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THIN-LAYER CHROMATOGRAPHY OF CHLORPROMAZINE METABOLITES

ATTEMPT TO IDENTIFY EACH OF THE METABOLITES APPEARING IN BLOOD, URINE AND FECES OF CHRONICALLY MEDICATED SCHIZOPHRENICS*

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SUMMARY

Data are presented for thin-layer chromatographic behavior of chlorpromazine and thirty-five of its metabolites, as well as for the thin-layer chromatographic behavior of forty-two still unidentified metabolites found free or as glucuronides in plasma, erythrocytes, urine or feces. Tables are given on the frequency of occurrence of each compound in each source. The presence and identification of several new phenothiazine metabolites and their methoxylated analogs are reported.

INTRODUCTION

The metabolic fate of chlorpromazine (CPZ)*** has been explored with increasing specificity and precision since 1954[1,2]. The possibility that its metabolism would be related to its clinical effects has always been under consideration. FORREST *et al.*[3] have calculated that there are a theoretical 168 metabolites derived by formation of sulfoxides, by hydroxylation, by N-oxidation, by mono- and didesmethylation, by oxidation of the side-chain and by conjugation with glucuronic acid or with sulfate. POSNER *et al.*[4] raised the possibility that oxidation at positions 3 and 8, as

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*** For abbreviations of the various phenothiazine derivatives studied, see APPENDIX.

well as dihydroxylation, might occur. DALY AND MANIAN[5] reported on the evidence for occurrence of O-methylation of one of the two hydroxy groups in 7,8-di-OH-CPZ. GOLDENBERG AND FISHMAN[6] have reviewed the field up to 1970.

For the separation, identification, and quantification of the individual metabolites, thin-layer chromatography (TLC) has become the technique of widest applicability, which moreover permits gas chromatography and mass spectrometry on the isolated compounds. This report summarizes the chromatographic data on 35 standards of phenothiazines, each of which is a proven or potential metabolite of chlorpromazine or promazine, and compares this with the chromatographically located metabolites found, during several years of work, in blood, urine, and feces of chronic schizophrenics on chlorpromazine and no other drugs.

EXPERIMENTAL

Materials

Standards were generously supplied by the Psychopharmacology Research Branch, National Institute of Mental Health, and by J. Cymerman Craig, Ph.D. of the School of Pharmacy, San Francisco Medical Center, San Francisco, Calif.

Blood, urine and feces of chronic schizophrenics were obtained in the course of therapy with chlorpromazine as Thorazine® liquid concentrate.

TLC materials have been previously described[7]. TLC plates of various manufacturers have been used from time to time but this report will deal only with Brinkmann F_{254} Silica gel plates, 250 mμ thick, 20 × 20 cm.

Methods

Blood plasma and erythrocytes have been separately extracted by methods previously described[7–9].

Urine, collected in glass, frozen at once and stored at −10 to −20° until analysis, has been treated in a variety of ways. In every instance individual specimens were analyzed for non-phenolic and phenolic metabolites according to a previously published method[10] and an aliquot taken for extraction contained 5 μmoles of total metabolites. In some instances apple-jacked urine was extracted. No material which had been subjected to adsorption on charcoal or ion-exchange resin was used in preparing this report. To obtain all of the identifiable metabolites from urine it was essential to conduct extraction serially at various pH levels; adequate extractions were made first with three volumes of dichloromethane (DCM) at pH 12 thrice, then at pH 8.5 with three volumes of DCM–isoamyl alcohol (98.5:1.5) (DCMI), and finally thrice at pH 2 with three volumes of DCM. These extractions were followed by hydrolysis. β-Glucuronidase (Ketodase, Warner-Chilcott) for 48 h permitted total extraction by DCM of the remaining metabolites, first at pH 12, then at pH 8.5 with DCMI. Traces of further acidic metabolites were occasionally found at pH 2, particularly in apple-jacked concen trates[11].

Plasma has been extracted by the method of CURRY[12], by the same method modified by use of DCM, or by ether extraction, both before and after β-glucuronidase hydrolysis. Extracts have been prepared at pH 12, 10, 9 and 8.5. Within these variations no real qualitative differences have been found. Quantitatively, no higher

yields of CPZ and each of the metabolites present have been recovered than at pH 9–9.5 with DCMI.

Erythrocytes have been hemolyzed and extracted also in a variety of ways, including the ether method of ZINGALES[13,14], both before and after β-glucuronidase hydrolysis.

Feces have been extracted by ethanol and ether until a further extraction yields no color reaction with 10^{-3} *M* $FeCl_3$ in 18 *N* H_2SO_4, and when no other means of extraction, before or after hydrolysis, produces a substance acting like a phenothiazine. Separation from non-phenothiazine compounds has been carried out in a variety of ways, which we propose to report on at a later date.

In every instance, extracts have been finally brought into methanol or ethanol

TABLE I

AVERAGE R_F RANGE OF CPZ DERIVATIVES

Compounds identified by number, see APPENDIX.

Compound	*Solvent*			
	I	*III*	*II*	*III*
After two-dimensional chromatography				
1	0.33–0.42	0.6 –0.68	0.26–0.32	0.73–0.80
2	0.22–0.28	0.38–0.44	0.20	0.55–0.6
3	0.17–0.20	0.35–0.43	0.14–0.17	0.45–0.5
4	0.08	0.2 –0.25	0.08–0.1	0.26–0.30
5	0.54–0.6	0.5 –0.6	0.46–0.50	0.6 –0.8
6	0.42–0.46	0.26–0.35	0.35–0.38	0.46–0.48
7	0.04	0.2 –0.24	0.02–0.06	0.2 –0.25
8	0.02	0.08–0.12	0.02–0.04	0.16–0.20
9	0.39–0.42	0.54–0.56	0.26–0.34	0.56–0.62
10	0.20–0.22	0.25–0.28	0.20–0.25	0.22–0.27
11	0.11–0.14	0.29	0.11–0.15	0.27–0.33
12	0.06–0.07	0.02–0.07	0.06–0.08	0.05–0.09
13	0.48–0.51	0.42–0.45	0.43–0.48	0.4 –0.5
14	0.38–0.42	0.1 –0.12	0.38–0.43	0.08–0.12
16	0.39–0.42	0.47	0.27–0.33	0.47–0.51
After one-dimensional chromatography				
15	0.28–0.32		0.30–0.34	0.77–0.81
17	0.27–0.33		0.18–0.22	streak
18	0.27–0.33		0.27–0.33	0.43–0.48
19	0.27–0.33		0.27–0.33	0.26–0.30
20	0.25–0.3		0.28–0.33	0.76–0.80
21	0.25–0.3		0.26–0.3	streak
22	0.27–0.31		0.28–0.32	0.77–0.81
23	0.17–0.22		0.14–0.19	0.20–0.25
24	0.4 –0.45		0.47–0.52	0.25–0.3
25			0.78–0.83	0.77–0.82
26			0.77–0.81	0.98–1.0
27			0.50–0.55	0.95–1.0
28			0.84–0.89	0.95–1.0
29	0.83–0.87		0.75–0.8	0.93–0.98
30	0.98–1.0		0.85–0.9	0.98–1.0
31	0.98–1.0		0.98–1.0	0.98–1.0
32	0.09–0.13		0.02–0.04	0.40–0.45
33	0.95–0.98		0.78–0.82	0.98–1.0
34			0.60–0.65	0.80–0.85
35	0.88–0.92		0.87–0.91	0.95–1.0

for spotting on TLC plates, as has been described by TURANO AND TURNER[7]. Best separations were obtained by development in two dimensions with the following solvent systems: (I) Acetone–methanol–triethanolamine (100:30:1); (II) acetone–methanol–ammonia (100:40:1); (III) isopropyl–alcohol 95% ethanol–ammonia (11:1:1) .Solveuts I and II were first dimension alternates. Solvent III was used for the second dimension.

Identification has been in the first instance dependent upon use of synthetic standards run side-by-side with unknowns. Occasionally a standard has been added to an aliquot of unknown, particularly when a spot suggested presence of two components. Metabolites have been calculated as R_F values in the usual way, as well as with reference to an internal standard, R_s.

Examination of plates has been by direct examination in visible and under UV light before and after spraying with the alcohol–H_2SO_4–$FeCl_3$ reagent of WECHSLER AND FORREST[15] and after heating the sprayed plates.

In some instances gas chromatography (GC) has been used in parallel with TLC to follow the breakdown of highly unstable compounds such as 7,8-di-OH–CPZ.

TABLE II

MOBILITIES ON SILICA GEL TLC PLATES OF CPZ AND 35 METABOLITES RELATIVE TO EACH OF THE FOUR INTERNAL STANDARDS (R_s)

See APPENDIX for compound corresponding to each number.

Compound	R_{CPZ}			$R_{7\text{-}OH\text{-}CPZ}$			$R_{7\text{-}OH\text{-}CPZSO}$			$R_{7\text{-}OH\text{-}Nor_8CPZSO}$		
Solvent	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
1				1.03	0.94	1.37	3.1	1.6	3.86	1.9	0.71	10.63
2	0.71	0.69	0.67	0.91	0.65	0.92	2.2	1.1	2.59	0.85	0.49	7.12
3	0.9	0.53	0.55	0.93	0.5	0.76	2.8	0.85	2.14	1.08	0.38	5.88
4	0.55	0.28	0.27	0.57	0.26	0.37	1.7	0.45	1.05	0.65	0.2	2.88
5	1.87	1.56	0.66	1.93	1.47	0.90	5.8	2.5	2.55	2.23	1.1	7.00
6	1.32	1.19	0.32	1.37	1.12	0.44	4.1	1.9	1.23	1.58	0.84	3.38
7	0.16	0.13	0.28	0.17	0.12	0.39	0.5	0.2	1.09	0.19	0.09	3.00
8	0.06	0.06	0.12	0.07	0.06	0.16	0.2	0.1	0.45	0.08	0.04	1.25
9	0.97	1.06	0.73				3.0	1.7	2.82	1.15	0.76	7.75
10	0.32	0.63	0.26	0.33	0.59	0.35				0.38	0.44	2.75
11	0.42	0.47	0.32	0.43	0.44	0.43	1.3	0.75	1.23	0.5	0.33	3.37
12	0.19	0.19	0.06	0.2	0.18	0.08	0.6	0.3	0.23	0.23	0.13	0.63
13	1.29	1.69	0.4	1.33	1.59	0.55	4.0	2.7	1.54	1.54	1.2	4.25
14	0.84	1.4	0.09	0.87	1.3	0.13	2.6	2.3	0.36			
16	0.97	1.03	0.6	1.0	0.97	0.8	3.0	1.65	2.3	1.15	0.73	6.38
30	3.23	2.72	1.18	3.3	2.56	1.61	10.0	4.35	4.55	3.85	1.93	12.5
31	3.25	3.13	1.18	3.3	2.94	1.6	10.0	4.35	4.55	3.85	1.93	12.5
33	3.1	2.5	1.18	2.8	2.35	1.6	9.6	4.0	4.5	3.7	1.78	12.5
32		0.06			0.06			0.1			0.04	
17		0.06			0.06			0.1			0.04	
23		0.5			0.47			0.8			0.36	
22		0.94			0.88			1.5			0.67	
18		0.94			0.88			1.5			0.67	
19		0.94			0.88			1.5			0.67	
20		0.97			0.91			1.55			0.69	
21		0.88			0.82			1.4			0.62	
15		1.0			0.94			1.6			0.71	
24		1.53			1.44			2.45			1.09	
29		2.43			2.29			3.9			1.73	
35		2.78			2.62			4.45			1.98	

RESULTS

Table I lists the R_F values in each of the three solvent systems described above of all 35 synthetic phenothiazines which we had available. Table II lists mobilities for the non- and monohydroxylated compounds, relative to an internal standard (R_s) in each of the three solvents (the standards were CPZ, 7-OH-CPZ, 7-OH-CPZSO, and 7-OH-Nor_2CPZSO); and for the dihydroxylated substances their R_s in Solvent II.

Some standards and their derivatives changed character more or less rapidly in solution. Tables III and IV condense results of examinations of these unstable compounds, chiefly by recording observations: (1) in long and short wave UV before spraying with any reagent; (2) by color of each spot in visible light before and after spraying with the color developing reagent described above, but before heating; and (3) by color in visible light after heating the sprayed plate.

Table IV, which details the characteristics of each of the metabolic compounds, is an appalling sight, but workers with these compounds must experience vast frustration and confusion, which this table may alleviate. This table requires some explanation. The pure compounds were dissolved in absolute ethanol previously purged with N_2. The solutions were immediately spotted under N_2 and run in sandwiches, and again spotted at intervals of hours or days thereafter. Each standard was made up repeatedly, with or without N_2, spotted with or without N_2. All were run in the dark, at temperatures varying from 22° to 30°. Previous work had revealed no effects on R_F, on resolution, or breakdown of other standards[7] by change of room temperature or humidity.

On the first run of the dihydroxylated compounds a variable number of spots appeared, and these tended strongly to increase in number and intensity on the first 24–48 h, even with the solution maintained at −20° in the dark. Further, the presence of the unstable compounds are given in Table IV, columns 2 and 3. In columns 2 and 3 are also given the R_F values in Solvent I (first dimension) and Solvent III (second dimension). Each value throughout the table is the average of at least five chromatographic runs. Columns 4 and 5 give the mobilities referred to CPZ (R_s), columns 6 and 7 to (9)*; 8 and 9 to (10); and 10 and 11 to (14). The colors given under column 1 refer to color in visible light after heating the sprayed plate. LUV refers to a spot, usually fluorescent, seen under long wave UV before heating. Superior [a] indicates the main spot due to parent compound. SF refers to solvent front. Frequently a spot other than that due to the parent compound was seen only once or twice in five or more experiments; these are identified by the symbols 1 × or 2 ×.

Table V provides data on GC of the unstable compounds. GC was carried out with a Packard gas chromatograph**, 8-ft. glass column, packed with 3% OV-17 using N_2 as carrier gas, and a flame ionization detector. It was programmed to start at 250° for 1 min, then to rise 2.5°/min to maximum 285°, and hold. Sensitivity was 1×10^{-10}. Twice the runs were made as soon as the standard was prepared in ethanol; a third time the run was made 8 days later. Major peaks are underlined. It appears

* Numbers in brackets refer to the numbered compounds in the APPENDIX.

** Dual flow controller, Model 824, temperature point programmer Model 846, deviation temperature controller Model 873, dual electrometer Model 843, and Dual Bipolar HV Supply Model 834.

TABLE III

SOME CHARACTERISTICS OF DIHYDROXY DERIVATIVES OF CPZ ON TLC, AFTER DEVELOPMENT IN SOLVENTS I AND III

Compound	*Long wave UV*	*Solvent I*				*Solvent III*		
		Visible before spray	*After spray and before heat*	*After spray and after heat*	*Long wave UV*	*Visible before spray*	*After spray and before heat*	*After spray and after heat*
24	bright rose			purple	bright rose	gray-pink	green to purple	purple
23	not visible			purple	not visible	gray-pink	green	purple
31				purple		pink	purple	purple
35	bright rose			1 pink to green 2 pink	peach	peach	yellow-green	pink to green
21	1 light		blue	2–3 blue	blue	blue	lavender	1 lavender
22	2 blue		blue	3 turq. blue			turquoise	1 bright blue
18			green to blue	purple		peach	lavender	purple
32	streak blue to peach			purple	pink	peach	pink-lavender	lavender-pink
17	peach			purple	pink	peach	lavender	lavender-peach
19	light streak		blue to purple	purple		peach	lavender	purple
20	blue		blue	2 purple			turquoise	purple
28				pink				pink
34				pink				pink
26				bright pink				bright pink
27				pink				pink
25	light			purple	light			pink

TABLE IVA

AVERAGE R_F AND R_x VALUES OF ALL SPOTS RESULTING FROM THE CHROMATOGRAPHY OF EACH METABOLITE DISUBSTITUTED WITH CPZ, 7-OH-CPZ, 7-OH-CPZSO AND 7-OH-Nor_3CPZSO IN SOLVENTS I AND III

The presence of one metabolite frequently alters the mobility of others. This is illustrated by the variability of R_F of the four standards seen in this table. It is for this reason the R_x values had to be specifically determined. When many components are present these R_x values remain fairly stable and the relative positions on a plate are constant. Experience will enable an investigator to recognize when there has been "leap frogging". For details, see text.

Standard	*Color*	*R_F value*		*R_x value to*							
				CPZ		*7-OH-CPZ*		*7-OH-CPZSO*		*7-OH-Nor_3CPZSO*	
		1D	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*
[a]1,3-Di-OH-Pr											
CPZ		0.34	0.80								
7-OH-CPZ		0.34	0.65								
7-OH-CPZSO		0.19	0.28								
7-OH-Nor_3CPZSO		0.37	0.11								
	pink	0.26	0.54	0.65	0.71	0.67	0.87	1.23	1.87	0.61	5.1
	pink	0.16	0.46	0.41	0.63	0.52	0.66	0.60	1.62	0.49	4.2
	lavender	0.05	0.47	0.19	0.69	0.20	0.83	0.37	2.1	0.18	5.1
	lavender	0.38	0.45	1.05	0.61	1.07	0.75	1.9	1.8	1.05	4.4
	LUV and purple	0	0.64	0	0.83	0	1.03	0	2.1	0	6.3
	LUV and pink	0	0.29	0	0.4	0	0.5	0	0.9	0	2.7
	LUV and purple	0	0.11	0	0.15	0	0.19	0	0.38	0	0.98
	LUV and purple	0	0	0	0	0	0	0	0	0	0
7,8-Di-OH_3-CPZ											
CPZ		0.29	0.84								
7-OH-CPZ		0.30	0.68								
7-OH-CPZSO		0.18	0.27								
7-OH-Nor_3CPZSO		0.35	0.12								
	pink	0.2	0.64	0.63	0.73	0.6	0.92	1.04	2.11	0.57	5.33
	purple	0.19	0.51	0.64	0.62	0.62	0.81	1.03	1.9	0.55	4.7
	purple	0.19	0.40	0.61	0.47	0.64	0.6	1.07	1.42	0.58	3.6
	purple	0.19	0	0.64	0	0.62	0	1.04	0	0.54	0
	purple	0.07	0.51	0.26	0.61	0.25	0.74	0.42	1.77	0.20	4.45
	Long wave UV	0.2	0.16	0.64	0.23	0.62	0.28	1.1	0.65	0.55	1.6
	Long wave UV	0.2	0.06	0.66	0.08	0.63	0.09	1.07	0.27	0.56	0.65
	pink	0.32	0.55	1.25	0.65	1.3	0.8	1.8	1.9	0.97	4.6
	lavender	0.33	0.27	1.1	0.33	1.1	0.42	1.8	0.97	0.96	2 34
	LUV and 1 purple	0	0.73	0	0.79	0	0.97	0	2.35	0	5.4
	LUV and 1 purple	0	0.31	0	0.34	0	0.40	0	1.0	0	2.3
	LUV and 1 purple	0	0.12	0	0.13	0	0.16	0	0.4	0	1.0
	purple	0	0	0	0	0	0	0	0	0	0
7,8-Di-OCH_3-CPZ											
CPZ		0.33	0.87								
7-OH-CPZ		0.33	0.65								
7-OH-CPZSO		0.21	0.28								
7-OH-Nor_3CPZSO		0.39	0.13								
	purple with 7-OH-CPZ[a]	0.33	0.65	1.0	0.79	1.0	1.0	1.7	2.5	0.84	5.3
	purple[a]	0.33	0.48	0.98	0.54	0.98	0.7	1.6	1.73	0.78	3.9
	pink	0.20	0.7	0.66	0.76	0.67	0.88	1.1	2.35	0.51	5.7
	LUV 1 ×	0.16	0.3	0.54	0.38	0.54	0.49	0.76	1.13	0.45	2.57
	LUV 1 ×	0.9	0.32	2.47	0.35	2.4	0.51	4.27	1.1	2.18	2.3
	LUV 1 ×	0	0.69	0	0.83	0	1.0	0	2.45	0	5.4
	purple 1 ×	0	0.31	0	0.38	0	0.44	0	1.1	0	2.4
	purple 1 ×	0	0.14	0	0.18	0	0.21	0	0.52	0	1.13
3,7-Di-OH-CPZ											
CPZ		0.37	0.82								
7-OH-CPZ		0.37	0.70								
7-OH-CPZSO		0.22	0.27								
7-OH-Nor_3CPZSO		0.38	0.11								

(Continued on p. 284)

TABLE IVA *(continued)*

Standard	*Color*	*R_F value*		*R_x value to*							
				CPZ		*7-OH-CPZ*		*7-OH-CPZSO*		*7-OH-Nor$_2$CPZSO*	
		1D	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*
	purple with 7-OH-CPZ[b]	0.37	0.67	0.96	0.81	0.96	1.0	1.6	2.3	0.9	5.8
	purple 1 ×	0.11	0.52	0.29	0.63	0.3	0.78	0.5	1.9	0.24	4.58
	pink	0.25	0.76	0.68	0.96	0.64	1.1	1.1	2.7	0.55	6.36
	pink on 2 D SF	0.29	1.0	0.87	1.22	0.87	1.4	1.6	3.5	0.81	8.5
	LUV 1 ×	0	0.7	0	0.84	0	1.04	0	2.55	0	6.1
	LUV 1 ×	0	0.31	0	0.36	0	0.44	0	1.1	0	2.9
	LUV 1 ×	0	0.15	0	0.18	0	0.22	0	0.55	0	1.3
	LUV 1 ×	0	0	0	0	0	0	0	0	0	0
7-OH-8-OCH$_3$-CPZ											
CPZ		0.33	0.83								
7-OH-CPZ		0.34	0.69								
7-OH-CPZSO		0.19	0.25								
7-OH-Nor$_2$CPZSO		0.38	0.11								
	purple[a]	0.34	0.50	1.0	0.6	0.99	0.73	1.7	1.07	0.9	4.7
	pink 1 ×	0.25	0.41	0.69	0.63	0.69	0.78	1.2	2.08	0.64	4.9
	lavender 1 ×	0.08	0.45	0.21	0.58	0.21	0.72	0.38	1.9	0.2	4.55
	LUV 1 ×	0	0.65	0	0.84	0	1.04	0	2.77	0	6.55
	LUV 1 ×	0	0.26	0	0.32	0	0.39	0	1.11	0	2.25
	LUV 1 ×	0	0.12	0	0.15	0	0.19	0	0.5	0	1.18
	LUV 1 ×	0	0	0	0	0	0	0	0	0	0
2-Cl-7-OH-8-OCH$_3$Ph											
CPZ		0.34	0.79								
7-OH-CPZ		0.34	0.64								
7-OH-CPZSO		0.34	0.25								
7-OH-Nor$_2$CPZSO		0.37	0.11								
	green[a] (both SF)	1.0	1.0	3.03	1.25	3.03	1.55	5.04	4.11	2.75	9.58
	pink	0.24	0.54	0.67	0.67	0.70	1.81	1.5	2.6	0.56	6.3
	pink 1 ×	0.34	0.50	1.07	0.58	1.07	0.68	1.8	1.88	0.96	4.29
	lavender and LUV	0.07	0.47	0.24	0.57	0.24	0.69	0.39	1.85	0.22	4.7
	LUV and lavender	0	0.69	0	0.81	0	0.98	0	2.65	0	6.11
	LUV and lavender	0	0.30	0	0.31	0	0.38	0	1.01	0	2.34
	lavender	0	0.11	0	0.13	0	0.16	0	0.43	0	1.0
	lavender	0	0.01	0	0.01	0	0.01	0	0.04	0	0.08
3,7-DI-OCH$_3$-CPZ											
CPZ		0.33	0.81								
7-OH-CPZ		0.32	0.66								
7-OH-CPZSO		0.18	0.26								
7-OH-Nor$_2$CPZSO		0.35	0.12								
	blue (partial with 7-OH)[a]	0.32	0.75	0.98	0.94	1.0	1.1	1.84	2.66	0.92	5.9
	blue[a]	0.24	0.62	0.73	0.82	0.77	0.95	1.4	2.3	0.7	4.7
	blue and LUV	0.02	0.15	0.05	0.23	0.05	0.25	0.1	0.63	0.05	1.44
	blue and LUV	0.01	0.08	0	0.12	0	0.14	0	0.35	0	0.80
	lavender and LUV	0.08	0.47	0.25	0.59	0.27	0.71	0.47	1.7	0.25	3.7
	LUV 1 ×	0	0.59	0	0.8	0	0.89	0	2.4	0	5.38
	LUV 1 ×	0	0.31	0	0.41	0	0.46	0	1.24	0	2.77
	LUV 1 ×	0.23	0.34	0.69	0.46	0.71	0.51	1.35	1.38	0.69	3.08
	LUV 1 ×	0.11	0.36	0.33	0.49	0.35	0.54	0.65	1.48	1.33	3.31
2-Cl-7-OH-10-PA-Ph											
CPZ		0.33	0.83								
7-OH-CPZ		0.34	0.68								
7-OH-CPZSO		0.20	0.28								
7-Oh-Nor$_2$CPZSO		0.36	0.12								
	purple[a]	0.95	0.96	2.78	1.16	2.67	1.56	4.8	3.3	2.4	8.5
	purple	0.70	0.52	2.15	0.65	2.13	0.80	3.88	1.72	2.02	5.04
	purple	0.67	0.38	2.2	0.46	2.1	0.55	4.0	1.3	2.0	3.1
	LUV 1 ×	0	0.66	0	0.8	0	0.96	0	2.0	0	5.2

TABLE IVA *(continued)*

Standard	*Color*	*R_F value*		*R_s value to*							
				CPZ		*7-OH-CPZ*		*7-OH-CPZSO*		*7-OH-Nor$_3$CPZSO*	
		1D	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*
	LUV 1 ×	0	0.53								
	LUV 1 ×	0	0.31	0	0.38	0	0.46	0	0.95	0	0.47
	LUV 1 ×	0	0.14	0	0.17	0	0.21	0	0.44	0	1.13
7-OCH$_3$-8-OH-CPZ											
CPZ		0.32	0.81								
7-OH-CPZ		0.33	0.66								
7-OH-CPZSO		0.19	0.26								
7-OH-Nor$_3$CPZSO		0.38	0.11								
	purple[a]	0.34	0.39	1.0	0.42	0.99	0.51	1.7	1.3	0.91	3.04
	purple	0.31	0.22	0.66	0.35	0.66	0.44	1.29	1.09	0.61	2.5
	LUV 1 ×	0	0.22	0	0.34	0	0.43	0	1.06	0	2.43
	LUV 1 ×	0	0.13	0	0.26	0	0.33	0	0.81	0	1.81
	pink 1 ×	0.21	0.45	0.90	0.60	0.90	0.74	1.5	2.1	0.74	4.9
	purple 2 ×	0.11	0.45	0.30	0.60	0.30	0.74	0.5	2.0	0.26	5.4
7-OH-8-OCH$_3$-Nor$_3$CPZ											
CPZ		0.34	0.87								
7-OH-CPZ		0.34	0.72								
7-OH-CPZSO		0.19	0.27								
7-OH-Nor$_3$CPZSO		0.37	0.11								
	purple[a]	0.42	0.29	1.44	0.35	1.43	0.42	2.45	1.16	1.29	2.73
	LUV 1 ×	0.08	0.53	0.23	0.61	0.23	0.78	0.42	2.08	0.2	5.17
	LUV 1 ×	0	0.67	0	0.77	0	0.98	0	0.6	0	6.5
	LUV 1 ×	0	0.28	0	0.33	0	0.41	0	1.1	0	2.8
	LUV 1 ×	0	0.11	0	0.13	0	0.16	0	0.43	0	1.1
	LUV 1 ×	0	0.01	0	0.01	0	0.01	0	0.03	0	0.08

[a] Main spot.

TABLE IVB

AVERAGE R_F AND R_s VALUES OF ALL SPOTS RESULTING FROM THE CHROMATOGRAPHY OF EACH METABOLITE DISUBSTITUTED WITH CPZ, 7-OH-CPZ, 7-OH-CPZSO AND 7-OH-NOR$_3$CPZSO[a] IN SOLVENTS II AND III

Standard	*Color*	*R_F value*		*R_s value to*							
				CPZ		*7-OH-CPZ*		*7-OH-CPZSO*		*7-OH-Nor$_3$CPZSO*	
		1D	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*
7-OH-8-OCH$_3$-Nor$_1$CPZ											
CPZ		0.32	0.72								
7-OH-CPZ		0.27	0.60								
7-OH-CPZSO		0.17	0.23								
7-OH-Nor$_3$CPZSO		0.39	0.1								
7-OH-Nor$_1$CPZ		0.10	0.27								
	purple 1	0.1	0.19	0.34	0.26	0.40	0.32	0.65	0.86	0.28	1.4
	purple 2	0	0.15	0	0.23	0	0.26	0	0.72	0	0.95
	purple 3	0.05	0.03	0.15	0.04	0.19	0.05	0.3	0.11	0.13	0.15
2-Cl-10-AA-Ph											
CPZ		0.34	0.93								
7-OH-CPZ		0.30	0.83								
7-OH-CPZSO		0.17	0.28								
7-OH-Nor$_3$CPZSO		0.40	0.16								
	spot	0.86	1.0	2.5	1.07	2.84	1.2	4.9	3.5	2.2	8.7
2-Cl-7-OH-10-Pa-PhSO											
CPZ		0.35	0.83								
7-OH-CPZ		0.32	0.67								

(Continued on p. 286)

TABLE IVB *(continued)*

Standard	*Color*	*Rf value*		*Rx value to*							
				CPZ		*7-OH-CPZ*		*7-OH-CPZSO*		*7-OH-Nor$_3$CPZSO*	
		1D	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*	*1D*	*2D*
7-OH-CPZSO		0.22	0.31								
7-OH-Nor$_3$CPZSO		0.4	0.13								
	spot	0.63	0.83	1.8	0.97	1.97	1.2	2.8	2.6	1.5	6.1
2-Cl-10-PA-Ph											
CPZ		0.35	0.96								
7-OH-CPZ		0.31	0.85								
7-OH-CPZSO		0.18	0.32								
7-OH-Nor$_1$CPZSO		0.39	0.15								
	spot	0.79	1.0	2.24	1.05	2.5	1.18	4.27	3.11	3.0	6.6
2-Cl-10-PA-PhSO											
CPZ		0.31	0.78								
7-OH-CPZ		0.25	0.63								
7-OH-CPZSO		0.16	0.23								
7-OH-Nor$_3$CPZSO		0.33	0.13								
	spot	0.52	0.96	1.68	1.3	2.1	1.57	3.26	4.24	1.59	7.7
N-Ac-7-OAc-Nor$_1$CPZSO (purple)											
CPZ		0.30	0.84								
7-OH-CPZ		0.25	0.67								
7-OH-CPZSO		0.17	0.24								
7-OH-Nor$_3$CPZSO		0.31	0.11								
	purple	0.81	0.79	2.74	0.9	2.2	1.15	4.8	3.3	2.6	7.1

[a] See heading to Table IVA.

TABLE V

CHARACTERISTICS OF 11 DIHYDROXYPHENOTHIAZINES ON GAS CHROMATOGRAPHY WITH A FLAME IONIZATION DETECTOR

Inlet and detector temperatures are 265°. Temperature program column settings are 250° for 1 min, linear increase of 2.5°/min to 285° and hold. The N_2 rate is 40 ml/min, H_2 rate is 40 ml/min, and air flow-rate is 400 ml/min. Underlined figures (in °C) are the main peaks. For details, see text.

Compound	*Standard prepared on*											
	July 6th, 1971			*July 7th, 1971*			*July 7th, 1971*					
	Run on											
	July 6th, 1971			*July 7th, 1971*			*July 15th, 1971*					
23	263		272	263		272	263		271			
24			274	264		274	263		273			
18			269	263		269	262		268			
19	264		270	264		269	263		270			
26	no peak						264	273	274			
35	263		265	259	264	266	259	263				
4	253		278	264	no major peak		273	276				
22	266	273		266	273	276	250	265	266	272	275	
17	no major peak			261	no major peak		no major peak					
20	268			263	265	268	261	267				
32	no peak							no peaks				

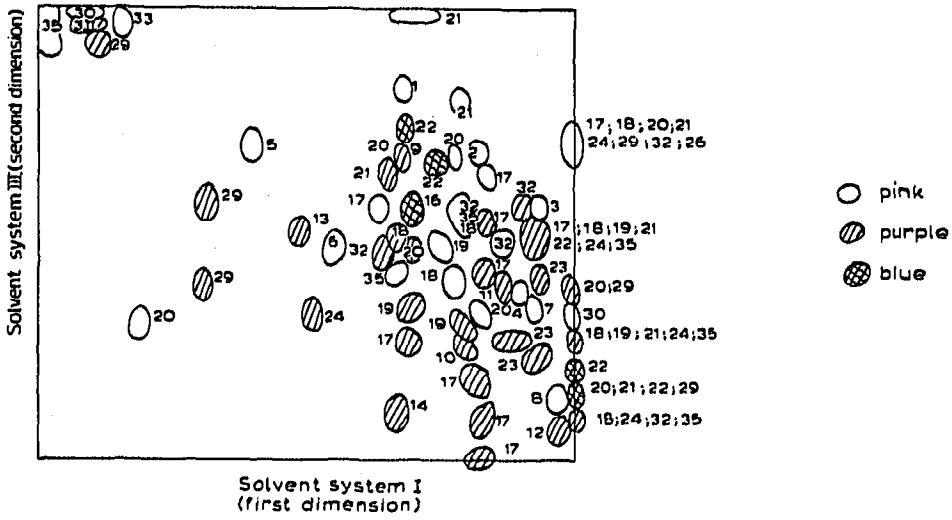

Fig. 1. This is a composite of all standards (25, 27, 28, and 34 excluded) run in Solvent I for the first dimension and Solvent III for the second multiple spots (*e.g.*, compound number 35). 15 and 9 occupy the same space. The color of the spot after spraying with the Forrest spray is indicated by the key. Details are given in the text.

that the minor peak at 263°–264° is derived from 7,8 disubstituted 2-chlorophenothiazines, including one with no adduct at N-10, where indeed it is the major peak (35). Possibly then the other compounds (18), (19), (20), (23), (24), break down by loss of the N-10 adduct. In the case of (35) the minor peak at 259° could be derived by methylation and the peaks at 265° and 266° by loss of one molecule of H_2O.

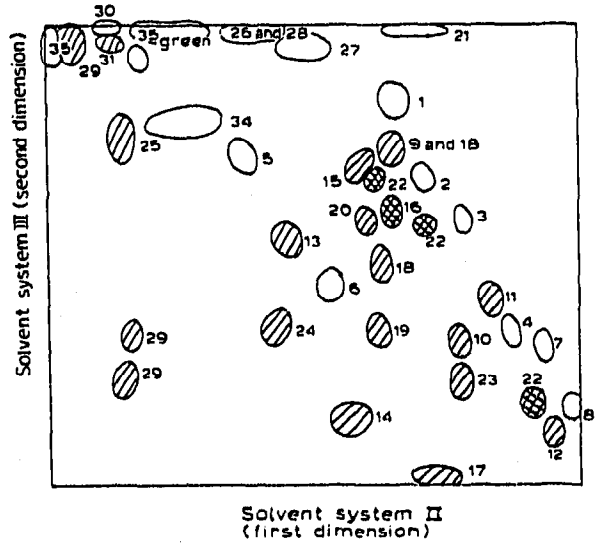

Fig. 2. A simplification of Fig. 1. Main, constant spots of each of the 35 standards studied in this report as run in Solvent II in the first dimension and Solvent III in the second dimension; color of spots are given in the key in Fig. 1.

TABLE VI

REWORKING OF R_s VALUES TO CPZ IN TABLE IV

Groups 1–9 are R_{CPZ} values of spots common to the selected CPZ derivatives. Group 10 gives the R_{CPZ} values of spots unique to each derivative. Solvents I and III were used.

Compound No.	*Common R_{CPZ}*									*Unique R_{CPZ}*
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 5*	*Group 6*	*Group 7*	*Group 8*	*Group 9*	*Group 10*
32	0.65–0.71	1.05–0.61	0.19–0.69	0–0.83	0–0.4	0–0.5	0–0			0.41–0.63
17	0.63–0.73	1.25–0.65	0.26–0.61	0–0.79	0–0.34	0–0.13	0–0	1.1–0.33		0.64–0.62
										0.61–0.47
										0.64–0.23
										0.64–0.08
										0.64–0
20	0.66–0.76	1.0–0.79		0–0.13	0–0.34	0–0.18		0.98–0.54	2.47–0.35	0.54–0.38
21	0.68–0.96	0.96–0.81	0.29–0.63	0–0.84	0–0.36	0–0.18	0–0			0.87–1.22
22	0.69–0.46	0.98–0.94	0.25–0.59	0–0.8	0–0.41	0–0.12				0.73–0.82
										0.33–0.49
										0.05–0.23
18	0.69–0.63	1.0–0.6	0.21–0.58	0–0.84	0–0.32	0–0.15	0–0			1.0 –0.6 (Group 2)
24			0.23–0.61	0–0.77	0–0.33	0–0.13	0–0.01			1.44–0.35
19	0.66–0.35	1.0 –0.42	0.3 –0.6		0–0.34					0.9 –0.6
29				0–0.8	0–0.38	0–0.17				2.78–1.16
										2.15–0.65
									2.2 –0.46	
35	0.67–0.67	1.07–0.58	0.24–0.57	0–0.81	0–0.31	0–0.13	0–0.001			3.03–1.25

Table VI is a reworking of the data in Table IV showing that there are evidently a number of compounds which appear as common derivatives of diphenolics. It is beyond the scope of this paper to propose what these derivatives are, but in so far as any of them may be found in biological sources, we propose to attempt identification.

Fig. 1 is a composite of all primary spots on TLC of pure standards available to us by the use of Solvent I for the first dimension, Solvent III for the second. The numbering of spots agrees with the numbering of compounds in the APPENDIX. Fig. 2 is a composite of all primary spots in TLC of the pure standards using Solvent II for the first dimension, Solvent III for the second. The improved separation is evident.

Fig. 3 is a composite of all still unidentified spots obtained from biological sources. Table VII gives their R_F values and Table VIII lists sources, percentage frequency of occurrence of each of 17 identified and 19 "unidentified" metabolites

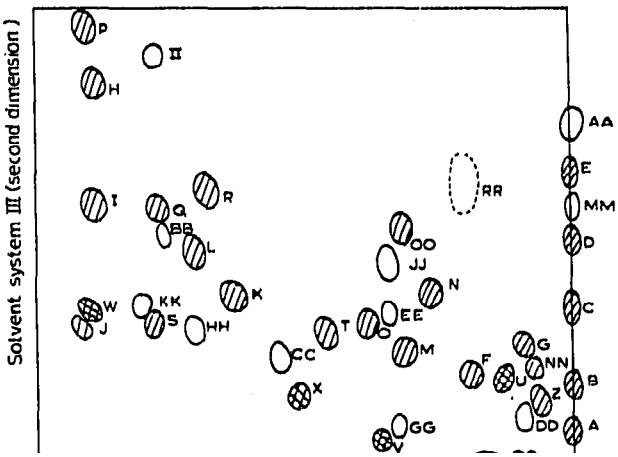

TABLE VII

R_F VALUES OF UNKNOWN SPOTS IN SOLVENT I FOR THE FIRST DIMENSION AND IN SOLVENT III FOR THE SECOND DIMENSION

Compound	*Solvent I*	*Solvent II*	*Compound*	*Solvent I*	*Solvent III*
	First dimension	*Second dimension*		*First dimension*	*Second dimension*
A	0	0.07	EE	0.34	0.33
B	0	0.18	V	0.35	0.43
C	0	0.34	O	0.39	0.31
D	0	0.48	T	0.46	0.27
MM	0	0.56	X	0.51	0.15
E	0	0.64	CC	0.55	0.23
AA	0	0.74	K	0.63	0.36
Z	0.07	0.18	BB	0.68	0.61
NN	0.08	0.21	HH	0.71	0.28
DD	0.09	0.14	L	0.71	0.47
G	0.09	0.26	Q	0.78	0.56
U	0.13	0.19	II	0.78	0.90
QQ	0.16	0	S	0.79	0.30
F	0.19	0.20	KK	0.81	0.35
RR	0.21	0.61	I	0.89	0.57
PP	0.27		H	0.89	0.83
N	0.28	0.38	W	0.90	0.33
M	0.32	0.25	J	0.92	0.30
OO	0.32	0.51	P	0.92	0.97
GG	0.33	0.09			

TABLE VIII

PERCENTAGE OF IDENTIFIED AND UNIDENTIFIED SPOTS OCCURRING IN TLC PLATES FROM URINE EXTRACTS OF 35 CHRONIC SCHIZOPHRENICS

The following compounds appeared only once: G, L, 9 (pH 12); R, EE (pH 8.5); 5, 8, 1, 33 (pH 2); M, BB, H and Q, after hydrolysis (pH 12); and 1, 4, II, 0, 30 and 2, after hydrolysis (pH 8.5).

Compound	*Non-hydrolyzed, pH 12(%)*	*Non-hydrolyzed, pH 8.5(%)*	*Non-hydrolyzed, pH 2(%)*	*Hydrolyzed, pH 12(%)*	*Hydrolyzed, pH 8.5(%)*	*Tentative identification*
30	44	12				
M or P	26	30			35	24
3	68	0				
33	56	9		64		
6	94	68			6	
1	100	41		41		
2	85	44				
4	97	74		32		
8	44	15				
14	26	53		73	94	
AA	59	6				32
H	12	26		55	76	29
II	12	56		95	68	
5	21	24				
7	15	0				
II	29	9		23		26–27
G		15		55	59	23
13				82	47	
QQ		79		59	94	17

TABLE VIII *(continued)*

Compound	*Non-hydrolyzed, pH 12(%)*	*Non-hydrolyzed, pH 8.5(%)*	*Non-hydrolyzed, pH 2(%)*	*Hydrolyzed, pH 12(%)*	*Hydrolyzed, pH 8.5(%)*	*Tentative identification*
U		47			29	22
10		50		59	32	
12		41		82	6	
31		6		18		
V		6		23		
PP		12			9	21
P		6			29	
9		26		86		
OO		12		18	15	18
O		15		27	9	19
W		6				
SS			84			
TT			35			
UU			24			
E				9		17
D				9		17
C				9		17

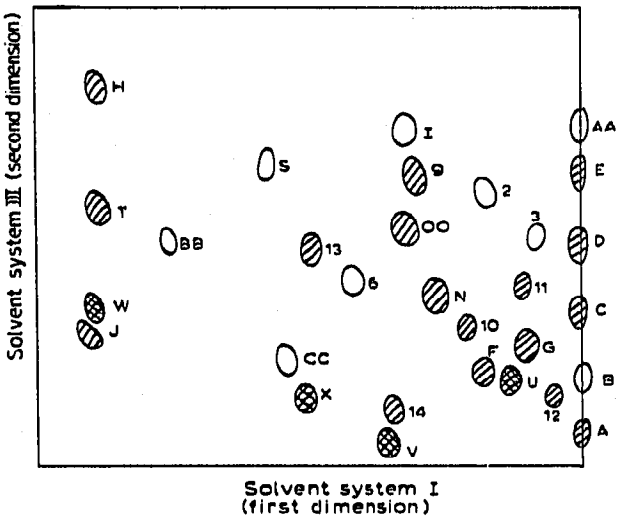

Fig. 4. Phenothiazine spots on TLC, which have been derived from fecal samples of patients on long term chlorpromazine therapy, as seen in Solvent I in the first dimension and Solvent III in the second dimension. Color of spots as in Fig. 1.

once evident. The major interest of clinicians has been in the blood and tissue levels of the unchanged drug. However, these metabolites are evidence of many cellular activities of sequences involving N-demethylation, N-oxidation, S-oxidation, oxidation of the side-chain, hydroxylation and O-methylation.

Our study supports the previously suggested[4,6] presence of, and identifies for the first time, various dihydroxylated phenothiazine metabolites and their methoxylated analogs from biological samples from chronic schizophrenics on CPZ therapy only. Support for 3,7-dihydroxylation of CPZ *in vitro* had been made by COCCIA AND

APPENDIX

C.A. numbering for phenothiazines. Hydrogen on positions 2, 3, 7, and 8 unless otherwise noted. CPZ = Chlorpromazine; PA = propionic acid; Pr = promazine; AA = acetic acid; Ph = phenothiazine; Ac = acetyl.

No.	*Abbreviation*	*2*	*3*	*5*	*7*	*8*	*10*
1	CPZ	Cl					$(CH_2)_3-N(CH_3)_2$
2	CPZSO	Cl		→O			$(CH_2)_3-N(CH_3)_2$
3	Nor_1CPZ	Cl					$(CH_2)_3-NH(CH_2)$
4	Nor_1CPZSO	Cl		→O			$(CH_2)_3-NH(CH_2)$
5	Nor_2CPZ	Cl					$(CH_2)_3-NH_2$
6	Nor_2CPZSO	Cl		→O			$(CH_2)_3-NH_2$
7	CPZNO	Cl					$(CH_2)_3-N(\rightarrow O)-(CH_3)_2$
8	CPZNOSO	Cl		→O			$(CH_2)_3-N(\rightarrow O)-(CH_3)_2$
9	7-OH-CPZ	Cl			OH		$(CH_2)_3-N(CH_3)_2$
10	7-OH-CPZSO	Cl		→O	OH		$(CH_2)_3-N(CH_{32})$
11	7-OH-Nor_1CPZ	Cl			OH		$(CH_2)_3-NH(CH_3)$
12	7-OH-Nor_1CPZSO	Cl		→O	OH		$(CH_2)_3-NH(CH_3)$
13	7-OH-Nor_2CPZ	Cl			OH		$(CH_2)_3-NH_2$
14	7-OH-Nor_2CPZSO	Cl		→O	OH		$(CH_2)_3-NH_2$
15	7-OCH_3-CPZ	Cl			OCH_3		$(CH_2)_3-N(CH_3)_2$
16	8-OH-CPZ	Cl				OH	$(CH_2)_3-N(CH_3)_2$
17	7,8-Di-OH-CPZ	Cl			OH	OH	$(CH_2)_3-N(CH_3)_2$
18	7-OH-8-OCH_3-CPZ	Cl			OH	OCH_3	$(CH_2)_3-N(CH_3)_2$
19	7-OCH_3-8-OH-CPZ	Cl			OCH_3	OH	$(CH_2)_3-N(CH_3)_2$
20	7,8-Di-OCH_3-CPZ	Cl			OCH_3	OCH_3	$(CH_2)_3-N(CH_3)_2$
21	3,7-Di-OH-CPZ	Cl	OH	OH			$(CH_2)_3-N(CH_3)_2$
22	3,7-Di-OCH_3-CPZ	Cl	OCH_3		OCH_3		$(CH_2)_3-N(CH_3)_2$
23	7-OH-8-OCH_3-Nor_1CPZ	Cl			OH	OCH_3	$(CH_2)_3-NH(CH_3)$
24	7-OH-8-OCH_3-Nor_2CPZ	Cl			OH	OCH_3	$(CH_2)_3-NH_2$
25	N-Ac-7-OAc-Nor_1CPZSO	Cl		→O	CH_3COO		$(CH_2)_3-N(CH_3COO)$
26	2-Cl-10-PA-Ph	Cl					CH_2CH_2COOH
27	2-Cl-10-PA-PhSO	Cl		→O			CH_2CH_2COOH
28	2-Cl-10-AA-Ph	Cl					CH_2COOH
29	2-Cl-7-OH-10-PA-Ph	Cl			OH		CH_2CH_2COOH
30	2-Cl-Ph	Cl					H
31	2-Cl-7-OH-Ph	Cl			OH		H
32	2,3-Di-OH-Pr				OH	OH	$(CH_2)_3-N(CH_3)_2$
33	2-Cl-PhSO	Cl		→O			H
34	2-Cl-10-AA-PhSO	Cl		→O			CH_2COOH
35	2-Cl-7-OH-8-OCH_3-Ph	Cl			OH	OCH_3	H

WESTERFELD[16] utilizing rat and human liver microsomal enzyme systems. The finding of 7,8-di-OH-CPZ, and of 7-OH-8-OCH_3CPZ and its N-dimethylated forms, and of 8-OH-7-OCH_3CPZ establishes the existence of a hydroxylation metabolic pathway in humans, similar in part to the one developed and confirmed *in vitro* by DALY AND MANIAN[17,18]. These investigators utilized preparations of rabbit and rat liver microsomes and employed an analytical method specific for only vicinal dihydroxy compounds. What possible role these disubstituted phenothiazine metabolites play in the psychotropic activity of CPZ should be considered and further biological evaluation studies on their activity may be warranted.

The fact that more than 75% of the administered CPZ has rarely been accounted for by the color tests in use[3,4,7], suggests that in some proportion of mole-

cules, the phenothiazine structure is lost. FORREST[18] claimed to recover essentially 100% of radioactive CPZ as metabolites in urine and feces. CURRY *et al.*[20] suppose that the loss occurs in the upper gastrointestinal tract, a supposition we share, since some 30% of CPZ is destroyed by incubation for 1–2 h with gastric or duodenal washings, but less than 2% is lost by 2-h incubation with feces[21]. There is no positive evidence that degradation or rupture of the phenothiazine ring takes place *in vivo*, but the possibility cannot be excluded that CPZ has, in part, been converted to a metabolite or metabolites not responsive to the color tests in use. It will be necessary to perform experiments with tagged CPZ to locate and identify the "lost CPZ".

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