J Forensic Sci, Oct. 1972, Vol. 17, No. 4

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Phenothiazines: Metabolism and Analytical Detection

In the early 1950's chlorpromazine (Thorazine), the first phenothiazine drug for clinical use in man, was synthesized in France, was introduced in Europe and Canada without delay, and has been used on a large scale in this country since 1954. Since its introduction chlorpromazine (CP) has been administered to about 100 million people all over the world, and, therefore, metabolic and toxicologic studies have been conducted in many laboratories. There are more than 10,000 papers on record dealing with clinical, pharmacological and biochemical aspects of its use.

Metabolism and mode of action of the phenothiazine drugs have been a major concern since the mid-1950's, when it became obvious that continued use of this group of psychoactive drugs in medicine and psychiatry was likely. While the mode of action is still the subject of controversy, a complicated but orderly pattern of physiological drug metabolism has emerged from the investigations of various specialized research laboratories over the past 15 years.

It took more than a decade to work out the data for CP, which turned out to be a broad spectrum antipsychotic, on which most of the chemical investigations have been conducted. This is surprising, since a good deal of information was available for unsubstituted phenothiazine, which had been used on a large scale in veterinary medicine as an antihelminthic, especially in Canada, Australia, and New Zealand. Much of this information, however, could not be directly applied to CP with its two substituents at very strategic sites of the molecule. The dimethylaminopropyl side-chain at position 10 makes a water-soluble drug out of the nearly insoluble phenothiazine, while the introduction of chlorine at position 2 imparts unique electronic properties, which relate to its versatility as a drug.

These structures are shown in Fig. 1 which illustrates the sequence of biotransformations affecting the nucleus of the chlorpromazine molecule. The scheme was developed by Piette et al [*1*] on the basis of data obtained by methods of electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR), in analogy to the scheme of Craig [*2*] for unsubstituted phenothiazine. From Fig. 1 it is obvious that some of the intermediary metabolites may function as redox systems. The first metabolic derivative, Precursor I, which has lost one electron and has become a positive ion radical and hence a highly reactive compound, is the only one among all of the drug metabolites in this scheme to show free radical content by EPR [*3,4*]. Its formation also represents the first obligatory step in the oxidation of CP by any method used, chemical, electrochemical, or photolytic.

Presented at the Twenty-fourth Annual Meeting of the American Academy of Forensic Sciences, Atlanta, Ga., 1 March 1972. Received for publication 1 March 1972; accepted for publication 5 June 1972.

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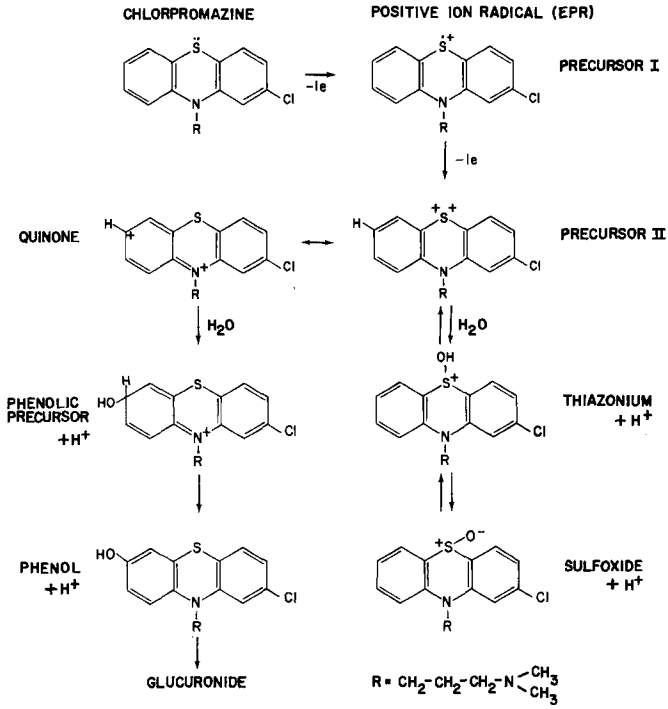


FIG. 1—*Biotransformations of chlorpromazine: scheme of changes in the nucleus.*

In physiological drug metabolism, Precursor I loses a second electron and is transformed into Precursor II. Two alternate pathways are then available. The straight line down, on the right side of the scheme, represents the sequence leading to sulfoxide. It should be noted that this is a reversible process. In an appropriate biological medium, sulfoxide may again be reduced to the lower oxidative level of Precursor II, either by enzymatic action or by other reducing conditions.

The alternate metabolic pathway from Precursor II to a quinone, two phenolic precursors, and finally to a glucuronide is shown on the left side of the scheme. Hydroxylation in position 7 has been selected for illustration, as it is the major site of hydroxylation in man [5], but the same mechanism would apply to the alternate probable positions 3 or 8, or to both positions 3 and 7, or 7 and 8.

The mixture of glucuronides constituting the majority of the urinary drug metabolites contains mostly monoglucuronides and some diglucuronides not only of the unoxidized drug, but also of the sulfoxide and the numerous metabolites of both of these, resulting from demethylation of the side-chain at position 10. The conjugated drug fraction, furthermore, includes a small amount of ester sulfates [5].

The metabolic changes are by no means limited to the nucleus. Figure 2 shows the sequence of metabolic events affecting the side-chain. *N*-oxidation at the amino nitrogen [6] is followed by successive demethylation of both methyl groups [7]. Oxidative deamination yields a postulated but unstable propionic aldehyde (in brackets), and a propionic acid derivative thereafter. Further β-oxidation then shortens, and finally eliminates the remaining side-chain, yielding 2-chlorophenothiazine [8].

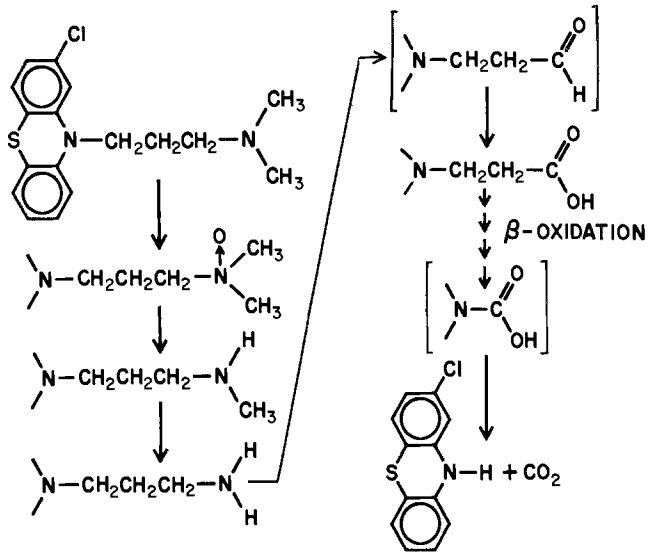


FIG. 2—*Biotransformations of chlorpromazine: side-chain transformations.*

This scheme is complex and on theoretical grounds it has been estimated that 168 different chlorpromazine metabolites could be formed [*9*]. By now, it appears that the number of potential metabolites was underestimated. In urine, several dozens of metabolites are seen in thin layer chromatograms of extracts of the urinary drug metabolites, while in tissues and serum the number is much smaller [*8*].

Qualitative Demonstration of Urinary Phenothiazines and Related Drugs

Qualitatively, there is a very simple means of demonstrating the large fraction of urinary drug metabolites, regardless of its complexity. Long before anything was known about the structure of these drug metabolites, a series of rapid urine color tests was devised [*10*] to demonstrate them in the urine of patients. Empirically, it had been found that the color intensities of these sensitive reactions were proportional to the drug dosage given, bu' that the color reactions were not caused by the unoxidized drugs as such, nor by the sulfoxides. It was concluded that the intense color resulting from these tests were due drug metabolites of intermediate oxidation level, that is, phenolic derivatives and t O-glucuronides.

These procedures are rapid, simple, convenient, and inexpensive and furnish a deal of information with a minimum of effort. For example, on disturbed wards, or i follow-up of patients on self-medication, it becomes immediately obvious whethe patients have conformed to the prescribed routine, and the same is true of outpa after their release from the hospital. Toxicology laboratories make ample use of simple tests, and they also have proved helpful in drug evaluation studies. There is al a certain percentage of patients who, for various reasons, inherent to their illness, do ingest the drugs, and thus contribute to the margin of error in studies concerned with evaluation or comparison of drugs based on oral drug administration. Such tests are r performed routinely in many hospitals in this country and abroad. They require less ti a minute. One milliliter of urine is mixed with an equal volume of test solution, and

resulting color is read immediately against the color chart. These rapid tests also yield useful preliminary data in other contexts. If, for example, the very sensitive FPN[2] test is negative, no further elaborate assay procedure will yield satisfactory information. If it is positive, the intensity of the resulting color may serve as a measure for the volume of urine to be extracted in a specific assay, as submicrogram quantities of phenothiazine drugs per milliliter of urine can be visually recognized.

Quantitative Determination of Urinary Chlorpromazine Metabolites

Quantitative assays of urinary CP derivatives of the most varied types and degree of sophistication are available. All of them are based on preliminary solvent extraction and subsequent determination by various methods of spectrophotometry, chromatography (including gas chromatography), spectrophotofluorometry, radioquantitation, ion exchange adsorption, or combinations of these procedures. These have been recently reviewed by Usdin [*11*] in a comprehensive paper with references. The state-of-the-art up to late 1971 is clearly presented, and this discussion will be limited to methods considered practical by the criteria of simplicity, comprehensiveness, and ease of performance in laboratories lacking elaborate equipment such as a gas chromatograph-mass spectrometer interface. Obviously, the selection of methods to be employed will be dictated by the type of information desired. In addition, some very recent developments, not yet covered in Usdin's review, will be mentioned.

Many reasonably quantitative procedures have been reported for the assay of the readily extractable, unconjugated, moderately polar chlorpromazine metabolites, such as the nonphenolic sulfides and sulfoxides and their demethylated derivatives, as well as the *N*-oxide. This entire group, however, constitutes no more than 20 percent of the total urinary chlorpromazine fraction. With subchronic and chronic drug administration about 80 percent is present in the form of conjugates, mostly O-glucuronides. A single, initial dose is metabolized differently from a chronic dose. It takes about 3 weeks for the average patient to fully develop the enzymatic potential for efficient drug detoxication by glucuronic conjugation [*12*]. It is believed that initial CP metabolism is an example of how drug metabolism correlates with pharmacological effects. Initially, drug metabolism consists primarily of sulfoxidation and demethylation, with very little hydroxylation and glucuronic conjugation manifested. During the initial phase of CP therapy patients feel tired and apathetic, but in about 3 weeks these side-effects subside completely. The metabolites primarily produced during the initial drug therapy are still pharmacologically active, whereas the polar glucuronides as end products of efficient detoxication are considered inactive. An acute single dose is also processed differently in respect to rate of excretion. Due to a higher degree of tissue binding, only about 15 to 25 percent of the dose will appear in a 24-h urine collection, while almost twice as much may be expected at a later steady state.

Methods suitable to establish these findings were reported a few years ago [*13,14*]. In a 2-step procedure, all unconjugated CP metabolites are extracted from alkaline urine with either dichloromethane, or dichloromethane containing 15 percent propanol according to Kaul [*15,16*], and this drug fraction is then assayed as a group by reaction with a final concentration of 50 percent sulfuric acid and measurement of their resulting common maximum absorptions at 530 nm. The conjugated metabolites are then assayed as a group by passing a small volume of urine through an IRC 50 (sodium form) resin column, which

[2] FPN reagent [*10*] consists of 5 parts 5 percent vol/vol ferric chloride solution, 45 parts 20 percent vol/vol perchloric acid and 50 parts 50 percent vol/vol nitric acid.

will retain all unconjugated metabolites with the exception of trace amounts of deaminated compounds. The conjugates in the effluent are reacted with sulfuric acid, and the common maximum absorption at 550 nm serves to assay this fraction. The efficiency of the solvent extraction for the unconjugated metabolites and the separation on the ion exchange column were checked by means of radioquantitation using tritiated CP, and found to be satisfactory, that is, accounting for 90 percent or more of the compounds.

Fecal Excretion of Chlorpromazine

Very few data are available for fecal excretion of CP in any species. According to unpublished data from various laboratories, it approximates 5 percent or less during a steady state. The Rhesus monkey was found to be a very good model for human CP metabolism, and both the qualitative and quantitative aspects of urinary drug excretion were nearly identical in these two species [*17*]. If this holds true for the fecal excretion as well, then it should be expected that an average of 5 percent of a daily dose is excreted by this route in the case of man, with essentially the same conjugated and unconjugated metabolites as present in urine.

Metabolism of Piperidine- and Piperazine-Linked Phenothiazines

Not all phenothiazine drugs are metabolized exactly as CP; thus, thioridazine (Mellaril®) with an SCH_3 group in position 2 and a piperidine ring instead of CP's aliphatic tail, produces less and fewer glucuronic conjugates, but more unconjugated drug metabolites, with a preponderance of sulfoxides, as illustrated in Fig. 3 [*18*]. Very little is known about the metabolism of the piperazine-linked phenothiazine drugs, which are given in much smaller daily doses. These would comprise, for example, perphenazine (Trilafon®), prochlorpromazine (Compazine®), trifluoperazine (Stelazine®), and fluphenazine (Prolixin®).

Most recently, ^{3}H-prochlorperazine metabolism in rabbit and guinea pig was studied [*19*], using radioquantitation in the assay of total urinary and fecal drug excretion, including the period of time required for total excretion of the label. Figure 4 shows the structural formula of prochlorperazine, while Table 1 summarizes the total excretion of the labeled compound in the two species studied for 17 days. Compared to CP metabolism in the same two species, there was a remarkable reversal of rates for urinary and fecal excretion; rabbit and guinea pig excreted the majority of CP derivatives via the urine,

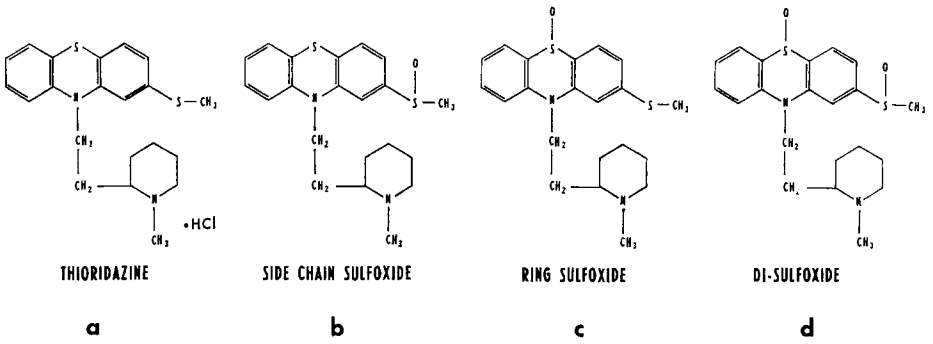


FIG. 3—*Thioridazine and its known sulfoxidized derivatives.*

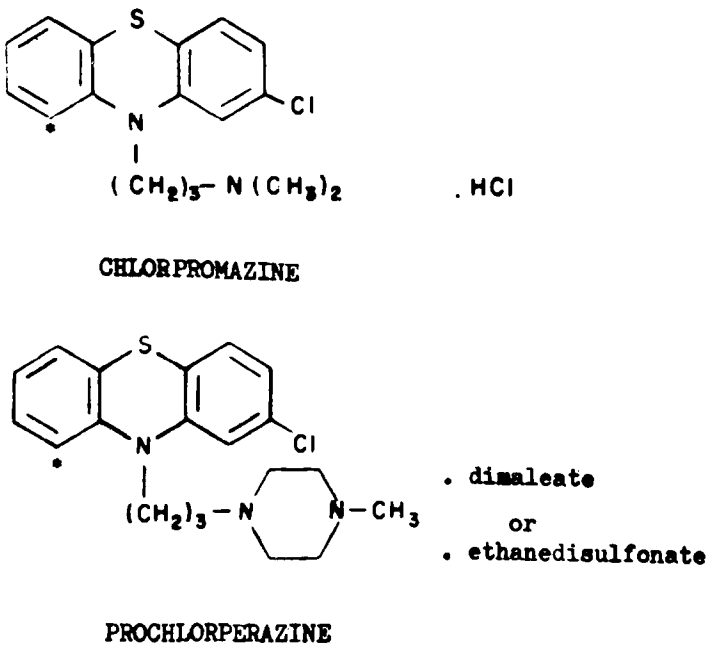


FIG. 4—*Structures of chlorpromazine and prochlorperazine with asterisk (*) at position 9, the site of the tritium label.*

like man, but in the case of prochlorperazine the ratio was 4.3 to 1 in rabbit, and 7 to 1 in guinea pig, in favor of fecal excretion. There are no data for prochlorperazine excretion in man, but it is suspected that this unexpected reversal would equally apply to man. Hence, the frequently voiced clinical opinion that all pehnothiazines are essentially alike in their therapetuic as well as toxicological effects, has no basis in fact with regard to drug metabolism.

In the cases of extremely low daily doses of phenothiazine drugs administered, for example, with trifluoperazine or fluphenazine, it may be possible to devise suitable methodology for their assay by using an "applejack" process, that is, controlled thawing of a frozen specimen and collecting the initial melting volume, as shown in Fig. 5. In this case, a pool of CP-containing urines (400 to 600 mg per day) was used. Approximately 10 percent of the volume will contain 50 percent of the drug content, and 50 percent of the original volume will account for 95 percent. Obviously, at any practical cut-off point, for example, after collection of the first 10 percent of volume, the process could be repeated. The rates of appearance in the melting volume were very similar for conjugated and unconjugated metabolites [*20*].

Drug Content in Cerebrospinal Fluid

Literature data on the presence of CP in CSF are scarce. According to unpublished data from various investigators, the concentrations in this fluid are low. We have been unable to detect any CP metabolites in specimens of patients receiving less than 600 mg/day by conventional methodology. Unpublished data indicate that 1 ml of CSF in a Rhesus monkey, chronically dosed with 30 mg/kg CP, contained 0.001 percent of a ^{3}H-CP label,

TABLE 1—*^{3}H-prochlorperazine excretion in rabbit and guinea pig.*[a]

	Rabbit		Guinea Pig	
Day	Urine	Feces	Urine	Feces
1	9.59	47.81	4.92	33.05
2	4.05	11.84	1.50	18.17
3	1.69	5.61	0.83	7.92
4	0.25	2.31	0.59	3.78
5	0.31	1.98	0.31	0.89
6	0.21	2.11	0.24	0.65
7	0.21	0.50	0.21	0.60
8	0.19	0.34	0.11	0.38
9	0.14	0.28	0.15	0.26
10	0.14	0.17	0.08	0.24
11	0.10	0.14	0.09	0.23
12	0.09	0.09	0.07	0.20
13	0.11	0.12	0.07	0.38
14	0.07	0.07	0.07	0.20
15	0.08	0.08	0.08	0.12
16	0.08	0.05	0.08	0.13
17	0.05	0.05	0.05	0.14
Cumulative Totals	17.36	73.55	9.44	67.34
Total % Excreted	91		77	

[a] Expressed as percent of total administered radioactivity.

administered 3 hr earlier. A meaningful study will be possible only when ultrasensitive methods now being developed have been perfected.

Drug Content in Blood

Phenothiazine concentration in blood is probably the most obscure area of physiological drug metabolism. A vast discrepancy exists between reported plasma or serum drug concentrations which are usually in the nanogram range and rarely in the microgram range per milliliter of medium, after intake of 100 to 1000 mg CP per day. The corresponding urine concentrations would normally be a thousand times higher in 24 h urine collections with volumes of up to 2 l. (Excessively large volumes such as 8 l per day, however, are by no means uncommon at chronic administration of phenothiazines, due to large fluid intake of patients.)

The methods for assaying CP metabolites in blood have generally suffered from the inadequacy of the initial step, that is, the solvent extraction from whole blood or any subfraction thereof. The drug metabolites are particularly tightly bound to the endogenous protein constituents, and solvent extraction procedures for separation of the drug content, even of spiking material added to control sera, gave notoriously poor recoveries. Recently, some improvements have been reported by Kaul [*16*] with regard to the solvent extraction procedures. Usdin's review [*11*] deals adequately with the various currently employed sophisticated techniques such as combined gas chromatography/mass spectrometry as advocated by Hammar and Holmstedt et al, GC using an electron capture detection system (Curry et al) or flame ionization detection (Spirtes and Berman et al), radioactive derivative formation (Efron et al) or fluorescent derivative formation (Chan, Gershon et al, Kaul et al, and Forrest et al).

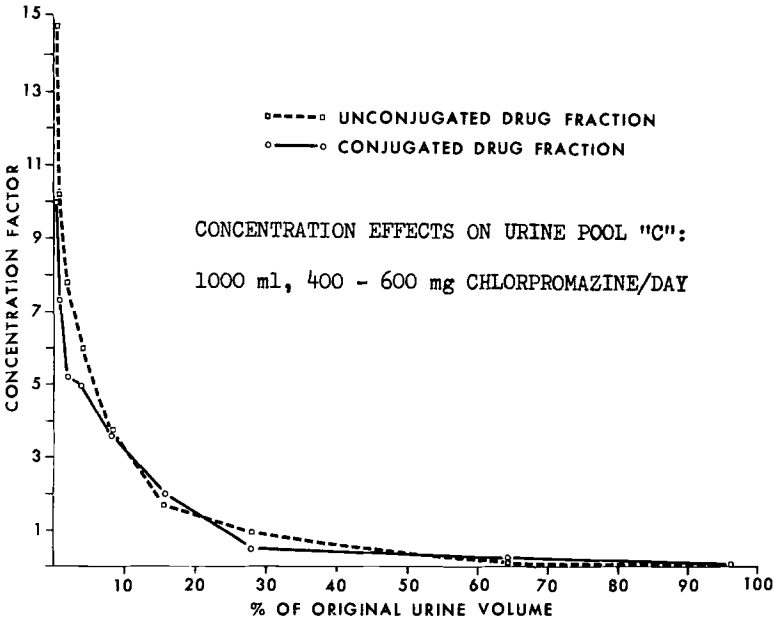


FIG. 5—"*Applejacking*" *for concentration of urinary drug content.*

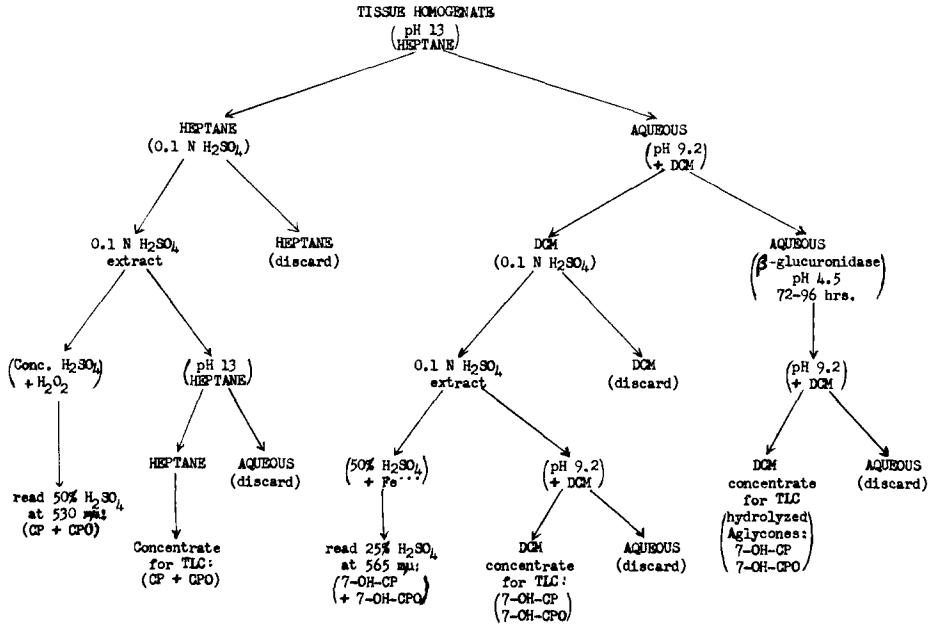
Until recently, it had been assumed that CP metabolites in blood are essentially concentrated in the serum. Kaul [*16*] reported increasing extractability of drug metabolites for serum, plasma, and whole blood, in that order. An earlier report by Zingales [*21*] on CP and thioridazine metabolites in human erythrocytes supports this concept.

None of the more sophisticated methodology is as yet thoroughly developed for routine analysis in the average hospital laboratory, and it seems premature to single out any one of them as the most promising.

Chlorpromazine Determination in Autopsied Tissues

As this area has been studied in detail and is of interest to forensic toxicologists and coroners' offices in general, data and methods will be discussed in some detail. Wechsler and Forrest [*22*] reported a procedure for extraction and assay of CP metabolites from animal tissues in 1959, and a modification thereof was used in the first comprehensive study of human autopsied tissues [*23*]. In analogy to the data from animal studies, the highest concentrations of drug metabolites were seen in lung and liver, with lesser amounts in the kidney and other visceral organs, and much lower concentrations in the various brain areas studied. Surprisingly high concentrations were seen in the keratinous tissues, hair, and nails. In general, the glandular tissues showed higher amounts than muscular tissues or fat. The high accumulation of drug derivatives in hair was later found due to interaction of CP derivatives with melanoprotein.

However, the methods were unsatisfactory, as they did not permit determination of individual drug metabolites as they accumulated locally in individual tissues. This was subsequently remedied by using more comprehensive solvent extraction procedures, at different pH's, whereby two groups of unconjugated drug derivatives which account for the vast majority of the compounds present were separated, as shown in Fig. 6. Extraction



Some overlap in the extraction of nonphenolic and phenolic metabolites at pH 13 and pH 9, respectively, is unavoidable.

FIG. 6—*Scheme for extraction of chlorpromazine metabolites from tissues.*

at high alkalinity (pH 12 to 13) with heptane containing 1.5 percent isoamylalcohol removes all nonphenolic material, while extraction at pH 9 to 10 yields additional phenolic CP metabolites. These two groups of derivatives were spectrophotometrically determined by use of their respective absorbancies at 530 and 565 nm, after reaction with sulfuric acid. Aliquots of the solvent extracts were also analyzed by TLC. The number of metabolites in each fraction was much smaller than in urine, and all metabolites were identified by means of reference compounds, in two solvent systems. The results for selected tissues from autopsies of six psychiatric patients were reported in 1968 [*24*], and are summarized in Tables 2 to 4.

A number of conclusions can be drawn from these data. Tissue storage of CP metabolites is essentially effected in the form of unconjugated metabolites. In most visceral organs, both phenolic and nonphenolic metabolites are present. Whether the lung or the liver will have the highest drug content, appears to be dependent upon the time elapsed between last drug administration and death. Drug concentration in the liver may be absolutely or proportionately higher than in the lung, when a short interval between last drug administration and death has elapsed. In the single patient suffering from side-effects of CP therapy, the most remarkable finding was her apparent inability to dispose of stored drug content, as manifested by the disproportionately large amount of CP metabolites stored in the liver. Subsequent TLC examination of the drug fraction also revealed the presence of some derivatives of levomépromazine and perphenazine derivatives, both of which had been discontinued 4 months prior to her death.

Significance of Drug Metabolism for Clinical Response and Drug Toxicity

It seems reasonable to ask whether elucidation of the complex biotransformation products is indeed relevant to the understanding of the mode of action of CP. One such

TABLE 2—*Description of autopsy cases.*

				Medication During Last 4 Months				
Patient	Age Years	Wt. lbs	Time Lapse from Death to Autopsy	Last CP[a] Dose, hrs before Death	CP mg/day	Other Phenothiazine Drugs (Occasionally)	Other Drugs (Occasionally) (& PRN)[c]	Post-Mortem Diagnosis
I (M)[a]	36	170	24-36 hrs	6	1050	none	Biperiden	Chest & Lung Pathology; Bronchial Asthma
II (M)	47	148	24-36 hrs	11	600	none	Amitriptyline Trihexyphenidyl	Congestive Heart Failure; Arterio-sclerosis
III (M)	49	161	24-36 hrs	1	600	none	Digitalis Sodium Amytal Mercuhydrin Reserpine Laxatives Acetylsalicylic Acid Antacids	Congestive Heart Failure; Coronary Thrombosis
IV (M)	60	170	72 hrs	10	400	none	Secobarbital Chloral Hydrate Laxatives Antispasmodics	Myocardial Infarct; Congestive Heart Failure
V (M)	44	175	24-36 hrs	1	200	Promethazine	Pentobarbital Meperidine Hydromorphone Heparin Neomycin Sodium Warfarin Stilbesterol Scopolamine Acetylsalicylic Acid Laxatives	Cancer Metastases to Lung, Liver, Left Adrenal; Embolus due to Thrombo-phlebitis
VI (F)[a]	32	144	24-36 hrs	6 or 18[b]	600	Levomépromazine & Perphenazine: both discontinued 4 months prior to death	Phenobarbitone Phenytoin Benztropine	Acute Intes-tinal Obstruc-tion; Volvulus of Sigmoid Colon; Pul-monary Ate-lectasis

[a] Abbreviations used: M (male); F (female); CP (chlorpromazine)

[b] Medical Records equivocal on this point.

[c] When required.

attempt was made by Spirtes, Guth, and coworkers, on the basis of local accumulations in selective tissues. This group reported in their review [*25*] that tranquilizing phenothiazines such as CP and prochlorperazine were stored in the hypothalamic areas of the dog brain to a greater extent than the nontranquilizing, antiemetic thiethylperazine, which accumulated preferentially in the cerebellum. Furthermore, the same group interpreted the corneal anesthesia observed in rabbits after CP administration on the basis of the significant accumulation of phenothiazines in the ocular tissues, reported by various investigators [*26,27*].

TABLE 3—*Drug content found in selected autopsied tissues.*

			Patient											
	% Recoveries		I 13.6 mg/kg		II 8.9 mg/kg		III 8.2 mg/kg		IV 5.2 mg/kg		V 2.5 mg/kg		VI[e] 9.2 mg/kg	
Tissue	CP[a]	7-OHCP[a]	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP	CP	7-OHCP
Liver	80	91	22	33	38	6.2	8.1	3.8	2.3	1.5	13	Tr[b]	190	37
	81	88	21	33	37	5.9	8.1	3.7	2.3	1.5	12	Tr	190	36
Lung	80	82	25	18	79	59	29	23	11	5.2	8.5	4.6	-[d]	
	80	84	25	18	77	58	29	24	11	5.2	8.5	4.5		
Kidney	89	84	7.0	5.6	2.3	2.3	9.1	8.2	0.6	0.7[c]	0.7	0.4	-	
	88	81	7.1	5.6	2.3	2.4	8.8	8.1						
Spleen	82	78	6.2	5.1	7.6	2.0	1.3	0.8	1.3	1.7	2.8	1.0	-	
	81	78	6.0	5.2	7.1	2.0	1.4	0.7	1.3	1.7	2.7	0.9		
Heart Muscle	80	78	7.3	4.0	Tr	Tr	1.1	0.4	1.5	Tr	0.5	Tr	-	
	78	75	7.3	3.7					1.5	Tr				
Pancreas	89	80	21	14	3.3	1.3	8.8	6.2	Tr	0.8	Tr	0.9	-	
	91	82	21	14	3.0	1.3	8.7	6.0	Tr	0.8	Tr	0.9		
Adrenal	88	82	4.4	Tr	1.7	1.7	1.5	Tr	1.6	0.8	-		-	
	87	81	4.4	Tr			1.6	Tr	1.6	0.8				
Thyroid	78	98	1.1	Tr	1.5	1.8	0.9	Tr	-		-		-	
	77	95	1.1	Tr	1.5	1.7	0.8	Tr						
Mesentery and Fat	89	50	-		4.4	Tr	1.6	Tr	-		-		-	
	87	50			4.2	Tr	1.4	Tr						
Intestinal Tissue	87	65	4.3	Tr	1.7	Tr	0.8	Tr	-		-		-	
	84	64	3.8	Tr	1.6	Tr	0.9	Tr						
Testes	79	64	-		-		1.0	1.6	1.6	2.3	-		-	
	81	62							1.6	2.3				

[a] The drug metabolites derived from chlorpromazine (CP) are expressed in µg of CP.HCl per g of wet tissue, and the metabolites derived from 7-hydroxychlorpromazine (7-OHCP) are expressed as µg of 7-OHCP, free base, per g of wet tissue. [b] Tr = trace.
[c] Material available for single determination only. [d] Tissue was unavailable. [e] Patient VI had hyperpigmentation of the skin

TABLE 4—*RF's of chlorpromazine metabolites in liver tissues.*

Organic Phase	Case II (no side-effects)		Case VI (hyperpigmented)		Reference Compound		Comment
	SS:A[+]	SS:B[+]	SS:A	SS:B	SS:A	SS:B	
Heptane (unconjugated, non-phenolic metabolites)	.50	.45	.50	.46	.51	.47	nor_1CP
	.33	.67	.32	.68	.33	.70	CP
	.65	.54	.64	.54	.65	.56	nor_2CP
			.42	.59	?	?	nor$_1$levomé-promazine
	.15*	.26*	.11*	.27*	.14	.28	nor_1CPO
	.07*	.53*	.07*	.55*	.07	.58	CPO
	.25*	.32*	.26*	.34*	.25	.37	nor_2CPO
			.18*	.49*	.18	.50	perphenazine
			.03*	.39*	.03	.40	perphenazine 5-oxide
Dichloromethane (unconjugated, phenolic metabolites)	.48	.15	.44	.17	.45	.18	$nor_1$7-OHCP
	.33	.38	.28	.38	.30	.40	7-OHCP
	.62	.25	.58	.24	.59	.25	$nor_2$7-OHCP
	.20*	.04*	.18*	.04*	.20	.04	$nor_1$7-OHCPO
	.10*	.10*	.07*	.10*	.10	.12	7-OHCPO
Dichloromethane (aglycones of hydrolyzed glucuronide fraction)	**	**	.43	.16	.45	.18	$nor_1$7-OHCP
	**	**	.28	.38	.30	.40	7-OHCP
			.56	.24	.59	.25	$nor_2$7-OHCP
			.07*	.10*	.10	.12	7-OHCPO

[+] SS:A Solvent System A: ethanol - ethyl acetate - acetic acid - water (17 : 56 : 17 : 10)
SS:B Solvent System B: butanol - ethanol (absolute) - diethylamine (12 : 6 : 1)

* Trace

** Trace, not measurable

However, there is as yet no unequivocal explanation as to how the antipsychotic effect of CP is produced. The brain is certainly not the only or even the major site of its accumulation, but it has been possible to interpret relatively high accumulations of drug in preferential tissue areas in terms of side-effects of drug therapy.

The common denominator for all pharamcological actions of CP, desired or incidental, is most likely its effect on membrane permeability which, in turn, is mediated by its free radical derivative. The ease with which CP donates an electron to a receptive acceptor accounts for its interactions with all types of enzymes and biogenic compounds, by the formation of complexes, adducts, intercalations, or its surface adsorption to even inorganic compounds.

The desire to correlate metabolic data with pharmacological effects has prompted a number of studies on the significance of individual CP metabolites.

Correlation of Individual Drug Metabolites with Side-Effects

By screening the urinary CP metabolites produced by all mammals, including man, (and by patients as well as by normal controls) [*11*], it was found that all of them produced the metabolites which we grouped together as nonconjugated and nonphenolic. Their relative abundance or scarcity permitted no correlation with clinical or behavioral features.

One group of unconjugated phenolic drug metabolites, however, appears to be significantly correlated with the late side-effects of CP induced skin pigmentation [*28,29*]. Normally, such derivatives of 7-hydroxychlorpromazine (7-OHCP) occur in trace amounts in the fresh urines of all patients. On standing, a little more of these compounds are produced due to hydrolysis of the glucuronides by chance amounts of β-glucuronidase. (Amounts of this enzyme in urine vary with the diet.) Patients with skin pigmentation, however, show measurable amounts of these phenolic drug derivatives.

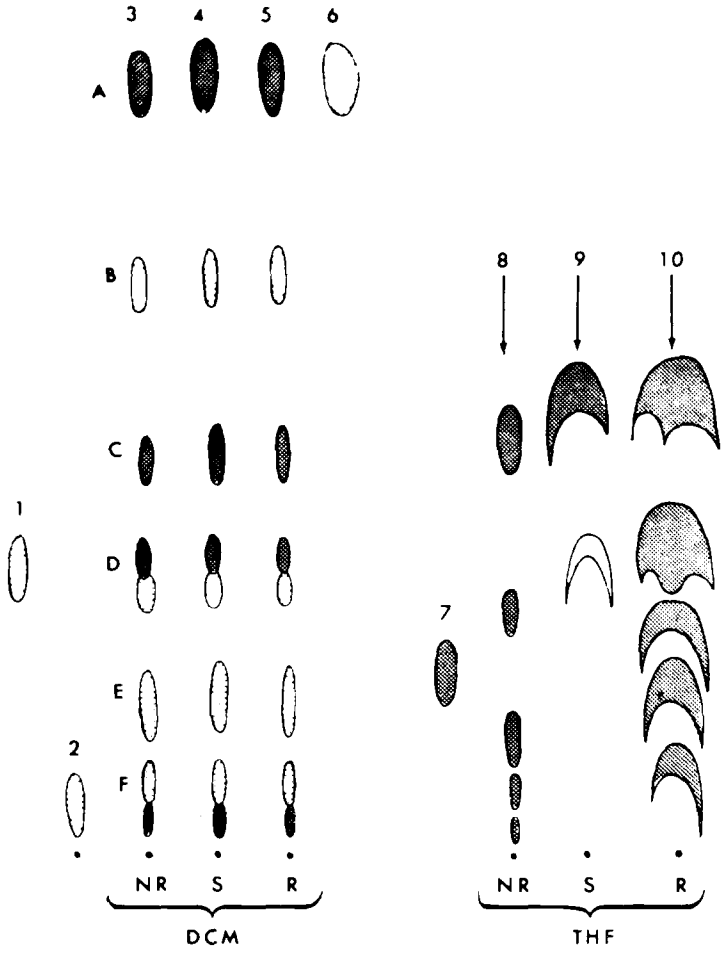
The most pertinent differences appear to be related to the fraction of conjugated drug metabolites. Figure 7, representing a thin layer chromatogram of the two groups of urinary CP metabolites from three different patients, illustrates this point. *R* stands for satisfactory response to drug therapy, *NR* for patients with unsatisfactory clinical response, and *S* for the side-effect of skin pigmentation.

Dichloromethane (DCM) is the solvent used for extraction of all unconjugated drug metabolites, while tetrahydrofuran (THF) is the solvent used to extract the glucuronides. The DCM fractions with unconjugated drug metabolites are nearly identical in all three patients, while the conjugates in the THF group show substantial differences. The satisfactory responder, *R*, has large spots indicating significant amounts of about five major groups of glucuronides. The unsatisfactory responders, *NR*, show the same five groups, but much smaller spots, signifying less drug metabolites to be present. Finally, *S*, the patient with skin pigmentation, shows only one normal group of glucuronides and one atypical one, possibly due to an *N*-glucuronide. It might be added that the patient used as prototype of a nonresponder for about a year, finally developed skin pigmentation, thus becoming the first to have a known pattern of urinary CP excretion "before and after" developing skin pigmentation.

It is believed that a urinary drug excretion pattern conforming to the *NR* might be used as a predictor of later side-effects or as a criterion to modify the chemotherapy.

Some differences in drug metabolism between males and females are certainly ascertainable. Thus female patients, in general, respond to somewhat lower doses of phenothiazines and also develop side-effects more frequently. This is probably attributable to the estrogen components of the ketosteroids, acting similarly to CP or being potentiated by CP. In summary, it is believed that such interpatient variations, as seen in urinary CP excretion patterns, were essentially limited to the fraction of drug-glucuronides. It appears that poor glucuronidation may be a genetic component. In extreme cases of clear-cut deficiency of glucuronic conjugation change to use of other phenothiazine drugs, making lesser demands on this detoxication pathway, might be advisable.

As previously mentioned, there is interaction between CP and melanin, and drug distribution studies in animal tissues have shown the highest drug concentration in tissues rich in melanin. This interaction is one of charge-transfer, between CP as the electron donor and melanin as the electron acceptor. This has been shown by three different techniques *in vitro* using conductimetric titration, UV and EPR spectroscopy [*30*], and it appears likely that the same mechanisms apply *in vivo*. In organisms with normal melanin distribution, this results in local concentration of the drug, for example, in hair and in the eyes, as well as in such tissues as substantia nigra in the brain, or certain



NR = non-responder to drug therapy.
S = patient with side effect of skin pigmentation.
R = responder to drug therapy.
DCM = dichloromethane extract from urine (non-polar and moderately polar drug metabolites).
THF = tetrahydrofuran extract from urine (highly polar drug metabolites).
1 = reference compound, nor$_1$-chlorpromazine.
2 = reference compound, chlorpromazine sulfoxide.
3 = DCM extract, non-responder to drug therapy.
4 = DCM extract, patient with side effect of skin pigmentation.
5 = DCM extract, responder to drug therapy.
6 = reference compound, 2-chlorophenothiazine sulfoxide.
7 = reference compound, 7-hydroxy-chlorpromazine.
8 = THF extract, non-responder to drug therapy.
9 = THF extract, patient with side effect of skin pigmentation.
10 = THF extract, responder to drug therapy.

Dark hatched spots are purple; lighter, stippled spots are pink.

FIG. 7—*Thin layer chromatography of urinary CP metabolites from three characteristic patients.*

glandular tissues, as has been demonstrated in experimental animals or in tissues from human autopsies.

In the rare instances in which CP produced skin pigmentation (about 0.5 percent of chronic mental patients after prolonged high-dosage therapy), Greiner and coworkers in Canada [*31–34*] assume that a genetic overproduction of melanin predisposes these patients to eventual development of this side-effect. In the experience of Forrest et al, this side-effect proved entirely, but slowly, reversible with appropriate therapeutic measures [*29*].

In view of the normal interaction of CP and melanin, it was thought to be of interest to study an albino patient. The urinary drug excretion studies showed the patient's excretion pattern entirely normal and conforming to that of drug responders [*35*].

Figure 8 illustrates the chemical scheme for the interaction of CP or 7-OHCP with melanin. The melanin-induced oxidation of the sulfides ultimately leads to the respective sulfoxides by way of several intermediate reactions, and may therefore be considered either a redox reaction or a complete charge-transfer. As 7-OHCP was found to react more rapidly with melanin, we compared the urinary excretion of this CP metabolite in various chronically dosed patients of different ethnic derivation [*36,37*]. The results are listed in Table 5. A scale of increasing order resulted, which apparently paralleled the peripherally circulating melanin available in the various patients. In the single albino patient located in the past 10 years, the excretion of unconjugated 7-OHCP derivatives amounted to traces only, while Caucasian patients without hyperpigmentation,Oriental and Negro patients, and Caucasian patients with hyperpigmentation followed in sequence.

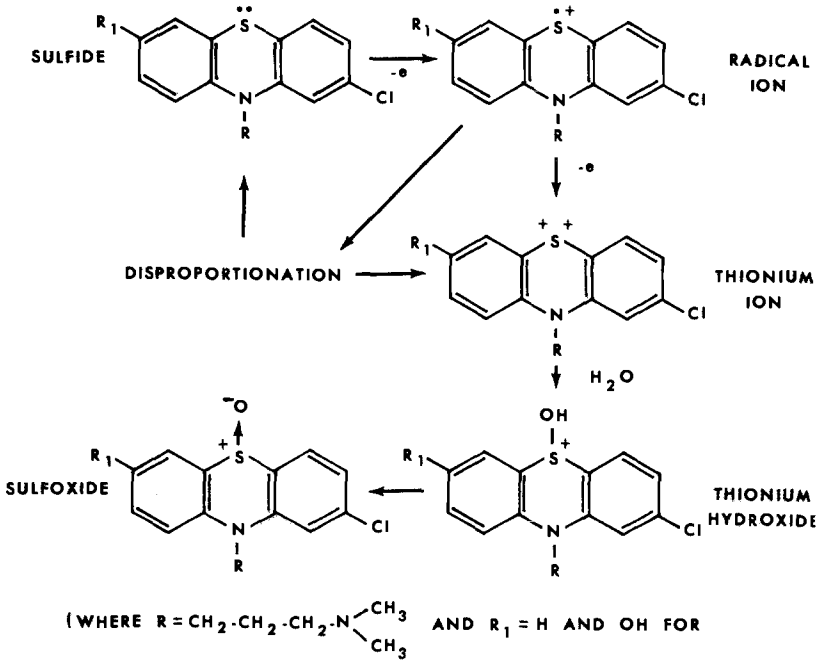


FIG. 8—*Scheme of the melanin-induced oxidation of chlorpromazine and 7-hydroxychlorpromazine.*

TABLE 5—*Excretion of unconjugated 7-hydroxychlorpromazine derivatives in 13 psychiatric patients.*

Patient	Dose mg/day	Ethnic* Group	Side** Effect	mg/24 hr***		% dose/*** 24 hr	
1	900	C	++	2.2	2.2	0.2	0.2
2	900	C	++	2.9	4.8	0.3	0.5
3	900	C	+	2.7	2.8	0.3	0.3
4	600	N	-	3.4	2.0	0.6	0.3
5	900	N	-	5.4	1.7	0.6	0.2
6	300	O	-	1.2		0.4	
7	600	O	-	0.3	0.4	0.05	0.07
8	900	C	-	0.8	1.0	0.1	0.1
9	600	C	-	0.4	0.4	0.07	0.07
10	1200	C	-	0.2	0.2	0.02	0.02
11	800	C	-	0.4	0.4	0.05	0.05
12	600	C	-	0.2	0.2	0.03	0.03
13	800	A	-	0.08	0.09	0.01	0.01

* The following abbreviations were used: A Albino; C Caucasian; N Negro; O Oriental

** Hyperpigmentation of exposed skin areas

*** Results of duplicate determinations in different 24 hr collections, except for Patient 6, from whom only a single 24 hr collection was available.

The next step was then to find an animal model, normally excreting significant amounts of unconjugated 7-OHCP derivatives. If the theory was correct, such an animal should be a suitable candidate for developing hyperpigmentation of the skin, when exposed to UV irradiation. This was indeed found to be true for pigmented rabbits [*38*]. As expected, albino rabbits lacking peripheral melanin did not produce hyperpigmentation. Dutch Belt rabbits were used primarily as they in fact have built-in control areas, combining the genetic features of their mixed albino and pigmented ancestry. Chronically dosed animals receiving 20 to 30 mg/kg CP per day had an area of their dorsal skin with albino and pigmented sections, measuring about 7 by 11 cm, shaved and thereafter clipped several times per week. They were exposed to daily UV irradiation for 30 min, with one-half of the shaved area covered at all times with aluminum foil held in place by tape (Fig. 9). After 3 weeks, all experimental animals had clearly developed hyperpigmentation of the exposed pigmented skin portions (Fig. 10), while exposed albino skin or covered control areas showed no hyperpigmentation. None of the other control animals, that is, dosed albino rabbits, or nondosed Dutch Belts exposed to UV irradiation, showed any hyperpigmenta-

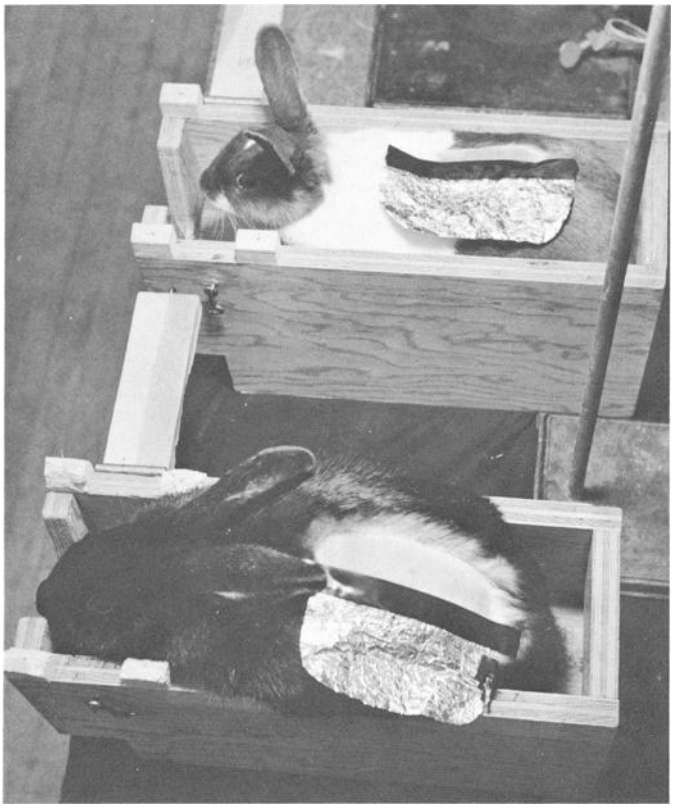
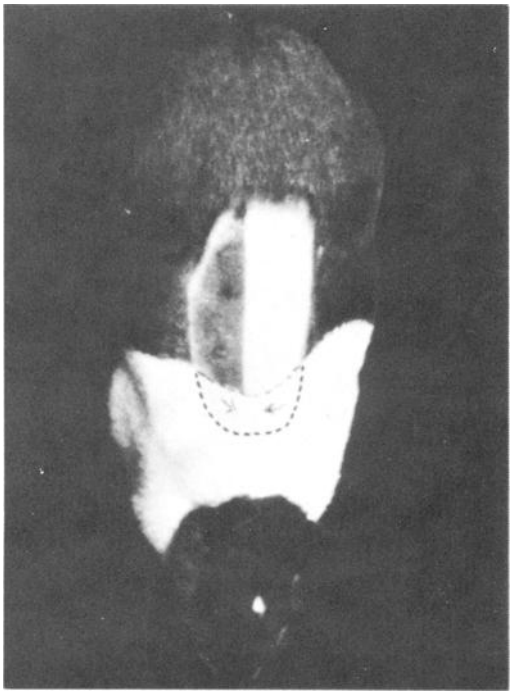


FIG. 9—*Rabbits in restraining boxes for ultraviolet irradiation of shaved dorsal skin.*

tion, or the characteristic histochemical correlate of the drop of the melanin granules from the epidermis into the dermis (Fig. 11). This satisfactorily demonstrated the *in vivo* mechanism of CP-melanin interaction, and also indicated that this side-effect is a cosmetic problem rather than a dangerous toxic reaction. The hyperpigmentation bleached uneventfully in all animals within a few weeks, when CP was discontinued, and somewhat more slowly at continuation of dosage. In a few animals phenobarbital was given to induce additional potential for glucuronic conjugation. These animals did indeed bleach more rapidly.

It should be remembered that the side-effect of hyperpigmentation of exposed skin areas affects very few patients, namely, somewhat less than 1 percent of the chronically dosed hospital patients. A genetic component which might either consist in overproduction of peripherally available melanin, or a slight metabolic imbalance of hydroxylation potential and the ability to conjugate all phenolic derivatives with glucuronic acid, appears to account for the incidence. This same genetic make-up, however, cannot explain the incidence of the much more frequently seen ocular pathology [*39*]. Opacities in lens and cornea appearing after long-term ingestion of CP in about 25 percent of patients appear to be unrelated to melanin interaction, as these tissues are devoid of melanin. To date we have been unable to find an animal model producing the same ocular manifestations. Ocular lesions produced in dogs or guinea pigs by various investigators under high and chronic



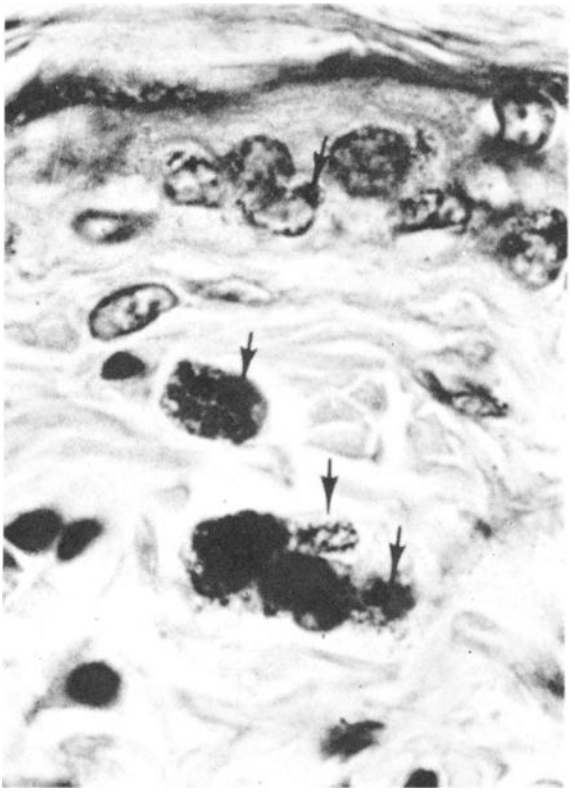
Chronic chlorpromazine-treated Dutch Belt rabbit showing hyperpigmentation of naturally pigmented area on the u.v. exposed portion of shaved skin. The albino area of shaved skin shows no difference between exposed and unexposed areas (arrows in area within dotted lines). Hyperpigmentation was produced with a 275 W General Electric sunlamp. Two small spots, each on the hyperpigmented and control sides, are the result of skin biopsies.

FIG. 10—*Chlorpromazine-induced hyperpigmentation of skin in rabbit.*

doses of CP, were not directly comparable to those seen in man. Efforts are now being made to study the Rhesus monkey in this context. A totally different mechanism may be at work in this instance. Elevated concentrations of β-glucuronidase [*40*] were reported to occur in the fluid of the ocular chamber, where they might hydrolize conjugated derivatives of the less soluble CP metabolites, for example, the deaminated derivatives of 2-chlorophenothiazine. It is hoped to prove or disprove this theory during the next 2 years, when Rhesus monkey tissues should become available.

In Vitro Tests

After studying *in vivo* CP metabolism in man, Rhesus monkey, cat, dog, rabbit, guinea pig, and sheep, it was concluded that a well-stocked zoo and several lifetimes would be needed to study all the species of interest as potential models for the various pharmacological effects of CP. *In vitro* tests could be performed in hours as compared to the months and sometimes years required for the *in vivo* studies. Although this is obviously reversing the natural sequence of a drug study program, a good deal was learned in the process [*41*] and some of the pertinent results will be summarized. Using conventional methods of preparing hepatic microsomal fractions, usually the 9000 g supernatants, and the con-



Chlorpromazine-treated rabbit, pigmented skin area after u.v. irradiation, H & E stain, × 1200 Pigment in both epidermis and dermis.

FIG. 11—*Drop of melanin granules from epidermis into dermis in hyperpigmented skin area.*

ventionally added cofactors in the microsomal incubations with ^{3}H-CP as the substrate [*42,43*], information was obtained on the various available drug-metabolizing pathways in the species studied.

Table 6 lists the results for 13 species of mammals. For 7 species *in vivo* data were available for comparison. It was found that all metabolites seen *in vitro*, had *in vivo* counterparts, usually roughly in the same proportions. The exceptions worth noting are the lack of glucuronide formation under the selected *in vitro* conditions, as well as higher percentages of *N*-oxidation seen *in vitro*. *N*-oxidation appears to be a primary type of biotransformation, which under *in vivo* conditions may be camouflaged by subsequent metabolic changes such as demethylation [*41*]. Basically, the information on the available drug metabolizing pathways in these various species should prove useful in the selection of test animals for known or new drugs of similar structure, as well as in the practice of veterinary medicine.

Another implication of these tests was the possible use of *in vitro* systems to prepare cold or labeled reference metabolites of phenothiazines or other structurally related heterocyclic drugs. Thus, the large percentage of CP *N*-oxide production by the hepatic microsomes of dog and guinea pig might be used for their preparation, while deaminated

TABLE 6—*Interspecies differences in* ^{3}H*-chlorpromazine metabolism* in vitro.

	Species												
Metabolite	Man, (5) M; (1) F	Cat, (2) M; (1) F	Sea lion, (2) M	Rhesus Monkey, (1) M	Squirrel Monkey, (3) M	Hog, (1) undetermined sex	Steer, (1) M	Horse, (1) F	Sheep, (2) undetermined sex	Rat, (4) M	Dog, (6) M	Rabbit*, (2) M; (4) F	Guinea pig*, (4) F
CP (unchanged)	80.9	78.5	68.1	61.4	56.0	56.2	50.2	44.6	30.7	23.4	14.1	14.0	11.6
nor$_1$CP	2.0	5.3	11.9	24.9	18.1	5.4	12.9	9.6	4.8	22.0	7.0	18.3	2.0
nor$_2$CP									2.3	0.8		14.5	
CPNO	0.5	1.6	1.7		2.7	23.0	9.8	6.6	17.9	25.0	63.0	2.2	60.1
CPO	8.5	7.6	10.8	3.4	14.5	5.0	16.5	18.4	5.9	11.8	6.6	15.8	9.4
nor$_1$CPO	Tr	1.0	0.6	0.7	2.5		2.9	1.4		1.6	2.1	5.9	5.4
nor$_2$CPO												2.0	
7-OHCP	0.6	1.6	1.6	3.2		7.1	4.6	13.2	34.0	4.5	1.8	2.9	0.3
nor$_1$7-OHCP	Tr		2.2	3.2				3.0	2.8	2.0		1.8	
nor$_2$7-OHCP												1.6	
7-OHCPO		0.3			1.3								
nor$_1$7-OHCPO				2.4						2.8			
Deam I	Tr							1.0		5.0	2.2	17.5	10.6
Deam II											0.8	0.6	0.3
UNP	1.5	1.4	0.5		1.1	0.4	0.6	1.6	0.4	0.2	1.6	2.1	0.2

*Values obtained were the same, using liver microsomes from either control or chronically CP-dosed animals. The numbers in parentheses following the species indicate the number of livers used. M = male; F = female. Numerical values represent percentages of unchanged chlorpromazine and metabolites formed. Tr = trace.

Abbreviations used: CP, chlorpromazine; nor$_1$CP, desmonomethylchlorpromazine; nor$_2$CP, desdimethylchlorpromazine; CPNO, chlorpromazine-N-oxide; CPO, chlorpromazine sulfoxide; nor$_1$CPO, desmonomethylchlorpromazine sulfoxide; nor$_2$CPO, desdimethylchlorpromazine sulfoxide; 7-OHCP, 7-hydroxychlorpromazine; nor$_1$7-OHCP, desmonomethyl-7-hydroxychlorpromazine; nor$_2$7-OHCP, desdimethyl-7-hydroxychlorpromazine; 7-OHCPO, 7-hydroxychlorpromazine sulfoxide; nor$_1$7-OHCPO, desmonomethyl-7-hydroxychlorpromazine sulfoxide; Deam I, unidentified deaminated I; Deam II, unidentified deaminated II; UNP, unidentified non-phenolic.

derivatives would be best prepared from rabbit liver microsomes. For present purposes, it was highly desirable to obtain ^{3}H-7-OHCP which is abundantly produced by sheep liver microsomes [*44*], but which requires a laborious multistep procedure for chemical synthesis.

In rabbit and guinea pig, chronic pretreatment of the animals with CP did not affect their drug-metabolizing potential.

Radioquantitation of Chlorpromazine Metabolites

Appropriately labeled CP, in this case tritiated at the metabolically insensitive position 9 of the nucleus, was most useful in numerous *in vitro* and *in vivo* studies. Thus, all CP metabolites produced in hepatic microsomal incubations, were quantitatively extracted by solvents and separated by TLC. The individual metabolites were scraped from the plates after spraying and counted directly in a Packard Tri-Carb scintillation spectrometer [*45,41*].

In vivo, the preparation was used to demonstrate placental transfer of CP in rabbits, guinea pigs, and sheep, by analyzing the CP metabolites in the various tissues of fetal and

neonatal guinea pigs and rabbits, or the urinary and fecal excretion in newborn lambs of dosed sheep [*44,46,47*]. Timing of the development of drug-metabolizing pathways in the fetal, prenatal and postnatal periods, as well as assay of the passage of CP into the milk [*47*] and into the hair of these animals [*48*] was also greatly facilitated by radioquantitation. Auxiliary drug detoxication by way of the hair is certainly a minor pathway, but in view of the fact that the balance sheet for input and output of chronically administered CP was very unsatisfactory [*11*], it was felt that all clues for the ultimate metabolic fate of the drug should be followed up. Table 7 lists the data for the fur of rabbit and guinea pig.

TABLE 7—*^{3}H-chlorpromazine in metabolites in the fur of rabbit and guinea pig.*[a]

	Rabbit		
After	Black Area	White Area	Guinea Pig
1 week	0.15	0.01	0.10
2 weeks	0.31	0.05	0.14
4 weeks	0.47	0.12	0.05
6 weeks	0.09	0.01	0.04
	1.02	0.19	
Total % in fur	1.21		0.33

[a] % of applied radioactivity.

The same two species also served in tracking the fate of a single dose of labeled CP in chronically dosed animals. Over a period of 15 days 95 percent of the label was excreted, 65 percent via the urine and 30 percent via the feces. The corresponding data for rabbit, within a 20-day period were 81 percent total excretion, of which 46 percent via the urine and 35 percent via the feces. The fate of a single small and acute dose of label in naive rabbits and guinea pigs has also been followed. Measurable excretion persisted for about 20 days, but appeared to be far from complete. The values for combined urinary and fecal excretion in five animals varied from 39 to 54 percent, and thus amounted to about one-half of the corresponding rate under chronic conditions. There was a shift toward diminished urinary excretion with a concomitant increase in percentage of fecal drug excretion noticeable in the metabolism of single drug doses [unpublished data].

In studies involving radioquantitation it was noted that special precautions must be taken to avoid spurious counts due to chemoluminescence [*49*]. They occurred in all types of biological material, when naturally alkaline preparations were counted, or if the recommended quaternary amines such as Hyamine or Soluene were used in the homogenization and solubilization of the preparations. Use of naphthalene-type scintillation cocktails instead of the toluene scintillation fluid, while avoiding high alkalinity, was adequate for direct counting of urines. Feces, certain tissues, and especially the keratinous materials hair and nail, however, clearly required oxidation of the material in the Packard Oxidizer, and counting of the resulting tritiated water. Some of these data are summarized in Table 8.

Fluorescent Labeling of Chlorpromazine Metabolites

While adequate methodology is available to assay urinary and fecal CP metabolites, as well as the drug content of most visceral tissues, certain biological materials such as blood and its subfractions, cerebrospinal fluid, saliva, brain, and fatty tissues contain very low

TABLE 8—*Effect of chemiluminescence on cpm in two scintillators.*

Material		Scint I		Scint II	
		Cycle 1	Cycle 2	Cycle 1	Cycle 2
Rabbit urine	Control	108 963	97 194	37	37
Guinea Pig urine	Control	5 062	4 639	36	34
^{3}H-CP, 100 μCi	Day 1	7 355	5 281	4 206	4 160
	Day 10	5 388	4 010	236	248
	Day 16	3 174	3 091	35	36
	Day 62	946	824	33	35
Sheep urine	Control	65 342	58 914	37	35
^{3}H-CP, 2 mCi	Day 1	85 222	80 082	11 083	11 072
Rhesus, male, urine	Control	26 520	25 696	35	34
^{3}H-CP 64 μCi, urine (3 day pool)		15 225	13 361	1 014	977
female, urine	Control	5 566	4 467	32	33
^{3}H-CP 64 μCi, urine (3 day pool)		9 997	8 537	1 164	1 156
male, serum	Control	57	54	36	36
Human urine					
male	Control	28 786	26 337	33	32
female	Control	6 168	5 323	32	33
neonate male	Control	13 996	11 684	37	36

Counting efficiency: 33–39%

Scintillator I
PPO 5 g; dimethyl POPOP 0.3 g; toluene to 1000 ml

Scintillator II
naphthalene 100 g; PPO 5 g; dimethyl POPOP 0.3 g; dioxane 730 ml; toluene 135 ml; methanol 35 ml

amounts of CP metabolites, which require very sensitive methods of determination. Using fluorescent labeling of suitable metabolites appears to be a promising approach. Simultaneous reports on the use of dansyl chloride for this purpose were made by Kaul et al [*15*] and Forrest et al [*50*]. Figures 12 and 13 show the structures of such dansylated derivatives of various CP metabolites and the related drugs desipramine and nortriptyline, as well as the mass spectral ions used in their identification by GC/MS. While Kaul is developing this approach into a workable assay for CP metabolites, we were primarily interested in using the fluorescence of these labeled drug metabolites for direct *in situ* demonstration of the compounds in microscopic studies. In most tissues, the background fluorescence and the fact that many endogenous constituents also produce dansylates, present problems not yet satisfactorily solved; however, in plasma smears on slides, it was very easy to distinguish between control specimens and patient samples. If this approach can be developed into at least a group assay for tricyclic drugs *in situ*, it might be useful to toxicologists in the future.

Chlorpromazine Assay using XAD-2 Non-Ionic Resin

Currently the use of XAD-2 resin is being explored for the adsorption and subsequent elution of CP metabolites. Urine, whole blood, and tissue homogenates have been tried to date. Tritiated samples were used in all instances for quantitative evaluation of each step, that is, spillage of compounds, elution by different solvents, and assay of label remaining tightly bound to the resin by Van der Waals forces.

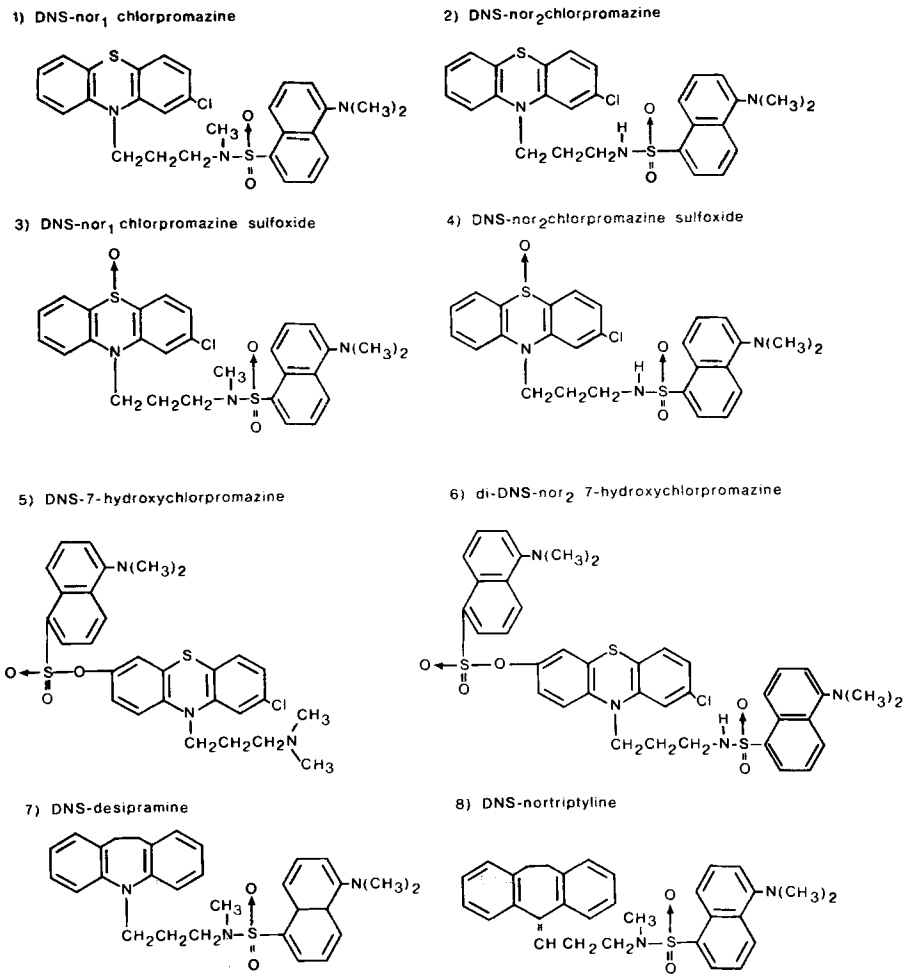


FIG. 12—*Structures of dansylated (DNS) drug derivatives.*

Using 10 ml of urine and 50 ml of resin suspension in both cases, all of the label was retained on the resin. Seventy-six percent of the label was found to be eluted by methanol, and subsequently 19 percent by ethyl acetate, and another 3 percent by tetrahydrofuran (THF). This accounted for 98 percent of the label applied. The various eluates have not as yet been analyzed by TLC to obtain a qualitative breakdown of the metabolites.

In the case of whole blood, a 5-ml aliquot was diluted with an equal volume of water, and added to 50 ml of resin suspension. In this instance there was an initial spillage of 10 percent which could not be eliminated by using more resin. Methanol eluted 60 percent of the label, ethyl acetate 17 percent thereafter, and THF another 8 percent. Including the spillage, 95 percent of the label was accounted for, but the final THF elution still contained 3 percent of the label, so that continuation of elution with this solvent might have yielded the missing 5 percent.

With 1 g of liver tissue homogenized in a total of 20 ml water and again using 50 ml of resin suspension, 3 percent of initial spillage was noted. Additional resin was not used,

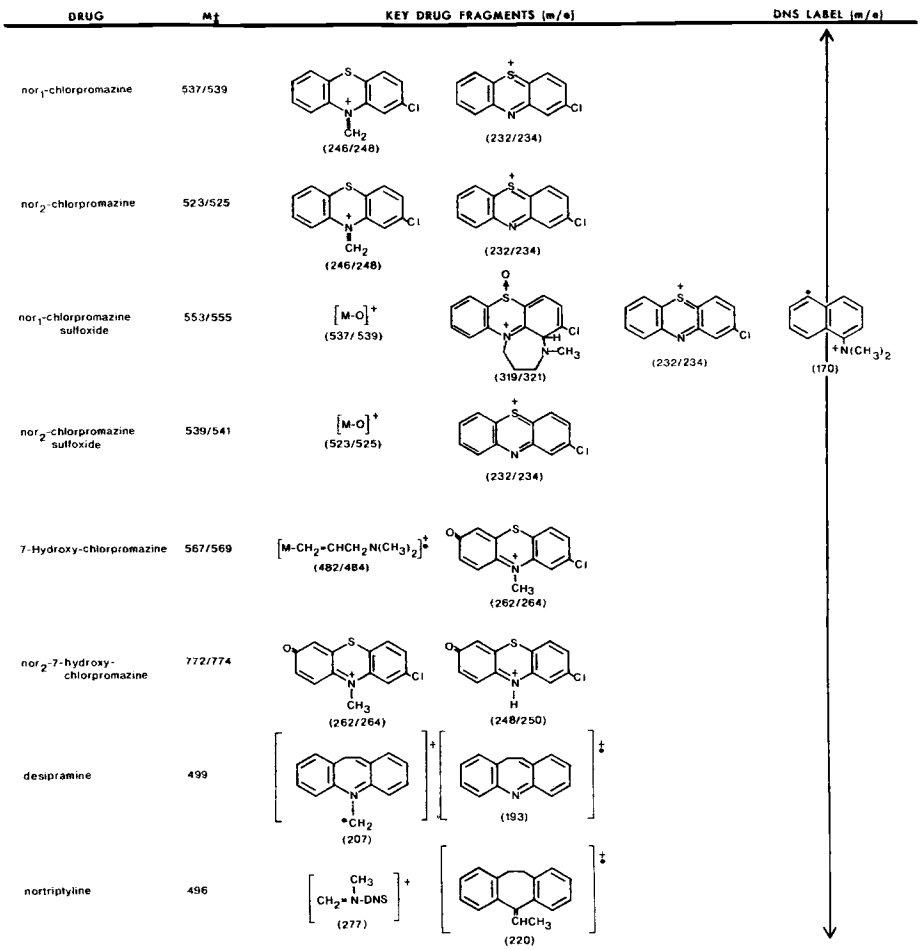


FIG. 13—*Mass spectral ions used for the identification of DNS drug derivatives.*

so it is not known whether this might have been avoided. Methanol eluted 38 percent of the label, ethyl acetate 52 percent, and THF another 7 percent, which, including the spillage, accounted for 100 percent of the label applied.

If the elution of individual drug metabolites, or groups of metabolites, is selective, as hoped and expected, this might indeed be developed into a practical new assay. Moreover, an efficient procedure to isolate the glucuronide fraction without procedural artifacts has been needed for many years, and the XAD-2 may be suitable for this purpose. There are no reference compounds for this important group of metabolites, and it is hoped that this approach will be useful for their preparation.

Assay of Chlorpromazine Metabolites by Mass Spectroscopy

Combined GC/MS has been employed by various groups of investigators to analyze CP metabolites in blood and urine. As mentioned previously, this elegant procedure suffers only from the inadequacy of the solvent extraction procedures used prior to injection of

the material. A still experimental, but most promising technique consists in introducing an unextracted sample of blood or urine into a mass spectrometer, by way of a selective membrane. The drug content is volatilized, and selected mass peaks which are uniquely characteristic of specific metabolites are monitored. This technique known as chemical vapor analysis (or CVA) is currently being developed [*51*]. Direct multiple mass fragmentography (DMMF) is a variation thereof [*52,53*]. Using CVA, some of the major urinary CP metabolites, notably the sulfoxides, have been detected in preliminary tests on unprocessed urines.

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