were used for immunization. The hapten-BSA conjugate (1 mg) was dissolved in 0.5 ml of sterile isotonic saline and emulsified with an equal volume of Freund's complete adjuvant. The emulsion (1 ml) was injected into guinea pigs intradermally at several sites over the

back and in the foot pads. Booster injections were given every two weeks for 6 weeks and then every four weeks for further two months. Blood was collected by cardiac puncture 7 days after each or the final booster injection, and the serum was separated and stored at -20° .

For antiserum titer determinations, 0.1 ml of ^3H -haloperidol solution (300 pg), 0.1 ml of 0.075 M phosphate buffer, pH 7.4 and 0.3 ml of diluted antiserum ($\times 100$, 1000, 2500, 5000 or 10000) were added to 0.5 ml of a buffer solution containing 0.06% of BSA and 0.04% of γ -Venin in a plastic assay tube. The contents were mixed and incubated at 4° for 24 hr. After addition of 1 ml of saturated ammonium sulfate, the tubes were mixed and stood for 10 min to precipitate the proteins, and then centrifuged at 3000 rpm for 20 min. One milliliter of the supernatant was pipetted into 10 ml of Bray's scintillator⁸⁾ and counted in a Packard Tri-Carb Model 2450 liquid scintillation spectrometer (Packard, Downers Grove, Ill.).

Assay Procedure

The following solutions were prepared prior to assay: (1) 0.075 M phosphate buffer, pH 7.4 (assay buffer), (2) a solution of ^3H -haloperidol in the assay buffer to give 1.86×10^4 dpm/300 pg/0.1 ml, (3) antiserum dilution 1:2000 with the assay buffer, (4) standard solutions containing 0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10 ng/0.2 ml of unlabeled haloperidol in the assay buffer, and (5) dextran-coated charcoal (DCC) suspension containing 500 mg of activated charcoal and 50 mg of dextran T-70 in 100 ml of the assay buffer.

The assay was carried out in plastic assay tubes. One tenth milliliter of sample or control serum and 0.2 ml of the assay buffer or each standard solution were added to 0.1 ml of ^3H -haloperidol solution. Then, 0.3 ml of the diluted antiserum was added to all tubes except for blank tubes to which normal rabbit serum ($\times 100$) was added. After adjusting the volume of each tube to 1.1 ml with the assay buffer, the contents were mixed and allowed to stand at 4° for 4 hr.* To separate

the antibody-bound and free drug, 0.2 ml of DCC suspension was added to each tube. The tubes were vortexed, stood at 4° for 10 min and centrifuged at 3000 rpm for 15 min. One milliliter of the supernatant was pipetted into a counting vial containing 10 ml of scintillation cocktail (Riafluor[®], New England Nuclear Corp., Boston, Mass.) and the radioactivity was counted in a scintillation spectrometer (Packard Tri-Carb Model 2450).

A standard curve was obtained by a logit-log plot of the relative percentage (B/B_0)⁹⁾ of bound labeled drug against the amount of unlabeled haloperidol added. The standard curve was obtained together with each series of unknown samples and all assays were made in duplicate.

Antibody specificity was evaluated by measuring the inhibition of the antibody-haloperidol binding caused by increasing amounts of the related compounds including the metabolites of haloperidol¹⁰⁾ and structurally related and unrelated neuroleptic drugs, together with antiparkinsonian agents.

Gas-Liquid Chromatography

A Yanagimoto G-180 gas chromatograph equipped with a 10-mCi ^{63}Ni electron capture detector was used. A glass column (100 cm \times 3 mm I.D.) was packed with 1% OV-25 on Chromosorb W 80-100 mesh. The column temperature was 255° , and the injector and detector temperatures were 300° . Ultra-pure nitrogen was used as the carrier gas with a pressure of 1.0 kg/cm².

The extraction procedure for haloperidol in serum was that reported by Forsman *et al.*³⁾

Human Study

The study was carried out under the supervision of physicians. Blood specimens from four male schizophrenic patients each receiving 6 mg of haloperidol in powder form, were taken at 1, 2, 3, 4, 6, 8, 10, 12 and 24 hr after dosing. The sera were separated and kept frozen until analyzed.

RESULTS

* Under these conditions, the binding of ^3H -haloperidol to the antiserum was constant for 2 to 24 hr at 4° .

Antibody Titer

A typical antiserum titer curve is shown in Fig. 2. The dilution of antiserum which exhibited 50% binding to the labeled antigen was 1:3500, showing an appropriate high-affinity to haloperidol.

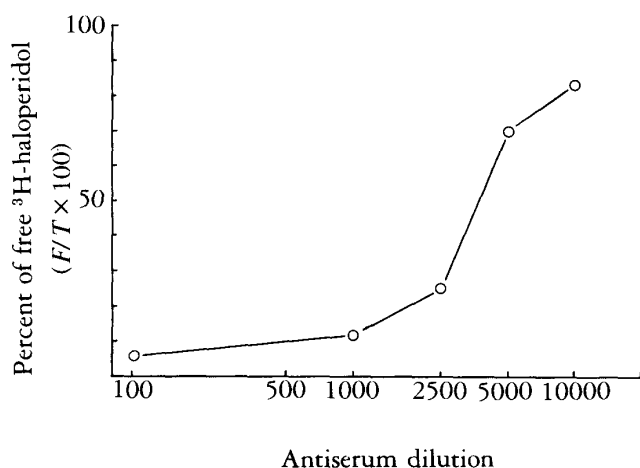


FIG. 2. Dilution Curve of Haloperidol Antiserum

The percentage of ³H-haloperidol (300 pg) unbound to the antibody is plotted against the dilution of the antiserum represented on a semi-logarithmic scale.

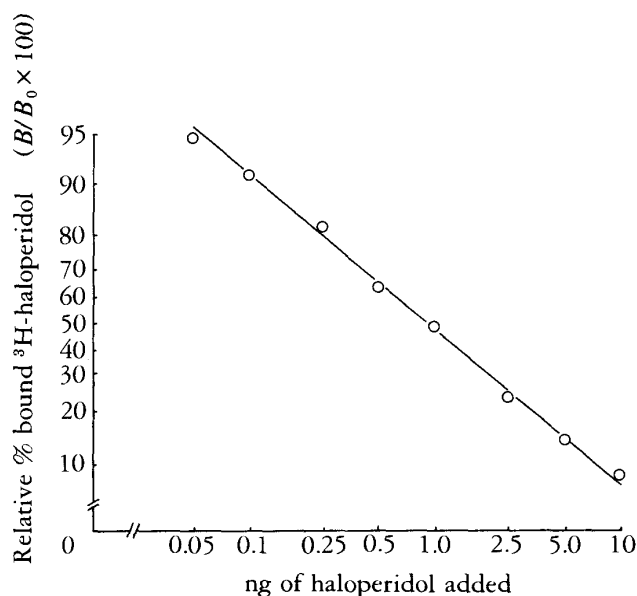


FIG. 3. Standard Curve of Haloperidol

The relative percentage of ³H-haloperidol bound to the antibody in the presence of various amount of unlabeled haloperidol is plotted on a logit-log scale.

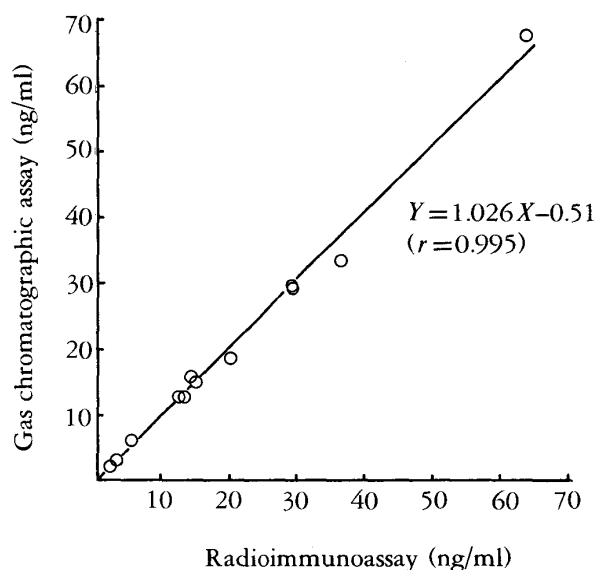


FIG. 4. Comparison of Radioimmunoassay and Gas Chromatographic Assay of Haloperidol in Serum

Serum samples were obtained from patients after oral administration of haloperidol. Line was drawn from the values calculated by the method of least squares.

X: Radioimmunoassay (ng/ml).

Y: Gas chromatographic assay (ng/ml).

r: Correlation coefficient.

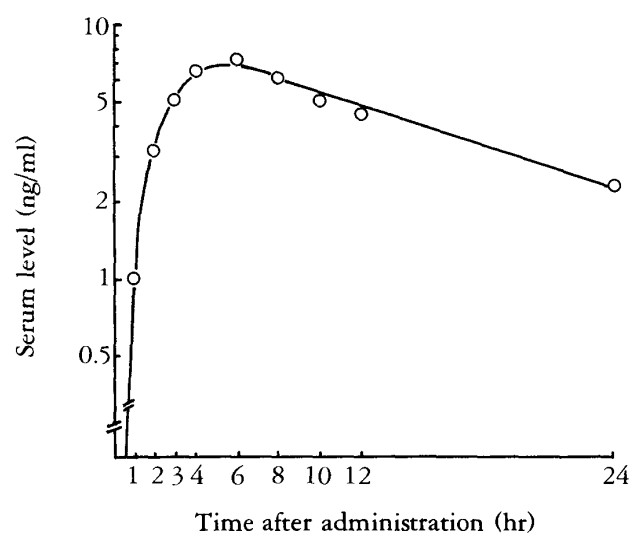
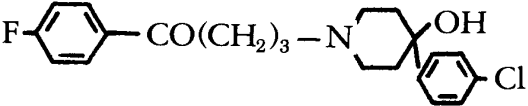
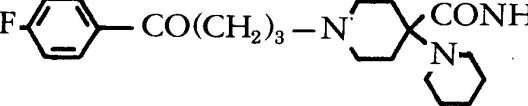
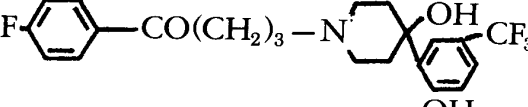
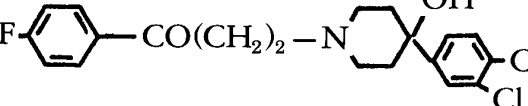
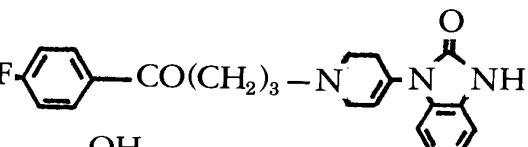
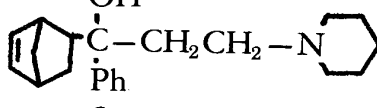


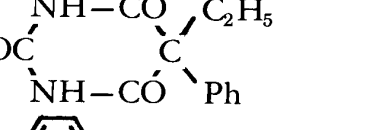

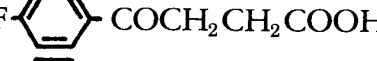

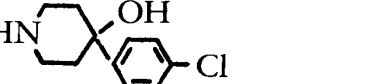


FIG. 5. Mean Serum Levels of Haloperidol in Patients after Single Oral Administration at a Dose of 6 mg/subject

Plottings are mean values from four patients.

TABLE I. *Specificity of Haloperidol Antiserum*

Generic name	Structure	Amount which reduces antibody binding of ^3H -haloperidol to 50% (ng)
Haloperidol		1.0
Floropipamide		1000
Trifluoperidol		60
Clofluperolol		125
Droperidol		> 1000
Biperiden		> 1000
Promethazine		> 1000
Chlorpromazine		> 1000
Phenobarbital		> 1000
Haloperidol Metabolite 1		> 1000
Metabolite 2		> 1000
Metabolite 3		> 1000
Piperidiny moiety of haloperidol		> 1000

The specificity of the antiserum was evaluated by incubating the antiserum ($\times 2000$, 0.3 ml) and ^3H -haloperidol (300 pg) with the test compound, the amounts ranging from 5 ng to 1000 ng per assay tube. The values are expressed at the amount of the test compound required for 50% inhibition of binding of ^3H -haloperidol to the antibody.

TABLE II. Serum Levels of Haloperidol in Four Patients after Single Oral Administration at a Dose of 6 mg/subject

Subject	Serum level (ng/ml)									Half-life ^{a)} (hr)
	1	2	3	4	6	8	10	12	24	
M.I.	0.4	1.1	1.3	3.7	4.5	4.4	4.8	4.0	2.8	24.2
T.I.	0.0	0.9	2.6	4.5	5.1	4.8	3.5	3.3	1.7	11.4
K.T.	3.5	9.6	14.0	14.0	14.0	11.0	8.4	7.1	3.5	9.5
X.M.	0.0	1.1	2.4	4.1	5.4	4.6	3.1	2.8	1.6	10.8
Mean	1.0	3.2	5.1	6.6	7.3	6.2	5.0	4.3	2.4	14.0
± S.E.	± 0.9	± 2.1	± 3.0	± 2.5	± 2.3	± 1.6	± 1.2	± 1.0	± 0.5	± 3.4

a) Serum elimination half-life: Calculated from the values at 6 to 24 hr.

Sensitivity of the Assay

A typical standard curve obtained with 1:2000 antiserum dilution and 300 pg of the label tracer, for non-labeled haloperidol added to control human serum is shown in Fig. 3. The detection limit of the assay is approximately 50 pg, and haloperidol can be reliably assayed in a range of 100 pg up to 5 ng contained in 0.1 ml of serum.

Intra- and Inter-assay Variation

Intra- and inter-assay variations were estimated from the repeated assay of human pooled sera. The coefficients of the variations were less than 10% over the concentrations tested.

Specificity of the Antiserum

The cross-reactivity of the antiserum with selected compounds is listed in Table I. Data are expressed at the amount required for 50% inhibition of antibody-binding of 300 pg of ³H-haloperidol. None of the compounds appreciably cross-reacted with the antiserum except for trifluoperidol (1.6%).

Comparison of Radioimmunoassay and Gas-Liquid Chromatography

Twelve human serum samples of haloperidol were determined by both radioimmunoassay and gas-liquid chromatography. The results are shown in Fig. 4. There is a good correlation between values obtained by the two methods (correlation coefficient, 0.995).

Serum Levels of Haloperidol in Patients

Table II represents the mean serum levels of

haloperidol in four male patients receiving orally 6 mg of haloperidol. The unchanged drug levels reached a maximum of 7.3 ng/ml at 6 hr after dosing, followed by a elimination half-life of 14.0 hr (Fig. 5 and Table II). The results are consistent with previous findings.²⁾

DISCUSSION

A radioimmunoassay was developed for haloperidol in unextracted human serum with a high sensitivity and specificity. Accurate determinations of haloperidol are possible with 0.1 ml serum samples over a concentration range from 1 to 50 ng/ml, corresponding to that reported by Clark *et al.*⁴⁾

The antiserum was prepared by use of III-BSA conjugate, simply due to easy preparation, as compared to II-BSA conjugate adopted by Clark *et al.*⁴⁾ The specificity of the antiserum is quite remarkable that any other butyrophenone neuroleptics and metabolites of haloperidol together with antiparkinsonian drugs, biperiden and promethazine, did not appreciably cross-react with the antiserum. In addition the antiserum showed virtually no cross-reaction with piperidinyll moiety of haloperidol, 4-hydroxy-4-(4-chlorophenyl)piperidine, which is a possible metabolite¹⁰⁾ resulting from oxidation of haloperidol, while that obtained by Clark *et al.*⁴⁾ cross-reacted to some extent. The present method is, therefore, more suitable for determination of

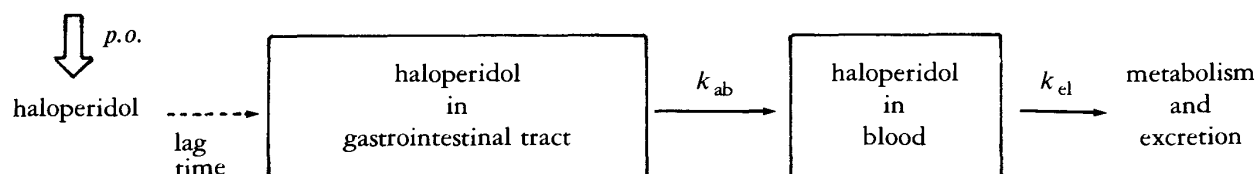


FIG. 6. Schematic Representation of Haloperidol in Blood as Linear Compartment Model in Patients

Lag time represents the time interval between ingestion of haloperidol and appearance of haloperidol in blood.

k_{ab} : Apparent absorption rate constant.

k_{el} : Apparent elimination rate constant.

TABLE III. Pharmacokinetic Parameters of Haloperidol in Patients after Oral Administration at a Dose of 6 mg/subject

Subject	Lag time (hr)	Absorption rate constant (hr ⁻¹)	Elimination rate constant (hr ⁻¹)	Steady-state level ^{a)}	
				Basal (ng/ml)	Peak (ng/ml)
M.I.	1.0	0.427	0.029	5.5	8.9
T.I.	1.5	0.701	0.061	2.0	6.3
K.T.	0.75	0.753	0.073	3.8	15.8
X.M.	1.5	0.621	0.064	1.7	5.7
Mean	1.2	0.626	0.057	3.3	9.2
± S.E.	± 0.2	± 0.072	± 0.010	± 0.9	± 2.3

a) Steady-state serum level of haloperidol in patients after oral administration at dose of 6 mg/subject once a day was calculated according to the model shown in Fig. 6.

haloperidol in serum.

The radioimmunoassay developed here offers distinct advantages, as compared to the gas chromatographic method;³⁾ accurate and sensitive determination with a small volume of serum as little as 0.1 ml, in contrast to 5 ml needed for gas chromatography, and a simple procedure with intact serum enabling an assay of a large number of samples, in contrast to tedious extraction procedures.

The mean serum level of haloperidol in patients who received orally 6 mg of the drug was maximal at 6 hr after dosing, followed by a first-order kinetic elimination (Fig. 5). Therefore, one-compartment pharmacokinetic model shown in Fig. 6 was adopted and parameters were determined by reference to the analysis of Forsman *et*

*al.*¹¹⁾ (Table III). The calculated steady-state serum level of haloperidol in oral dose of 6 mg once a day showed 3.3 ng/ml at basal level and 9.2 ng/ml at peak level on the fifth day.

Despite the wide-spread use of haloperidol in schizophrenic patients, a little is known concerning the relations between serum levels of haloperidol and its clinical effects or side effects, due to the lack of simple and accurate methods to measure the serum levels. The present radioimmunoassay procedure is simple and rapid enough to facilitate the monitoring of serum levels of haloperidol in patients, possibly leading to further insight into the relationships described above. The studies along this line will be reported elsewhere.

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