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SHORT COMMUNICATION

7-Hydroxychlorpromazine in the urines of schizophrenics receiving chlorpromazine*

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ALTHOUGH evidence suggesting hydroxylation to be a metabolic route for chlorpromazine in man was reported as early as 1959,[1–3] the lack of synthetic standard compounds has prevented positive identification of any of the presumed hydroxylated metabolites. Huang *et al.*[4] reported the presence of what appeared to be such a compound in the urines of chronic schizophrenic patients and estimated that it accounted for approximately 5% of the administered doses of chlorpromazine.

Synthetic 7-hydroxychlorpromazine has recently become available,† thus enabling positive identification and quantitative analysis for this compound. We report here the results of analyses of urines of five chronic male schizophrenics receiving chlorpromazine, showing 7-hydroxychlorpromazine to be a urinary product of chlorpromazine metabolism.

PROCEDURE

Five male chronic schizophrenics receiving maintenance-level daily doses of 400 to 1,000 mg of chlorpromazine (thorazine; Smith, Kline and French Labs.; Philadelphia), with no other drugs, were chosen for this study.‡ A 250-ml aliquot of a pooled 3- to 5-day total urine from each patient was analyzed by the procedure described below.

The urine was concentrated to the consistency of a syrup, at room temperature, with a Rinco rotary evaporator used with a high-vacuum pump and a dry ice–acetone trap. The residue was stirred with 100 ml of methanol, then filtered. The filtrate was evaporated to near-dryness, the residue having the consistency of a wax at refrigerator temperatures. A 1-g sample of the wax was dissolved in 50 ml of pH 6·8 phosphate buffer (0·2 M). The resulting solution was extracted with two 50-ml portions of methylene chloride, and the aqueous phase discarded. The two 50-ml organic phases were pooled, extracted with 50 ml of 0·1 N HCl, then discarded. The HCl extract was brought to pH = 10 with NaOH, and extracted with two 50-ml portions of methylene chloride. The methylene chloride layers were pooled, then evaporated to dryness. The residue was taken up in 4–8 ml of 0·1 N HCl–10^{-3} M $FeCl_3$. Visible spectra were run in 1-cm path-length cuvets in a Cary model 14 recording spectrophotometer, and the 7-hydroxychlorpromazine concentrations were calculated from absorption at 545 mμ and 515 mμ, based on a calibration curve prepared from authentic 7-hydroxychlorpromazine. This procedure, which appears to give nearly quantitative recovery of 7-hydroxychlorpromazine when added to urines, is outlined in Fig. 1.§

In addition, the dried methanolic extracts were chromatographed directly on Whatman 3MM filter paper strips in the *n*-butanol – ethanol – water (5 : 2 : 2 v/v) solvent system used by Huang *et al.*[4] Phenolic compounds were detected by spraying the chromatograms with 2% $FeCl_3$ in *n*-butanol. Nonhydroxylated phenothiazines do not form colored products with this treatment.

RESULTS AND DISCUSSION

The visible absorption spectrum of 7-hydroxychlorpromazine in 0·1 N HCl–10^{-3} M $FeCl_3$ is

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§ Attempts to extract 7-hydroxychlorpromazine directly from urine with methylene chloride have been unsuccessful, although the material seems to be readily extracted from aqueous solutions of the methanol-soluble fraction of urines.

shown as curve A in Fig. 2. The double band is characteristic of this compound under these conditions, and only single bands have been observed with nonhydroxylated phenothiazine derivatives. Virtually identical spectra were obtained with extracts from urines of all five patients except J. M.; a typical one is shown as curve B in Fig. 2.

Paper chromatograms of authentic 7-hydroxychlorpromazine in the *n*-butanol – ethanol – water (5 : 2 : 2 v/v) solvent gave a band with an R_f of about 0·8 which showed a blue color when sprayed with 2% $FeCl_3$ in *n*-butanol. A similar band was seen in the urines of all of the patients except J. M. In addition, a band at R_f of 0·6 was seen in all five patients. This appears to be the "$CPOH_1$," reported by Huang *et al.*¶ Both bands (R_f 0·6 and 0·8), when eluted with 0·1 N HCl–10^{-3} M $FeCl_3$, gave visible spectra identical with 7-hydroxychlorpromazine treated similarly. This supports the contention of Huang *et al.* that $CPOH_1$ is a hydroxylated derivative of chlorpromazine. This compound (R_f 0·6) appears to have solubility properties different from 7-hydroxychlorpromazine, since it was observed in the urine of all five patients, but only four of the patients showed spectra characteristic of a hydroxylated phenothiazine in the methylene chloride extracts. We also observed a good correlation between the relative intensities of the chromatographic $FeCl_3$-positive bands at R_f 0·8 and the

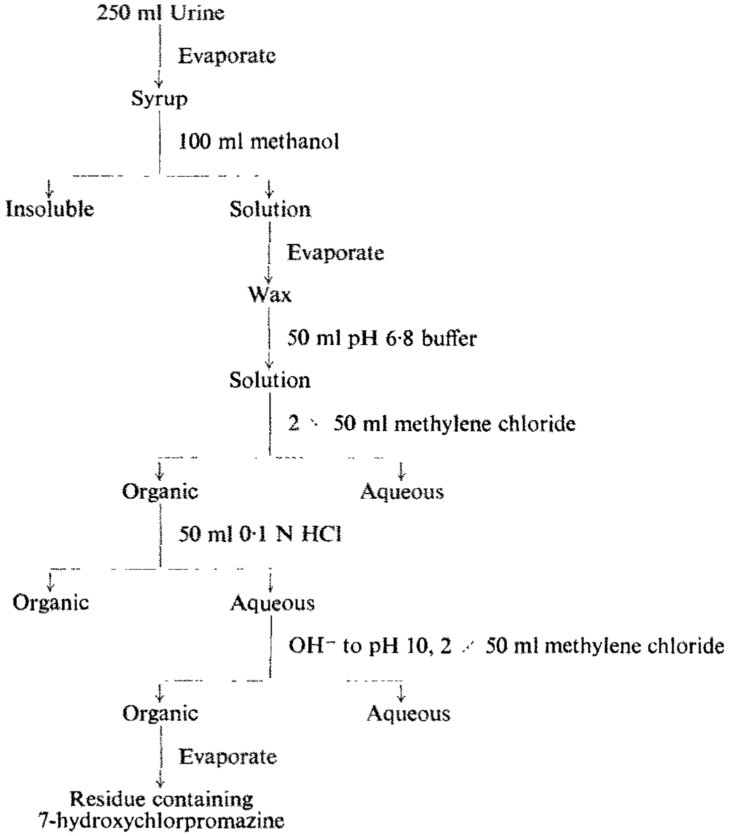

FIG. 1. Procedure for extraction of 7-hydroxychlorpromazine from urine.

¶ Neither the "R_f 0·6" nor the "R_f 0·8" band were observed in urines from individuals not receiving chlorpromazine.

amounts of $FeCl_3$-positive material extracted by our procedure (see Table 1). Thin-layer chromatograms on silica gel G, using acetone as a solvent, showed a band corresponding to 7-hydroxychlorpromazine in extracts. In one case an extract (patient R. F.) was acetylated and subjected to vapor-phase chromatography. A peak corresponding to acetylated 7-hydroxychlorpromazine was observed. This peak was absent in extracts of individuals not receiving chlorpromazine.

The results in Table 1 are reported as milligrams of 7-hydroxychlorpromazine per total 24-hr urine, and as a percentage of the total daily dose of chlorpromazine. The variation among the patients is striking. We have experienced difficulties in extracting 7-hydroxychlorpromazine from urines by several procedures which should, in principle, extract the compound. Thus we must consider the possibility that the variation in yield may be due either to metabolic differences between the individuals or to possible variable efficiencies of our extraction procedure on different urines.

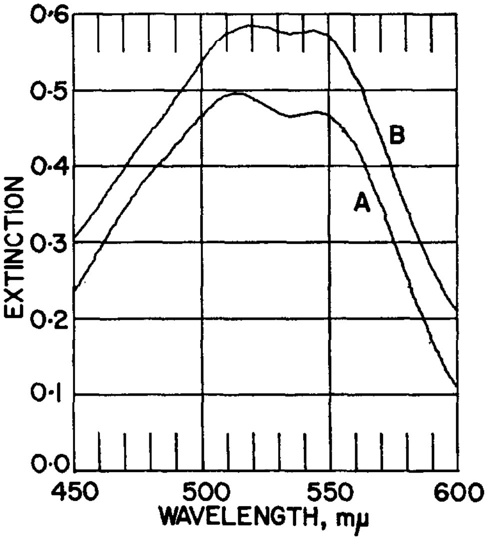

Fig. 2. Curve A is a visible spectrum of authentic 7-hydroxychlorpromazine in 0·1 N HCl–10^{-3} M $FeCl_3$. Curve B is the spectrum of the final residue obtained from extraction of urine from patient R. F., when dissolved in 0·1 N HCl–10^{-3} M $FeCl_3$. In both cases the reference cell contained 0·1 N HCl–10^{-3} M $FeCl_3$.

Table 1. Results of analyses of urines from five chronic male schizophrenics receiving chlorpromazine

Patient	Dose of chlorpromazine (mg/day)	Relative intensity of R_f 0·8*	Yield of 7-hydroxychlorpromazine	
			(mg/day)	(% of dose)
J. L.	1,000	++++	6·4	0·64
R. L.	600	+++	3·4	0·57
R. F.	1,000	++	0·7	0·07
D. S.	500	+	0·6	0·12
J. M.	400	Not detected	Not detected	

* Visual estimation of the color intensity of the band corresponding to 7-hydroxychlorpromazine on paper chromatograms.

From the data in Table 1, it appears that 7-hydroxychlorpromazine accounts for a relatively small fraction of the daily dose of chlorpromazine, amounting to less than 1% in the highest case.

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