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A Detailed Evaluation of Promazine Metabolism.* (29112)

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Unlike chlorpromazine and other phenothiazine tranquilizers which have been studied extensively over the last decade(1), promazine has received little attention as to its metabolism by man. Walkenstein and Seifter(2) reported that promazine is transformed by animals to yield nor$_1$promazine, promazine sulfoxide and nor$_1$promazine sulfoxide as urinary excretion products.† Additional products were also detected on their paper chromatograms but not identified. No evidence was found for didemethylation of the dimethylaminopropyl side chain. The possibility that promazine can undergo 3-hydroxylation as well as didemethylation was suspected from a more recent study(3), when promazine phenols were isolated whose properties proved to be analogous to the 7-hydroxychlorpromazine derivatives excreted during chlorpromazine therapy.‡

Confirmation of these preliminary indica-

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† The following abbreviations are used: TLC = thin-layer chromatography, P = promazine, CP = chlorpromazine, PSO = promazine sulfoxide, nor$_1$ = desmonomethyl, nor$_2$ = desdimethyl, PNO = promazine-N-oxide, HO = hydroxy, EdBzF = ethylene dichloride-benzene-formic acid-water (3/1/4/2), BuEt = n-butanol-ethanol-water (15/2/5) and BzA = benzene-acetic acid-water (2/2/1).

‡ Due to its symmetry, the 3- and 7-positions of promazine are equivalent (Fig. 1).

tions is given here. By means of thin-layer chromatography, many new metabolites of promazine have been isolated from human and animal urine and identified. It is shown that promazine differs quantitatively from chlorpromazine in its metabolism.

Experimental. Promazine hydrochloride was administered orally to 2 male and 3 female human adults in fixed doses ranging from 100 mg to 500 mg t.i.d., and to female mongrel dogs and rabbits (New Zealand whites) in doses of 4-4.5 mg/kg b.i.d. The animals were maintained on the drug for 6 days, the humans for 2-6 days. All urine samples (pH 5.5-7.5) were collected on a 24-hour basis, frozen, and stored for periods of 1-6 weeks before analysis.

Extraction procedure. The unconjugated metabolites of promazine (fraction AB), which include its nonphenolic and free phenolic derivatives, were extracted from urine at pH 9 with 3 volumes of ethylene dichloride (Eastman). Second and third extracts were prepared and discarded. The residual urine was divided into 3 portions, 2 for enzymatic hydrolysis of the glucuronide and sulfate conjugates, and the third to serve as a control. One portion was incubated overnight at pH 4.5 and 37° with one-tenth volume of β-glucuronidase (Ketodase, 5000 units/ml), then adjusted to pH 9 and extracted with ethylene dichloride (fraction C). The second portion was incubated at pH 6.2 with Mylase P (Wallerstein Co.) in 1% concentration and subsequently extracted in the usual manner (fraction D). The 3 organic extracts were washed with pH 9 ammonia buffer and dried over sodium sulfate. They were then taken to dryness and dissolved in methanol for chromatographic resolution.

Thin-layer and paper chromatography. A slurry containing 30 g silica gel G and 60 ml water was applied in a 250 μ layer to 20 × 20 cm glass plates using the Desaga-Brinkmann spreader. After standing for 15 minutes, the plates were dried in an oven at 110° for an hour and stored in a desiccator. Plates spotted for either 1- or 2-dimensional chromatography were developed in rectangular jars fitted with paper curtains. Chromatographic development was terminated when the solvent had risen 14 cm past the spotting point. In Table II and Fig. 2-7 are shown the solvent systems used. Some paper chromatographic separations were also carried out, based on systems employed previously (3).

The silica gel plates were sprayed with persulfate reagent(4) to locate the promazine derivatives. Secondary amines were detected by means of nitroprusside-acetaldehyde(2). To detect primary amines, a 0.2% solution of ninhydrin in n-butanol served as a selective and sensitive reagent. After thorough saturation with the solution, the plates were allowed to dry for 10 minutes *without use of heat*. Primary amines gave blue-purple spots against a white background. For paper chromatograms, the ninhydrin reagent was prepared with acetone.

Quantitative analyses. The metabolites were visualized on the chromatograms with persulfate reagent and compared to a series of graded method standards. These standards had been added to normal urine and treated in the same manner as the unknowns to correct for experimental losses and other factors. When appropriate standards were not available, suitable substitutions were made. Nor_2PSO, Nor_1PSO and PNO were determined by reference to PSO, while 3-HO-Nor_1P, 3-HO-Nor_2P, L4 and L5 were referred to the 3-HO-P standard. TLC was used for the following analyses: P and PSO (chloroform-acetone-diethylamine, 8.7/0.3/1); PNO (ethyl acetate-methanol-diethylamine, 14/4/3); 3-HO-P, 3-HO-Nor_1P, 3-HO-Nor_2P, L4 and L5 in fractions C and D (acetone-isopropanol-1% ammonia, 9/7/4); and 3-HO-P and 3-HO-Nor_1P in fraction AB (chloroform-acetone-diethylamine, 2/7/1 and ethyl acetate-acetone-methanol-diethylamine, 13.6/0.4/4/3). Paper chromatography was employed for analysis of Nor_1P (ammonia-methanol, 1/1) and Nor_1PSO and Nor_2PSO (EdBzF).

Preparation of derivatives. Promazine sulfides were converted to the corresponding sulfoxides by oxidation on filter paper with 3% hydrogen peroxide as described previously(3). The reverse reaction was accom-

plished with zinc and acetic acid(3). Phenols were O-methylated using diazomethane(3).

Results. Isolation of 3-HO-P (L3) and its sulfoxide and desmethyl derivatives. Evidence that promazine undergoes 3-hydroxylation was obtained in working up extracts prepared from glucuronide hydrolyzates (fraction C) of human urine. The material was chromatographed on paper using EdBzF in the first dimension to eliminate the faster-moving compounds, and BuEt in the second dimension. When stained with persulfate reagent, 1 periwinkle (purple-blue) and 3 lavender spots appeared. A similar pattern of metabolites has been described in our earlier chlorpromazine studies (Fig. 1, ref. 3). Their state of N-methylation was determined with the ninhydrin, nitroprusside and Dragendorff reagents. The 3 lavender phenols reacted as primary, secondary and tertiary amines, respectively, and are designated L1, L2 and L3. The periwinkle factor, Pwk 3, reacted as a tertiary amine. Ultraviolet studies on their chromatographic eluates revealed a sulfoxide spectrum for Pwk 3, and sulfide spectra for the lavender phenols.

L3 was identified as 3-HO-P (Fig. 1) by comparing it with authentic 1-HO-P, 2-HO-P, 3-HO-P and 4-HO-P. Of the 4 isomers, only 3-HO-P gave a lavender persulfate stain, and an absorption maximum in sulfuric acid similar to that obtained with L3 (564 mμ, Table I). L3 and 3-HO-P showed similar chromatographic behavior on paper in 3

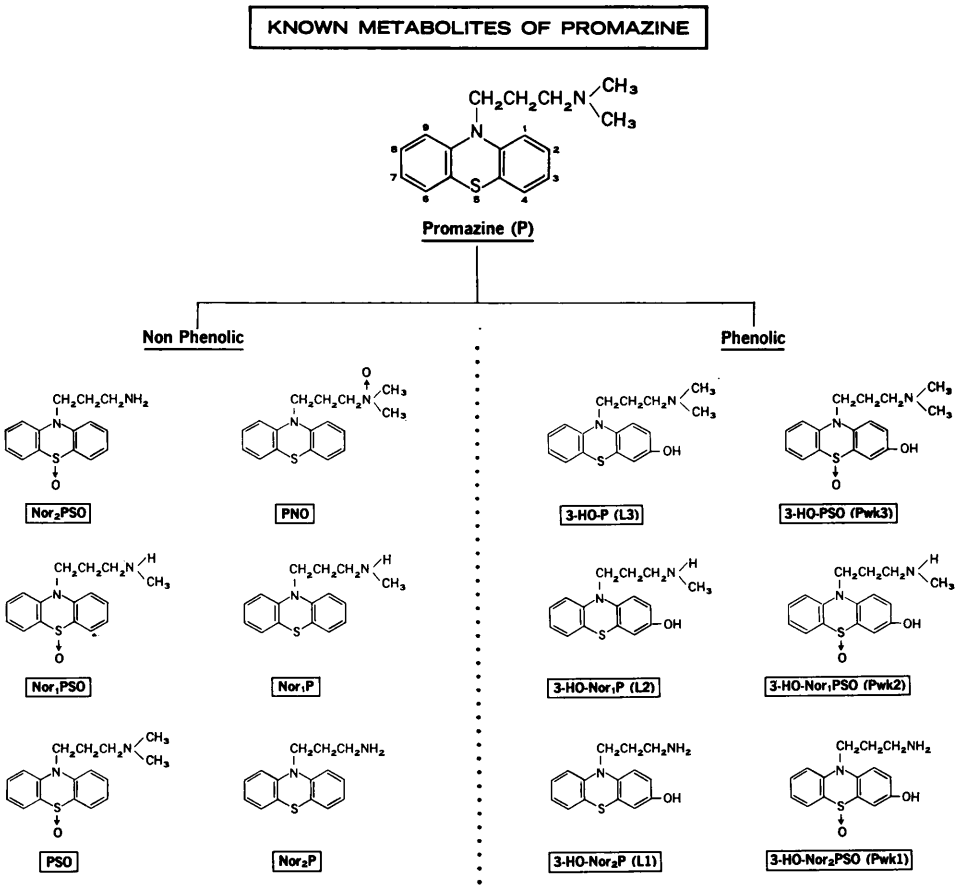

FIG. 1. Structures of promazine and its known human metabolites. The phenolic derivatives are excreted primarily as their glucuronic acid conjugates, but some also appear as ethereal sulfates, or in unbound form. The major metabolites are Nor_2PSO and Nor_1PSO, also 3-HO-P glucuronide, 3-HO-Nor_1P glucuronide, L4 and L5.

TABLE I. Color Reactions of Urinary and Synthetic Hydroxypromazines.

Substance	On chromatograms*			In 25% sulfuric acid	
	Pers	Nin	N-a	Color	Absorption max (mμ)
L3	lavender	–	–	lavender	564
L2	"	–	+	"	564
L1	"	+	–	"	564
1-HO-P	tan-peach	–	–	tan	435
2-HO-P	orchid	–	–	orchid-pink	548
3-HO-P	lavender	–	–	lavender	564
4-HO-P	tan-peach	–	–	tan-peach	489†

* Pers = persulfate stain (paper); Nin = ninhydrin (paper and TLC); N-a = nitroprusside-acetaldehyde (paper and TLC).
† Broad, diffuse peak.

solvent systems (BzA, EdBzF and BuEt) and in 6 TLC systems (Table II). Further evidence for their identity was obtained by preparing derivatives. Upon methylating L3 and 3-HO-P with diazomethane, the reaction products formed (3-MeO-P) yielded identical UV spectra, color tests and chromatographic properties (Table II). A second derivative was made by oxidizing L3 and 3-HO-P with peroxide to form the sulfoxides. These products also proved to have identical chromatographic behavior (Table II), UV spectra and persulfate reactions (periwinkle).

Identification of Pwk 3, L1 and L2. Pwk 3 was indistinguishable from the sulfoxide of 3-HO-P in all respects, including persulfate reaction (periwinkle), UV spectra and R_f's in 6 TLC systems (Table II). Its identity as 3-HO-PSO (Fig. 1) was confirmed when it was reduced with zinc and acetic acid to give a compound similar to 3-HO-P by all known criteria.

Their reactions to acid (Table I) and to ninhydrin and nitroprusside suggested that L1 = 3-HO-Nor$_2$P and L2 = 3-HO-Nor$_1$P. An analogous relationship has been demonstated for 7-HO-CP and its congeners(3). An attempt was made to N-methylate L1 and L2 with methyl iodide and aqueous carbonate(3) to give authentic 3-HO-P, but the reaction proceeded all the way to the quaternary amine. 3-HO-P yielded a chromatographically similar product. This supports our structural assignments for L1 and L2 (Fig. 1).

Isolation of other urinary metabolites. A number of TLC systems were devised to resolve the promazine metabolites present in fractions AB, C and D. Typical results are given in Fig. 2-7. The phenols are represented by the dark spots in the Figures. TLC revealed some new phenols such as L4, L5, Pwk 1 and Pwk 2. L4 and L5 occur in human urine primarily as glucuronides (Fig. 3), but are also excreted in free form (Fig. 2). They have not been identified with certainty; however, studies now in progress suggest that they may be the N-acetyl derivatives of L1 and L2, *i.e.*, N-acetyl-3-HO-Nor$_2$P and N-acetyl-3-HO-Nor$_1$P. Pwk 1 and Pwk 2 were isolated from human glucuronide hydrolyzates by 1-dimensional TLC using acetone-isopropanol-25% ammonia (9/7/4) as the solvent (Fig. 6a). They yielded periwinkle

TABLE II. Identification of 3-Hydroxypromazine (L3) and Its Sulfoxide (Pwk 3) by Means of Thin-Layer Chromatography

Solvent system*	R_f						
			O-methylated		Oxidn product of		
	L3	3-HO-P	L3	3-HO-P	L3	3-HO-P	Pwk 3
1	.81	.81			.55	.55	.55
2	.45	.45			.15	.15	.15
3	.25	.25	.66	.66	.05	.05	.05
4	.45	.45	.70	.70	.04	.04	.04
5	.53	.53	.73	.73	.15	.15	.15
6	.08	.08	.70	.70			

* 1 = acetone-isopropanol-25% ammonia (9/7/4).
2 = acetone-isopropanol-1% ammonia (9/7/4).
3 = acetone-diethylamine (9/1).
4 = ethanol-dioxane-benzene-ammonia (5/40/50/5).
5 = ethyl acetate-acetone-methanol-diethylamine (13.6/0.4/4/3).
6 = chloroform-acetone-diethylamine (8.6/0.4/1).

colors on paper with persulfate reagent, and sulfoxide spectra in solution. When L1 and L2 were oxidized to the sulfoxide state, they behaved similarly to Pwk 1 and Pwk 2 (Fig. 6b). From this we deduce that Pwk 1 = 3-HO-Nor_2PSO and Pwk 2 = 3-HO-Nor_1PSO (Fig. 1).

Six nonpolar metabolites were identified in fraction AB of human urine, *viz.*, Nor_2PSO, Nor_1PSO, PSO, PNO, Nor_1P and Nor_2P (Fig. 1, 2 and 7c). Nor_1P, PSO and Nor_1PSO were previously found by Walkenstein and Seifter(2) in dog urine. The PSO, Nor_1P and Nor_2P isolated from human urine correlated with the corresponding authentic standards in their color reactions, UV spectra and by chromatography. Nor_2PSO and Nor_1PSO were identified by comparison with the sulfoxides of Nor_2P and Nor_1P, which were generated *via* peroxide treatment on paper. The structure of the N-oxide was deduced in a manner similar to that described for CPNO(5).

The peach (Pe) and orchid (Orc) factors

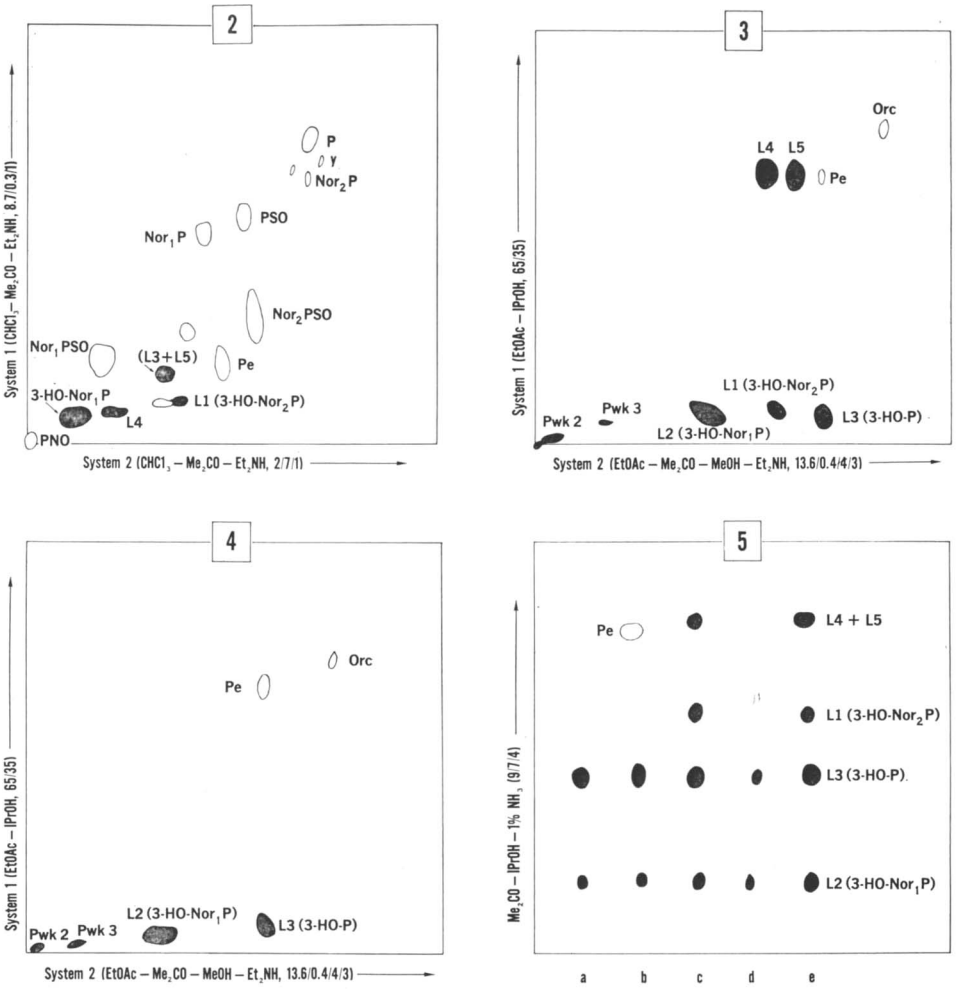

FIG. 2-5. Thin-layer chromatograms of promazine and its metabolites, stained with persulfate reagent. Dark spots indicate lavender color (phenols), all other spots pink except for Pe (peach factor), Orc (orchid factor), and y (yellow color): 2. Unconjugated human metabolites. 3. Human glucuronide hydrolyzate. 4. Dog glucuronide hydrolyzate. 5. Animal and human metabolites of promazine — a, unconjugated rabbit metabolites. b, dog glucuronide hydrolyzate. c, human glucuronide hydrolyzate. d, human sulfate hydrolyzate. e, mixture of eluted metabolites and synthetic 3-HO-P.

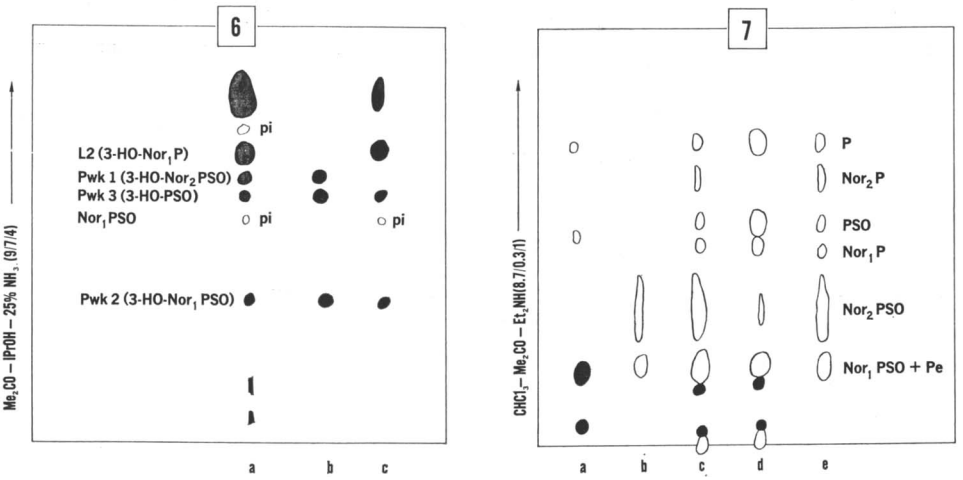

FIG. 6. Separation of 3-HO-PSO from its desmethyl derivatives by thin-layer chromatography: a, human glucuronide hydrolyzate. b, oxidation products of 3-HO-Nor$_1$P, 3-HO-P and 3-HO-Nor$_2$P, in ascending order. c, dog glucuronide hydrolyzate.

FIG. 7. Resolution of unconjugated metabolites of promazine: a, rabbit; c, man; d, dog. b, oxidation products of Nor$_1$P and Nor$_2$P, in ascending order. e, mixture of standards and some eluted metabolites (Nor$_2$PSO and Nor$_1$PSO + Pe).

and other trace metabolites of promazine have been detected in urine but not identified. Pe occurs in significant quantities in dog urine, which is especially suitable for demonstrating Pe and Orc due to the absence of L4 and L5 (compare Fig. 4 with Fig. 3). Chromatographic studies have shown that Orc is not 2-HO-P, and Pe is not 1-HO-P or 4-HO-P. Their structures remain to be determined. (See *Addendum.*)

Species differences. Some concept of the qualitative and quantitative differences between human and animal metabolite patterns may be obtained from Tables III and IV and Fig. 2-7. When taken orally, man excretes promazine to the extent of about 33% in urine. The remainder is doubtless distributed for the most part between the feces and body storage depots. Our estimates for the individual human fractions are as follows: nonphenols, 14%; phenol glucuronides, 15%; phenol sulfates, 3%; and free phenols, 1 or 2%. It is apparent from these data that the urinary metabolites are divided between phenols and nonphenols in the ratio of 19:14, or about 1.4 to 1. The major components of the glucuronide fraction are 3-HO-P, 3-HO-Nor$_1$P, L4 and L5. 3-HO-P and 3-HO-Nor$_1$P are also excreted as ethereal sulfates (Fig. 5d) along with small amounts of their sulfoxides. The predominant metabolites in the nonpolar fraction are the desmethyl sulfoxides, Nor$_2$PSO and Nor$_1$PSO.

The dog metabolite pattern differs from the human in 2 major respects: (a) dogs only appear to excrete about 13% of their medication in urine. The favored metabolites are the nonphenols, principally PSO and Nor$_1$PSO; (b) they do not demethylate as extensively as humans. This is shown by their decreased excretion of Nor$_2$PSO, their negligible excretion of 3-HO-Nor$_2$P glucuronide, and the absence of 3-HO-Nor$_2$PSO glucuronide and Nor$_2$P. Pe and Orc occur in dog urine, but not L4 and L5.

Rabbits were found to resemble neither dogs nor humans in their disposition of promazine. Only 10-13% of the drug could be detected in urine. This occurred almost wholly in the phenol fractions. As seen in Table IV, 3-HO-P is the principal metabolite and is distributed equally between the free phenol and the glucuronide, and in smaller amounts as the sulfate. The other significant product is 3-HO-Nor$_1$P. 3-Hydroxylation is clearly the major route of promazine metabolism in the rabbit.

Discussion. The several biochemical routes

TABLE III. Daily Urinary Excretion Values for Promazine and Its Metabolites.*
(Expressed as % of dose.)

Nonphenols	Man†	Dog‡
Nor_2PSO	6.3 ± .6	.6 ± .21
Nor_1PSO	5.2 ± .9	2.8 ± .9
PSO	.7 ± .18	3.8 ± 1.7
PNO	.8 ± .22	1.1 ± .9
P	.2 ± .15	.8 ± .35
Nor_1P	.4 ± .19	.2 ± .12
Nor_2P	trace	—
Total	13.7	9.3

	Unconjugated		Glucuronide	
Phenols	Man†	Dog‡	Man†	Dog‡
3-HO-P	trace	.1 ± .02	3.7 ± 1.1	.7 ± .22
3-HO-Nor_1P	.5 ± .14	.1 ± .04	3.1 ± 1.1	.8 ± .32
3-HO-Nor_2P	trace	—	.9 ± .32	trace
L4 + L5	trace	—	5.5 ± .42	—
3-HO-PSO	—	—	P/U	P/U
3-HO-Nor_1PSO	—	—	P/U	P/U
3-HO-Nor_2PSO	—	—	P/U	—
Total	~1	trace	~15	~2

	Fraction AB		Fraction C	
Other metabolites	Man†	Dog‡	Man†	Dog‡
Pe	P/U	P/U	P/U	P/U
Orc	trace	trace	trace	trace

* Values represent mean ± stand deviation; (—) represents absence of metabolite; P/U = present, but undetermined.

† Each subject received 300 mg promazine hydrochloride daily. Analyses are based on 2 urine collections from each of 5 subjects.

‡ Analyses are based on 6 urine collections from each of 2 dogs.

of transformation which have been demonstrated for promazine (demethylation, oxidation, hydroxylation and conjugation) are responsible for the numerous metabolites—possibly 26 to 30—that appear in human urine. Ignoring the different forms of conjugation, these metabolites reflect 16 to 18 basic variations on the native promazine molecule. Twelve compounds are identified in Fig. 1; the remaining ones include the L4 and L5 phenols, as well as Pe, Orc and some other trace substances. From a quantitative viewpoint, the important human metabolites are Nor_2PSO, Nor_1PSO, 3-HO-P glucuronide, 3-HO-Nor_1P glucuronide and L4 and L5 glucuronides. These substances total 24%, or about ¾ of the urinary promazine output.

TABLE IV. % Excretion of Promazine Phenols by the Rabbit.*

Urinary product	% Excreted as		
	Free phenol	Glucuronide	Sulfate
3-HO-P	2.7	2.4	1.4
3-HO-Nor_1P	.7	.6	.5

* Six 24-hour urine specimens from each of 2 rabbits were pooled for analysis.

The metabolism of promazine and chlorpromazine(3,5,6) is similar in some gross respects, as one would anticipate from their similar structures. Both drugs undergo side chain demethylation and N-oxidation. Like phenothiazine(7), both drugs orient *para* to the ring nitrogen atom; for example, they hydroxylate at the 3- or 7-position,§ and oxidize at position 5 to yield the sulfoxide. The significant differences between the drugs lie in their relative urinary output, and in their demethylation patterns. Dogs excrete less promazine than chlorpromazine. This is evident from the respective nonpolar fractions, which total 9% and 17%(6). In contrast, the human nonpolar fractions show the reverse trend, *viz.*, 14% for promazine *vs* 7%

§ Hydroxylation of chlorpromazine at the 3-position has not yet been demonstrated.

for chlorpromazine(6). The total urinary excretion also appears to favor promazine (33%) if we adopt Nadeau and Sobolewski's estimate for chlorpromazine of 20% or less (8). However, final judgment in this matter must be reserved because of conflicting estimates by other authors.

It is instructive to examine the demethylation patterns for the 2 drugs. Neither Nor_1CP nor Nor_2CP has been reported in human urine, whereas 7-HO-Nor_2CP glucuronide constitutes an important metabolite of chlorpromazine. Conversely, 3-HO-Nor_2P glucuronide is a minor promazine metabolite, but Nor_1P and Nor_2P can be detected as we have demonstrated above (Table III, Fig. 2 and 7c). A possible explanation for the absence of Nor_1CP and Nor_2CP is that they are formed as intermediates, but hydroxylate so readily that they do not appear in the urine. Further information might be obtained by comparing the metabolism of the desmethyl chlorpromazines with the desmethyl promazines.

Summary. Thin-layer and paper chromatography have been used to study the metabolism of promazine following its oral administration to man and animals. Humans excrete about 33% of the drug in urine, in the form of 26-30 derivatives. These compounds are distributed between nonphenolic (14%) and phenolic (19%) fractions in the ratio of 1 to 1.4. The principal metabolites are nor_2promazine sulfoxide (6.3%), nor_1promazine sulfoxide (5.2%), 3-hydroxypromazine glucuronide (3.7%), 3-hydroxy-nor_1promazine glucuronide (3.1%), and the glucuronides of L4 and L5 (5.5%). In addition to the desmethyl sulfoxides, the nonphenolic fraction contains promazine-N-oxide, promazine sulfoxide, unchanged promazine, nor_1promazine and traces of nor_2promazine. The phenolic fractions consist of glucuronides (15%), ethereal sulfates (3%) and unconjugated phenols (1-2%). The compounds bound to glucuronic acid include 3-hydroxypromazine and its 2 desmethyl derivatives, the 3 corresponding sulfoxides, and L4 and L5. 3-Hydroxypromazine and 3-hydroxy-nor_1promazine and their sulfoxides are also excreted as ethereal sulfates.

Relatively small amounts of the drug are voided by dogs (13%) and rabbits (10-13%). Furthermore, these animals exhibit markedly different excretion patterns. The metabolites in dog urine are present for the most part in the nonpolar fraction, principally as promazine sulfoxide and nor_1promazine sulfoxide, while rabbits tend to excrete the drug as free or conjugated phenols. The major rabbit metabolite, 3-hydroxypromazine, is distributed among all 3 phenol fractions. Neither animal produces primary amine metabolites in amounts comparable to man.

Addendum. The peach factor *(Pe)* has recently been identified in our laboratory as phenothiazine sulfoxide. This indicates that both man and dog can degrade the side chain of promazine down to the phenothiazine nucleus. *Orc* appears to be an oxidation product derived from 3-hydroxyphenothiazine. Chlorpromazine yields the corresponding 2-chloro derivatives as metabolites.

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