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ACCUMULATION OF PHENOTHIAZINE TRANQUILIZERS IN RAT BRAIN AND PLASMA AFTER REPEATED DOSAGE*

M. A. MAHJU†‡ and R. P. MAICKEL

Laboratory of Psychopharmacology, Departments of Pharmacology and Psychology, Indiana University, Bloomington, Ind. 47401, U.S.A.

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Abstract—After a single i.p. dose to rats, chlorpromazine, fluphenazine, promazine, triflupromazine and trimeprazine achieve brain/plasma ratios > 1, with half-lives in brain and plasma of < 10 hr. After five doses at 12-hr intervals, brain/plasma ratios of all compounds, except triflupromazine, are greater than after a single dose, due to accumulation in the brain. Triflupromazine appears to reach a maximum limit in the brain at about 11–12 μg/g. The half-lives of promazine and trimeprazine in brain and plasma are greater after five doses than after a single dose, while those of chlorpromazine, fluphenazine and triflupromazine are essentially the same after one or five doses.

SINCE early studies by Salzman and Brodie[1] showed that brain/plasma levels of chlorpromazine in animals were > 1, numerous papers have reported tissue localization of phenothiazine tranquilizers. A recent review[2] emphasized the diversity of results obtained in these studies of physiological disposition. For example, Weschsler and Roizin[3] failed to find measureable quantities of chlorpromazine in tissues of rats given a daily dosage of 6 mg/kg for 14–90 days, while Forrest and Forrest[4] found metabolites of chlorpromazine in the urine of patients as much as several months after cessation of drug therapy. Huang *et al.*[5] reported glucuronides of chlorpromazine in urine 30 weeks after discontinuing the drug. Using ^{35}S-chlorpromazine, Wase *et al.*[6] showed a continuous accumulation of the drug in brain over a 4-day dosage period, while Phillips and Miya[7] found repeated daily dosage of ^{35}S-prochlorperazine to rats for 13 days produced brain levels of the drug approximately double those after a single dose.

However, no comparative studies of drugs under similar experimental conditions have been reported. Any attempt to compare phenothiazines faces differences in species or strains of animals used, in methods of assay, or in experimental design. The present paper reports on the disposition, in brain and plasma, of five phenothiazine derivatives after administration of single or multiple doses to rats. The compounds represent a diversity of structural characteristics, with a spectrum of pharmacological activities from the anti-histaminic trimeprazine to the potent tranquilizer, fluphenazine.

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‡Present address: Department of Pharmacology, Nishter Medical College, Multan, Pakistan.

MATERIALS AND METHODS

All experiments used adult, male Sprague–Dawley rats (300–400 g) obtained from Hormone Assay Laboratories, Chicago, Ill., and maintained on Purina lab chow and tap water *ad lib.* for at least 7 days prior to experimental use, as well as during the course of the experiments. Animals were sacrificed by decapitation. Blood was collected into heparinized beakers, transferred to tubes and centrifuged. Plasma was removed and stored (at $-10°$) until use. Brains were removed, rinsed with distilled water, blotted dry and stored at $-10°$ until assay.

Drugs were given by i.p. injection as aqueous solutions of their salts, at dosages selected on the basis of similarity to clinical dose and lack of toxicity: chlorpromazine 18·0 mg/kg, fluphenazine, 8·6 mg/kg, promazine, 35·4 mg/kg; trifluprazine, 13·6 mg/kg; and trimeprazine, 13·3 mg/kg, all expressed as free-base weight. All chemicals used were reagent grade. Drugs were determined by separation of unchanged drug from tissues by a modification of the extraction procedure of Salzman and Brodie,[1] followed by fluorescent determination as described by Ragland and Kinross-Wright.[8]

Tissues were homogenized in 3 vol. of 0·01 N HCl using a motor driven Teflon pestle and glass homogenizer tube (Polytechnical Products). After transfer of 2·0 ml of the homogenate to 13-ml glass-stoppered centrifuge tubes, 0·5 ml of 10·0 N sodium hydroxide was added and the tubes were mixed with a vibrating mixer. After addition of 6·0 ml of *n*-heptane (Phillips Petroleum Co. "Pure", 99 Mol % minimum) containing 1·5% isoamyl alcohol, the tubes were stoppered, mechanically shaken for 5 min (IEC Bottle Shaker) and centrifuged for 5 min at 2000 rpm. An aliquot (5·0 ml) of the heptane phase was transferred to a centrifuge tube containing 2·0 ml of acetate buffer, 0·1 M, pH 5·6. These tubes were stoppered, shaken for 5 min and centrifuged for 5 min, after which 4·0 ml of the organic solvent phase was transferred to another centrifuge tube containing 2·0 ml of 50% acetic acid. After shaking for 5 min, the tubes were placed in the freezer for 10 min. The tubes were then shaken with a vibrating mixer and centrifuged. The organic solvent phase was removed by aspiration and discarded; 1·0 ml of the acid phase was transferred to 13 × 100 mm test tubes and 0·2 ml of 30% H_2O_2 added. After mixing with a vibrating mixer, the tubes were placed in a boiling water bath for 10 min, then removed and placed in cool tap water for 5 min. Fluorescence was measured in an Aminco–Bowman spectrophotofluorometer using 1-cm Quartz cuvettes. The instrument was equipped with a 1P28 phototube, activation and emission slits set at 3·0 mm and the phototube slit width was set at 1·0 mm. Activation and emission wavelengths (in mμ) for the various drugs were: chlorpromazine (330–380), promazine (330–370), trifluprazine (340–400), fluphenazine (340–400), and trimeprazine (330–370).

The procedure for the estimation of phenothiazines in plasma was almost the same as for brain tissues. Plasma samples (1·0 ml) were diluted with 1·0 ml of distilled H_2O and carried through all the steps except that these samples were not placed in the freezer for 10 min. Along with each set of determinations a sample blank was prepared by carrying a sample of brain tissue or plasma obtained form a control animal. In addition, tissue standards, water standards and a water blank were also run at the same time. A blank consisting of 50% acetic acid and H_2O_2 which had been heated was also read with each set of samples.

Recoveries of drug added to plasma *in vitro* were 90–95 for fluphenazine and trifluprazine and 98–102 per cent for chlorpromazine, promazine and trimeprazine.

Recoveries of drug added to brain *in vitro* were 85–90 per cent for chlorpromazine and trimeprazine, and 98–102 per cent for promazine. These recovery values are considerably higher than those initially reported by Salzman and Brodie[1]. Although no specific explanantion can be given, several possibilities must be considered. The increased sensitivity of the oxidation-fluorescence assay over ultraviolet absorbance is > 10-fold yet tissue blanks in the former assay are < 20 per cent of those in the latter. In addition, we are using a smaller volume of solvent and shorter shaking time, thus reducing the likelihood of oxidative destruction of the drugs during the analytical procedure. Sensitivity limits for accurate measurement (in μg/ml or μg/g) were: trifluprazine, 0·10; fluphenazine, 0·12; promazine, 0·17; trimeprazine, 0·21; and chlorpromazine, 0·44.

Specificity of the method was confirmed in random samples by use of thin-layer chromatography as described by Baumler and Rippstein.[9] Using silica gel G plates and a developing system of methanol : acetone : triethanolamine (50 : 50 : 1·5), only a single spot was observed in ultraviolet light corresponding in R_f to authentic drug, when the final acid extract was applied to the plate. By contrast, acetone deproteinized extracts of plasma or brain yielded at least three fluorescent spots under similar conditions. Similarly, > 90 per cent of the material after oxidation appeared as the sulfoxide by TLC.

Gross behavior was estimated by a modification of the system reported by Maickel[10] in an open field (36 in. × 36 in., with floor divided into 6 in. × 6 in. squares) and in home cages (6 in. × 12 in.). Observers were unaware of the drugs given to each animal; animals were identified by number only. Observations were made by three observers at 40, 60, 100 and 120 min after drug administration; the values give by the observers at 40 and 60 min and at 100 and 120 min were then averaged; these means were used to obtain the data in Table 1.

TABLE 1. DEPRESSANT EFFECTS OF PHENOTHIAZINE DERIVATIVES ON GROSS BEHAVIOR OF RATS*

Compound	Time (min)	Number of doses				
		1	2	3	4	5
Saline	60	2·0	2·5	1·7	2·0	2·2
	120	2·2	2·0	2·0	1·8	2·0
Chlorpromazine	60	0·2	0·3	0·2	0·2	0·3
	120	0·0	0·2	0·2	0·3	0·2
Fluphenazine	60	0·5	0·5	0·3	0·5	0·3
	120	0·5	0·3	0·5	0·5	0·5
Promazine	60	0·3	0·5	0·3	0·3	0·2
	120	0·3	0·3	0·3	0·5	0·5
Trifluprazine	60	0·3	0·5	0·5	0·8	1·3
	120	0·5	0·5	0·8	1·5	1·7
Trimeprazine	60	0·8	1·0	1·3	1·3	1·5
	120	1·0	1·0	1·3	1·7	1·5

*Drugs were given at the doses in the text and gross behavior was estimated as described in Methods.

Groups of four rats were given various dosages of drugs and rated, using an arbitrary scale of 0–3 as follows: 0 equals completely sedated, no motor activity; 1 equals less active than normal, some sedation; 2 equals normal exploratory activity (animal

moved around open field at random; rate approximately 5 squares in 5 min; 3 equals slight hyperactivity, increased exploration.

RESULTS

Determination of partition coefficients of phenothiazine derivatives. The distribution of the compounds used in this study was determined as described by Mayer *et al.*[11] using pH 7·4 phosphate buffer as the aqueous phase, and benzene, chloroform or *n*-heptane as the organic phase. The results (Table 2) indicate generally high lipid

TABLE 2. PARTITION COEFFICIENTS OF PHENOTHIAZINE DERIVATIVES*

Compound	Partition "K" for solvent		
	n-heptane	Benzene	Chloroform
Trifluprom azine	670 (1)	340 (1)	4750 (3)
Trimeprazine	310 (3)	790 (2)	3215 (4)
Chlorpromazine	370 (2)	500 (3)	1530 (5)
Fluphenazine	80 (4)	430 (4)	6660 (2)
Promazine	70 (5)	360 (5)	$>10^4$ (1)

*Distribution of compounds between organic phase and pH 7·4 phosphate buffer was performed as described by Mayer *et al.*[11] Each value is the mean of four separate determinations. Rank in each solvent is given in parenthesis.

solubility for all of the compounds, with some variability in rank order in the different solvents. Agreement between *n*-heptane and benzene is good, but with chloroform the apparent partition of fluphenazine and promazine is much greater. For all of the drugs however, the partition values increased with increasing polarity of the organic phase, i.e. chloroform > benzene > *n*-heptane.

Time course of brain and plasma levels of phenothiazine derivatives after a single dose. Measuring the brain and plasma levels of the various drugs at 1, 4, 8 and 12 hr after a single i.p. dose gives the data in Table 3. All of the compounds achieve within 1 hr, and maintain for at least 12 hr, brain/plasma ratios > 1. The lowest ratios (< 5·0) are seen with chlorpromazine at 4–12 hr; the highest ratios are seen with trifluprom azine (> 25·0) at 4 and 8 hr after dosage. In the case of chlorpromazine and fluphenazine, the ratios at the 1-hr point are higher than at the other time points.

Plotting this data on semi-logarithmic paper yields curves that fit the kinetics of first-order decay in both brain and plasma. The slopes of the best straight lines were obtained by computer regression analysis and used to estimate the half-lives of each drug in brain and plasma (Table 3). Chlorpromazine has the longest half-life in brain (7·3 hr) and in plasma (9·1 hr), while trifluprom azine has the shortest half-life, 4·3 hr in brain and 5·1 hr in plasma.

Time-course of brain and plasma levels of phenothiazine derivatives after multiple dosage. The levels of the various drugs in rat brain and plasma at 2, 4 and 8 hr after administration of five doses, spaced at 12-hr intervals, are seen in Table 4. The brain/plasma ratios, with the exception of trifluprom azine, are all higher than those obtained after a single dose. Comparison of the 4- and 8-hr time points after five doses with similar time points after a single-drug dose indicates markedly increased brain levels

TABLE 3. TIME-COURSE OF BRAIN AND PLASMA LEVELS OF PHENOTHIAZINE DERIVATIVES AFTER A SINGLE DOSE IN RATS*

Compound		Hours after dosage 1	4	8	12	Half-life (hr)
Chlorpromazine	B	15·50 ± 1·30	11·03 ± 1·46	7·12 ± 0·44	5·51 ± 0·92	7·3
	P	2·51 ± 0·21	2·24 ± 0·41	1·54 ± 0·40	1·13 ± 0·19	9·1
	B/P	6·15	4·91	4·62	4·69	
Fluphenazine	B	4·72 ± 0·41	3·11 ± 0·56	1·97 ± 0·28	1·07 ± 0·35	4·9
	P	0·80 ± 0·80	0·61 ± 0·08	0·38 ± 0·08	0·21 ± 0·19	5·8
	B/P	5·90	5·10	5·17	5·08	
Promazine	B	24·61 ± 0·41	17·22 ± 1·50	11·10 ± 1·07	6·49 ± 1·04	5·8
	P	2·49 ± 0·11	1·70 ± 0·24	1·20 ± 0·28	0·70 ± 0·04	6·2
	B/P	9·88	10·1	9·24	9·25	
Triflupromazine	B	11·20 ± 1·16	8·09 ± 0·51	4·21 ± 1·06	2·05 ± 0·33	4·3
	P	0·45 ± 0·09	0·28 ± 0·04	0·15 ± 0·06	0·10 ± 0·02	5·1
	B/P	24·8	28·7	28·1	20·5	
Trimeprazine	B	9·26 ± 1·36	6·83 ± 1·01	4·06 ± 0·81	2·74 ± 0·21	5·8
	P	0·81 ± 0·11	0·54 ± 0·14	0·35 ± 0·02	0·25 ± 0·07	6·3
	B/P	11·4	12·6	11·6	10·9	

*Drugs were given i.p. at dosages listed in the text. Each value is mean ± S.E.M. of brain (B, μg/g) or plasma (P, μg/ml) levels of drug in six rats killed at the times indicated after dosage.

TABLE 4. TIME-COURSE OF BRAIN AND PLASMA LEVELS OF PHENOTHIAZINE DERIVATIVES AFTER FIVE DOSES IN RATS*

Compound		Hours after fifth dose 2	4	8	Half-life (hr)
Chlorpromazine	B	29·41 ± 2·01	24·02 ± 3·17	15·70 ± 1·09	6·5
	P	2·29 ± 0·18	1·80 ± 0·24	1·26 ± 0·33	7·8
	B/P	12·9	13·3	12·5	
Fluphenazine	B	18·64 ± 1·04	15·50 ± 0·93	8·64 ± 1·11	4·8
	P	1·60 ± 0·09	1·43 ± 0·31	0·91 ± 0·19	7·1
	B/P	11·6	10·8	9·52	
Promazine	B	39·99 ± 2·68	37·06 ± 3·11	31·04 ± 4·67	16·0
	P	1·83 ± 0·17	1·65 ± 0·31	1·20 ± 0·43	9·7
	B/P	21·8	22·5	25·8	
Trifluprazine	B	12·06 ± 0·81	6·93 = 1·10	3·65 ± 0·14	3·5
	P	1·09 ± 0·14	0·63 ± 0·19	0·31 ± 0·08	3·6
	B/P	11·0	11·0	12·2	
Trimeprazine	B	30·65 ± 1·41	29·17 ± 2·64	27·93 ± 2·16	30·5
	P	2·19 ± 0·11	2·01 ± 0·11	1·80 ± 0·14	25·1
	B/P	14·9	14·5	15·5	

*Drugs were given i.p. at dosages listed in the text, repeated at 12-hr intervals. Each value is the mean ± S.E.M. of brain (B, μg/ml) or plasma (P, μg/ml) levels of drug in six rats killed at times indicated after the fifth dose.

of all drugs except trifluprazine. However, similar comparisons of the plasma levels show those of fluphenazine, trifluprazine and trimeprazine to be higher after the multiple dosage, while promazine is virtually identical and chlorpromazine is lower after multiple dosage.

Plots of the data in Table 4 also fit the kinetics of first-order decay curves. The half-lives in brain and plasma, given in Table 4 show chlorpromazine, fluphenazine and trifluprazine to differ only slightly from those obtained after a single-drug dose. The plasma half-life of promazine increases by only 56 per cent, while the brain half-life of this compound is increased almost 3-fold, from 5·8 to 16·0 hr. The effects of the multiple dosage schedule on the values for trimeprazine is even more pronounced; multiple dosage increases the plasma half-life about 4-fold (from 6·3 hr to 25·1 hr) while the brain half-life is lengthened by a factor of slightly more than five, from 5·8 hr to 30·5 hr.

Brain and plasma levels of various phenothiazine derivatives after single or multiple dosage. Table 5 shows the data obtained 2 hr after administration of one to five doses of the compounds. A cursory glance at the data suggests several trends. With the exception of fluphenazine, all of the compounds appear to continue accumulating in brain with each successsive dose. The levels of fluphenazine in brain do not increase significantly after the third dose; in one set of animals given eight doses of trifluprazine at 12-hr intervals, the brain level was 10·57 μg/g, virtually identical to the level after three, four or five doses.

Examination of the plasma levels with cumulative dosing also shows several patterns of accumulation. For all the compounds except promazine, plasma levels 2 hr after each dose continue to rise. However, promazine reaches a peak plasma level after two to three doses, then levels off and even begins to show a falling tendency by the fifth dose.

TABLE 5. ACCUMULATION OF PHENOTHIAZINE DERIVATIVES IN RAT BRAIN AND PLASMA AFTER ONE TO FIVE DOSES*

Compound		Number of doses				
		1	2	3	4	5
Chlorpromazine	B	13·03 ± 1·03	14·98 ± 3·74	17·43 ± 1·06	20·67 ± 2·86	24·01 ± 2·01
	P	1·67 ± 0·21	1·85 ± 0·19	1·95 ± 0·33	2·13 ± 0·21	2·26 ± 0·19
	B/P	7·80	8·09	8·93	9·71	10·6
Fluphenazine	B	5·03 ± 0·76	6·99 ± 0·47	10·06 ± 1·06	11·51 ± 0·67	14·87 ± 1·02
	P	0·71 ± 0·09	0·79 ± 0·06	0·90 ± 0·10	1·03 ± 0·06	1·35 ± 0·06
	B/P	7·08	8·83	11·1	11·1	11·0
Promazine	B	20·63 ± 1·79	28·28 ± 5·28	33·23 ± 2·14	36·01 ± 3·98	39·67 ± 4·72
	P	2·06 ± 0·46	2·23 ± 0·32	2·30 ± 0·41	2·15 ± 0·19	1·87 ± 0·11
	B/P	10·0	12·7	14·4	16·8	21·2
Triflupromazine	B	9·63 ± 0·91	10·06 ± 0·81	11·09 ± 0·93	11·75 ± 1·02	11·13 ± 0·80
	P	0·39 ± 0·09	0·43 ± 0·05	0·68 ± 0·10	0·89 ± 0·21	1·01 ± 0·29
	B/P	24·7	23·4	16·3	13·2	11·0
Trimeprazine	B	0·84 ± 0·85	13·11 ± 1·50	17·92 ± 1·86	22·67 ± 1·03	30·21 ± 1·73
	P	0·84 ± 0·14	1·06 ± 0·14	1·28 ± 0·19	1·63 ± 0·31	2·06 ± 0·13

*Doses (see text) were given i.p. at 12-hr intervals. Each value is the mean ± S.E.M. of brain (B, μg/g) or plasma (P, μg/ml) levels of drug in four rats killed 2 hr after administration of the last dose.

Depressant effect of single or multiple dosage of phenothiazine derivatives. The data in Table 1 give some estimation of the effects of repeated dosage on the tranquilizing action of the various compounds tested. Although these observations are limited by the rating system used and by the complexities associated with mere visual observation, the data do show several interesting trends. None of the drugs shows an increased potency with repeated dosage, although this would be the most difficult aspect to measure. However, in the case of triflupromazine and trimeprazine, the animals seem to show an adaptive phenomena; with succeeding doses the drugs have less sedative effect.

DISCUSSION

In a recent review, Maickel[12] re-emphasized the necessity of determining the physiological disposition of psychoactive drugs. Although reports of the physiological disposition of phenothiazine tranquilizers are consistent in showing accumulation of the drugs in adrenals, kidney, liver and lung,[1, 3, 13–17] discrepancies exist in the localization in brain. Some papers report high levels in brain,[1, 14–16] while others report brain/plasma values close to 1·0.[3, 13] A similar uncertainty surrounds the distribution studies of the other phenothiazine derivatives,[2] probably due to the diversity of species, techniques and experimental designs used.

Studies of the brain and plasma levels of the compounds reported here after a single dose to rats show clear localization in brain, with brain/plasma ratios ranging from 4·62 to 28·7 (Table 3). It may be pertinent to this single-dose distribution phenomenon that triflupromazine, the compound with the highest lipid partition tendency in two solvents (Table 2), also achieves the highest brain/plasma ratio, suggesting some correlation with lipid partition, not unlike the thiobarbiturates. The half-lives of the various compounds in brain and plasma after a single dose are rather similar (Table 3); brain half-lives range from 4·3 to 7·3 hr, while those for plasma range from 5·1 to 9·1 hr. Plots of the brain and plasma levels semi-logarithmically against time yield straight line decay curves, characteristic of first-order kinetics.

A similar study of the decay of brain and plasma levels of the compounds after administration of five doses at 12-hr intervals demonstrates the accumulation of the drugs in brain (Table 4), as brain/plasma concentration ratios range from 9·52 to 25·8. Semi-logarithmic plots of plasma and brain levels against time after the fifth dose again yield characteristic first-order decay curves. The half-life values for chlorpromazine, fluphenazine and triflupromazine in brain and plasma do not differ greatly whether measured after one dose or five doses (Tables 3 and 4). However, after five-drug doses, the plasma half-life of promazine is increased by about 60 per cent, while the brain half-life of this compound is increased by 176 per cent. Similarly, the plasma half-life of trimeprazine after five doses is 298 per cent greater than that after a single dose; the brain half-life after five doses is 425 per cent greater than after one dose. The changes observed in the half-lives of these compounds may reflect adaptive changes occurring in binding processes, a possibility awaiting further study.

The plasma and brain levels of these phenothiazine derivatives in rats given one to five doses of the drugs at 12-hr intervals (Table 5) show the plasma levels of drug to rise continuously for all the compounds except promazine. In this latter case, plasma levels rise for the first three doses, then decline progressively after doses 4 and 5. In contrast, the brain levels of all the compounds except triflupromazine continue to rise

progressively with each succeeding dose. The results with trifluprομazine demonstrate an apparent leveling-off at brain concentrations of 11–12 ug/g, suggesting possible saturation of binding mechanisms, as plasma levels continue to rise. These differences in accumulation tendencies are reflected in the brain/plasma ratios after each successive dose. Promazine shows a steady increase, from 10·0 to 21·2, while chlorpromazine increases at a more gradual rate, from 7·8 to 10·6. Fluphenazine and trimeprazine have similar patterns; the former increases from 7·1 to a plateau of 11·0 to 11·1 after three doses, while the latter drug starts at 9·5 and plateaus at 13·9 to 14·7 after three to five doses. Because of the plateau reached by brain levels of trifluprομazine, the brain/plasma values for this drug start at 24·7 and decrease to 11·0 after the fifth dose.

Despite the tendency of all of these compounds to accumulate in brain, none of them showed increased sedative effects with repeated dosage (Table 1). The effects of chlorpromazine, fluphenazine and promazine were relatively constant over the dosage period. In the case of trifluprομazine and trimeprazine, the sedative effects of the drugs became less pronounced with successive doses.

Presumably, the accumulation of these drugs represents binding to tissue constituents, a phenomenon suggested by many since the first studies of chlorpromazine distribution.[1] The observations presented herein on lack of increased tranquilizing effect despite increased brain levels of the various compounds suggests that these high levels represent binding to sites other than those having receptor potential. For example the compounds may be accumulating in brain areas not concerned with behavioral functions being measured in a specific test. However, one cannot eliminate the possibility of some sort of neuronal adaptation because of continued exposure to the drug.

In addition, one must consider possible changes in metabolism of the phenothiazine derivatives with repeated dosage. It is not unreasonable to assume that repeated doses of the phenothiazine derivatives may cause changes in the qualitative or quantitative pattern of drug metabolism,[17] or changes in absorption or distribution. In this regard it is of interest that chlorpromazine,[18–20] promazine[20] and trifluprομazine[18, 19] have shown to increase liver microsomal drug enzyme activity. The implications of such drug-induced changes in drug metabolism have been discussed in a recent review by Conney.[21] Further work is required to accurately assess the role of drug metabolism changes in the accumulation of the phenothiazine derivatives.

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