In other experiments the effects of sympathetic denervation on MAO activity in salivary glands was examined. Rats were subjected to unilateral superior cervical ganglionectomy, and both submaxillary glands were removed and assayed for monoamine oxidase activity 7 days after ganglionectomy (Table 3). There was a 28% decrease of enzyme activity in denervated glands as compared with the contralateral innervated glands. Similar findings have been obtained by Pöch.[10]

TABLE 3. EFFECT OF SUPERIOR CERVICAL GANGLIONECTOMY ON SALIVARY GLAND MONOAMINE OXIDASE ACTIVITY

Group	No. of rats	MAO Activity
Innervated side	7	4·67 ± 0·23
Denervated side	7	3·32 ± 0·35*

* $P < 0.01$.

The decrease in MAO activity after sympathetic denervation of tissues suggests that MAO is localized in sympathetic nerve endings. The marked effect of denervation on this enzyme activity in the pineal gland is in accordance with the high concentration of sympathetic nerve endings in this organ. These data are consistent with theories which hold that deamination of a portion of endogenous catecholamines can occur within the sympathetic nerves under normal conditions and especially after release by certain drugs, such as reserpine and guanethidine.[1] Catecholamines so released would then enter the circulation as deaminated and, consequently, inactive metabolic products.

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Comments on "7-hydroxychlorpromazine in the urines of schizophrenics receiving chlorpromazine"*

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IT WAS reported by Fishman and Goldenberg[1] early in 1963 that chlorpromazine is hydroxylated by man and animals at the 7-position to yield a family of closely related derivatives. These metabolites

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were isolated from urinary glucuronide hydrolyzates and identified† as 7-HO-CP, 7-HO-Nor$_1$CP, 7-HO-Nor$_2$CP, and 7-HO-CPSO. In a subsequent paper from our laboratory[2] the 7-position was established as the principal site of hydroxylation of chlorpromazine, and four new members of the family were reported: 7-HO-Nor$_1$CPSO, 7-HO-Nor$_2$CPSO, L4, and L5. Reference is made to Fig. 1 for structural formulas.

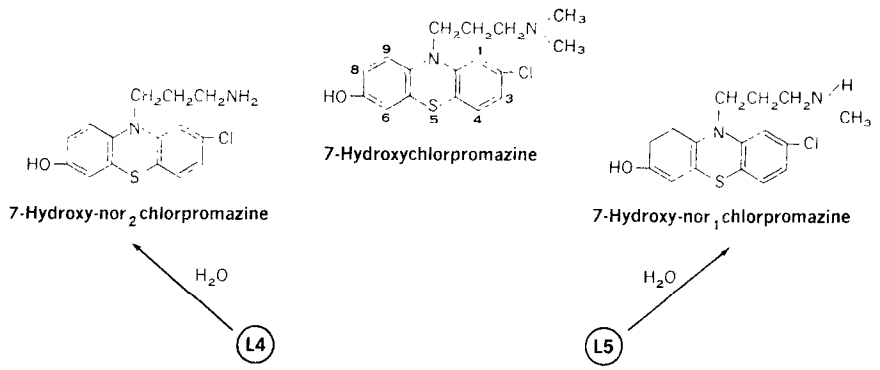

FIG. 1. Five metabolites of chlorpromazine which bear the 7-hydroxy group and yield similar color reactions with $FeCl_3$ (purple). L4 and L5 are derivatives of 7-HO-Nor$_2$CP and 7-HO-Nor$_1$CP, respectively, as shown by hydrolysis. Sulfoxide metabolites such as 7-HO-CPSO, 7-HO-Nor$_1$CPSO, and 7-HO-Nor$_2$CPSO, which are not shown here, do not react with $FeCl_3$ but respond to persulfate reagent with a periwinkle (purple-blue) color.

A recent communication to this journal by Price *et al.*[3] presented additional evidence for 7-HO-CP in the urines of schizophrenics receiving chlorpromazine. The metabolite was quantitated and found to average 0·3 to 0·4% of drug intake. We have reviewed the experimental methods employed by Price and co-workers and wish to point out the following (1) Their supporting data for identification of 7-HO-CP are equivocal in nature and could have been used equally well for claiming 3-HO-CP as a metabolite of chlorpromazine.‡ (2) The excretion values for 7-HO-CP are doubtful, since at least five different phenolic metabolites are detected by their analytical method. The values are also misleading because the authors have overlooked the major route of elimination of the phenols, viz., via bound forms such as glucuronic and sulfuric acid conjugates.

OBSERVATIONS

The claims of Price and co-workers[3] are based on the similarity in behavior between urine extracts and authentic 7-HO-CP with respect to the following properties: absorption spectra in ferric chloride solution, paper chromatography, thin-layer chromatography, and gas chromatography. The authors do not state whether any effort was made to establish the specificity of their methods. As we shall show below, the methods used were either partially or completely nonspecific.

Absorption spectra. The visible spectrum of 7-HO-CP in 10^{-3} M ferric chloride (0·1 N HCl) was found by Price to have absorption maxima at about 515 mμ and 545 mμ. Similar spectra were reported with extracts from patients' urine. There is no indication whether other hydroxyphenothiazine derivatives were studied. We therefore tested the reaction of several phenols with ferric chloride. It was of interest to note that 3-HO-CP also yielded a double band, with absorption maxima at 505 mμ

† The following abbreviations are used: CP = chlorpromazine, CPSO = chlorpromazine sulfoxide, CPNO = chlorpromazine-N-oxide, Nor$_1$ = desmonomethyl, Nor$_2$ = desdimethyl, 2-Cl-PhzSO = 2-chlorophenothiazine sulfoxide, pwk = periwinkle (purple-blue) and bl = blue.

‡ There is, in fact, some preliminary evidence in our laboratory for 3-hydroxylation as a minor (trace) route of metabolism.

and 535 mμ. The proximity of these values to the 7-HO-CP peaks raises some question as to the interpretation of the spectra obtained with urine extracts, which actually contain not one, but a number of chromogenic drug metabolites (see Fig. 2 and concluding paragraph below).

Chromatography. Urine specimens from schizophrenic patients on chlorpromazine therapy were extracted and chromatographed according to the procedures of Price and co-workers.[3] Two extracts are described in their paper: a crude methanolic one (I), prepared from syrupy urine concentrates; and a purified organic extract (II), made by taking (I) to dryness, partitioning the metabolites into methylene dichloride from pH 6·8 phosphate buffer, re-extracting with 0·1 N HCl, and finally drawing the metabolites back into methylene dichloride after adjusting the acid solution to pH 10. Both extracts were chromatographed by the authors' paper and thin-layer solvent systems (Table 1, footnotes); (II) gave cleaner results than (I), which was heavily contaminated with inorganic salts.

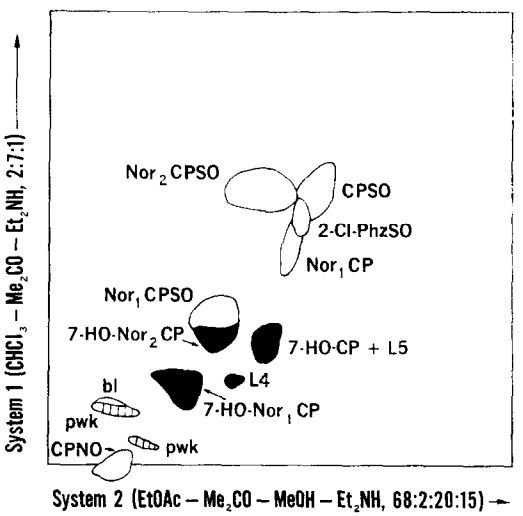

FIG. 2. Thin-layer chromatogram of R_F 0·8 band. The band was eluted from a paper chromatogram which had been streaked with urine extract and chromatographed with *n*-butanol:ethanol:water (5:2:2).

In agreement with the observations of Price *et al.*, urine extracts chromatographed on paper with *n*-butanol:ethanol:water (5:2:2) gave a band at R_F 0·8 which was equivalent in position to authentic 7-HO-CP. Similar agreement was obtained by thin-layer chromatography with acetone as solvent. We now proceeded to evaluate the significance of these results by studying the resolving power of the two chromatographic systems. As shown in Table 1, neither system could distinguish 7-HO-CP from such isomers as 3-HO-CP and 8-HO-CP. In fact, methylation of the hydroxyl group (7-MeO-CP) or its removal (CP) appeared to have little effect on mobility in either solvent. From these observations it is concluded that the two chromatographic systems did not serve the purpose for which they were used. Other solvents are available, however, for the separation of chlorpromazine phenols. For example, 7-HO-CP (R_F 0·52) and 3-HO-CP (R_F 0·37) can be resolved by thin-layer chromatography with our ethyl acetate mixture (system 2, Fig. 2); 7-HO-CP gives a typical lavender color on the chromatogram using persulfate reagent,[4] while a navy blue spot is noted for 3-HO-CP.

The authors[3] indicate that one urine extract was acetylated and gave a gas chromatographic peak corresponding to acetylated 7-HO-CP. No statement was made as to the column packing or the overall distribution of peaks. These urine extracts contain numerous chlorpromazine metabolites and, in our experience with several packings (XE-60 and SE-30 on Gas-Chrom P), it would be statistically unlikely if at least one component of the acetylated mixture did not correspond in approximate retention time to a proffered standard, in this case 7-AcO-CP.

The purity of the "7-hydroxychlorpromazine band" present on the paper chromatograms of Price and co-authors was assayed by eluting the area at R_F 0·8 with methanol and rechromatographing by thin-layer chromatography. For this purpose a two-dimensional system was used which was developed in our own laboratory. The chromatogram (Fig. 2) was stained with persulfate reagent. At least fourteen metabolites could be detected, and most were identified. The dark spots represent five

TABLE 1. RESOLVING POWER OF CHROMATOGRAPHIC SYSTEMS USED BY PRICE, MARTIN AND GUDZINOWICZ

Derivative	R_F	
	Paper*	Thin-layer†
CP	0·80	0·08
3-HO-CP	0·77	0·07
8-HO-CP	0·77	0·06
7-HO-CP	0·78	0·06
7-MeO-CP	0·78	0·08
7-HO-Nor$_1$CP	0·77	0·03
7-HO-Nor$_2$CP	0·77	0·12
7-HO-CPSO	0·64	0·00
L4	0·83	0·62
L5	0·83	0·64

* Ascending chromatography, Whatman 3MM paper; solvent system, *n*-butanol:ethanol:water (5:2:2).

† Solvent system, acetone.

phenolic sulfides of the 7-configuration, viz., 7-HO-CP, 7-HO-Nor$_1$CP, 7-HO-Nor$_2$CP, L4, and L5. All such compounds react with the ferric chloride reagent used by these authors[3] for quantitating their "7-hydroxychlorpromazine," and consequently contribute to the analysis. Additional colorimetric interference is offered by other trace phenolic metabolites present in the whole-urine extracts employed for analysis. The authors state that urinary 7-HO-CP amounts to less than 1% of the daily dose of chlorpromazine (average, 0·3 to 0·4%); since their color reagent is nonspecific, even these low figures would appear to be exaggerated. It is doubtful whether free urinary 7-HO-CP exceeds 0·1%. The bulk of the phenols are excreted in conjugate form.[2, 5–7] No effort was reported by Price and co-workers to identify or estimate the 7-HO-CP in these fractions.

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