Quantitative Studies of Urinary Excretion of Chlorpromazine Metabolites in Chronically-Dosed Psychiatric Patients

By A. G. BOLT, I. S. FORREST, and M. T. SERRA

A method was developed for the routine assay of conjugated and unconjugated chlorpromazine metabolites in human urine. The method could be adapted to excretion studies of related drugs, *e.g.*, other phenothiazines and imipramine derivatives. The unconjugated drug metabolites were extracted from alkaline urine into dichloromethane and assayed spectroscopically in 50 per cent sulfuric acid at 530 mμ. The conjugated chlorpromazine metabolites were determined after passing a sample of urine through an ion-exchange resin to remove the unconjugated metabolites and contaminating endogenous urinary constituents. The eluate was made up to 50 per cent sulfuric acid content, and drug metabolites estimated spectroscopically at 550 mμ. The total urinary drug excretion of 15 chronic mental patients receiving chlorpromazine in doses of 100 to 1400 mg. per day, varied from 21.1 to 70 per cent of the daily dose. The conjugated drug metabolites formed the major fraction of urinary metabolites. The ratio of conjugated to unconjugated drug metabolites ranged from 2.1 to 11.

ESSENTIALLY two groups of chlorpromazine metabolites are observed in human urine (1–6)—namely, (*a*) the unconjugated fraction consisting of unchanged drug, desmonomethylchlorpromazine, desdimethylchlorpromazine, their sulfoxides, and chlorpromazine-*N*-oxide; (*b*) the conjugated fraction containing the greater number and amount of urinary chlorpromazine metabolites (6, 7). The metabolites in this fraction are the *O*-glucuronides (and small amounts of ethereal sulfates) of the mono- and dihydroxy derivatives of chlorpromazine, their demethylated derivatives, and of the corresponding sulfoxides (6, 8). Trace amounts of other unknown conjugates may also be present.

The unconjugated metabolites have been consistently reported in the literature and represent from 5 to 10% of the daily dose. There are, however, discrepancies in the estimates in the major drug fraction containing the conjugated metabolites which may be due to the different methods used (4, 9, 10). Other methods reported in the literature involve chemical or enzymatic hydrolytic procedures which are time consuming and give low recoveries. The authors have previously described a method (11), using a strong cationic resin, which was less satisfactory than the present method. When the earlier procedure was proposed, hydroxlyated or methoxylated derivatives of chlorpromazine were not available as reference compounds for conjugated metabolites; in fact, these metabolites were later shown to be incompletely estimated.

Since significant inter-patient (7) and interspecies (12, 13) differences in urinary chlorpromazine metabolites have been noted, especially with regard to the large group of conjugated drug metabolites (7), simple and rapid procedures are needed for the separate estimation of the two groups of drug metabolites.

EXPERIMENTAL

Materials.[1]—Dichloromethane, ACS; 97% sulfuric acid, ACS; 0.1 *N* sulfuric acid solution; Sørensen's phosphate buffer (pH 6); 2 *N* sodium hydroxide solution; 2 *N* hydrochloric acid solution; 30% hydrogen peroxide solution; ion-exchange resin[2] IRC-50, analytical grade, 20–50 mesh; chlorpromazine HCl; 7-hydroxychlorpromazine; 7-methoxychlorpromazine HCl; chlorpromazine-5-oxide HCl.

Apparatus.—U.V. spectra were obtained using a Beckman DB recording spectrophotometer. The Beckman Zeromatic pH meter was used for pH measurements.

Determination of Unconjugated Drug Metabolites.—A 10-ml. aliquot of urine was adjusted to pH 9–9.5 and extracted with 3 × 10 ml. of dichloromethane (14). The extract was evaporated on a water bath under a stream of nitrogen, the residue was dissolved in 5 ml. of 0.1 *N* sulfuric acid solution, and the acid solution transferred to a 10-ml. volumetric flask. The flask was immersed in an ice bath and 5 ml. of concentrated sulfuric acid was added very slowly down the wall of the flask. The sulfuric acid mixture was heated for 15 min. at 65°, brought to room temperature, 1 drop of 0.1% H_2O_2 was added, stood for 15 min., and adjusted to 10 ml. with cold 50% sulfuric acid solution. The

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[2] Marketed as Amberlite IRC-50 by Rohm & Haas, Philadelphia, Pa.

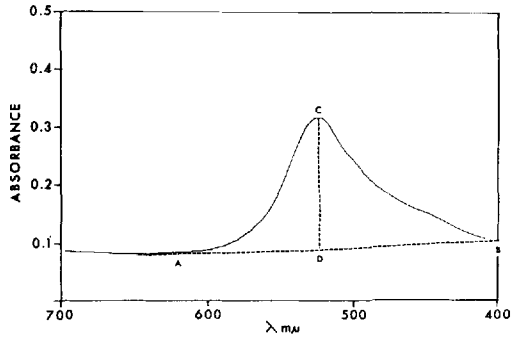


Fig. 1.—The spectrum of a typical dichloromethane urine extract after treatment with 50% sulfuric acid solution. Reading of absorbance at 530 mμ by the background cancellation method.

absorption spectrum of the acid solution was recorded between 400–700 mμ (see Fig. 1). Absorbance was measured at 530 mμ by a background cancellation technique as follows. A straight line AB was drawn between the points of minimum absorption on either side of the peak and a perpendicular line was drawn from the point of maximum absorbance, C, to intersect AB at D; the line CD was measured in absorbance units. A standard calibration curve was prepared from aqueous solutions of chlorpromazine sulfoxide hydrochloride. The absorbance of chlorpromazine sulfoxide after reaction with 50% sulfuric acid was proportional to concentrations up to 5×10^{-5} *M*.

Determination of Conjugated Drug Metabolites.—Chromatographic columns were prepared from the ion-exchange resin in the Na^+ form. One gram of resin was used for each ml. of urine, depending upon the quantity of metabolites in the urine.[3] The column was washed with a few ml. of Sørensen's pH 6 buffer which was discarded. From 0.5 to 2.0 ml. of urine[3] was then passed through the column, and the effluent was collected in a 10-ml. volumetric flask. The column was washed with pH 6 buffer to yield an effluent volume of 5 ml. The effluent was treated with concentrated sulfuric acid as described previously and maximum absorption read at 550 mμ by the background cancellation method. A standard calibration curve was prepared from aqueous solutions of 7-methoxychlorpromazine hydrochloride after treatment with 1 drop of 30% hydrogen peroxide (to form the corresponding sulfoxide). Absorption of the standard compound was read at 567 mμ after addition of concentrated sulfuric acid to give a final acid concentration of 50% by volume. Absorbance was proportional to concentration up to 5×10^{-5} *M* in 50% sulfuric acid.

Procedure.—Twenty-four hour urine collections from male chronic mental patients, receiving 100 to 1400 mg. of chlorpromazine hydrochloride per day, were assayed for conjugated and unconjugated chlorpromazine metabolites. Each determination was made in duplicate. At least two collections, about a week apart, were obtained from each patient. Additional collections were made in those cases in which urinary concentrations of chlorpromazine metabolites varied between samples. The patients were thoroughly supervised, both with regard to ingestion of medication and collection of 24-hr. specimens.

[3] An estimate of the quantity of urine to be used can be made according to a rapid urine color test (15); use 2 ml. of urine for a 1+ color reaction, 1 ml. for 2+, and 0.5 ml. for 3+ and 4+.

RESULTS

The results are summarized in Table I. Total urinary excretion of the combined groups of drug metabolites varied from 21.1 to 70%. Conjugated and unconjugated metabolites ranged from 16 to 51%, and 2.1 to 19% of the daily dose, respectively. All patients excreted much larger amounts of conjugated than unconjugated chlorpromazine metabolites; the ratio of the conjugated to the unconjugated drug metabolites varied from 2.1 to 11.

DISCUSSION

Method.—Since *O*-glucuronide derivatives of 7-hydroxychlorpromazine (II) were not available, 7-methoxychlorpromazine (I) was chosen as the reference compound for the determination of the conjugated metabolites, although the $\lambda_{max.}$ of 7-methoxychlorpromazine after reaction with 50% sulfuric acid was 567 mμ, compared with 550 mμ for the conjugated metabolites. 7-Methoxychlorpromazine was used for the following reasons.

(*a*) The absorptivity of 7-hydroxy- (the aglycone of 7-methoxy-) and 7-methoxy-chlorpromazines are nearly identical. (*b*) The conjugated drug metabolites consist mainly of *O*-glucuronides (II), in which the glucuronic acid residue is attached to the phenothiazine nucleus *via* an ether linkage (6) at the 7-position (16, 17). Thus, the structure of the chlorpromazine *O*-glucuronides is more closely related to that of 7-methoxychlorpromazine than to 7-hydroxychlorpromazine. (*c*) Under the experimental conditions 7-methoxychlorpromazine and the *O*-glucuronides of chlorpromazine reach maximum color development in 50% sulfuric acid solution faster than 7-hydroxychlorpromazine. (*d*) 7-Methoxychlorpromazine is more stable than 7-hydroxychlorpromazine and is more readily available.

In 50% sulfuric acid, the colored reaction products form more rapidly from the chlorpromazine and 7-methoxychlorpromazine sulfoxides than from the corresponding sulfides (11, 18), and therefore the sulfoxides were used as reference compounds.

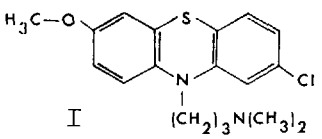
The present method was rapid and reproducible to 5%. Studies using thin-layer chromatography showed that all unconjugated drug metabolites were absorbed onto the exchange resin and that only conjugated drug derivatives remained in the effluents. These studies also showed that all unconjugated chlorpromazine metabolites were extracted into dichloromethane.

The present method determines approximately 95% of the chlorpromazine derivatives normally present in the patient's urine. However, the assay procedure does not estimate all the drug metabolites in urine. 7-Hydroxychlorpromazine, its demethylated derivatives, and corresponding sulfoxides, and the dihydroxychlorpromazine derivatives are not measured by the present procedure. 2-Chlorophenothiazine and its sulfoxide and other nonbasic (deaminated) metabolites are not completely extracted from urine into dichloromethane. However, only trace amounts of deaminated and hydroxylated metabolites are normally present in

TABLE I.—EXCRETION OF URINARY CHLORPROMAZINE METABOLITES IN 15 CHRONIC MENTAL PATIENTS ON CONTINUOUS DRUG THERAPY RANGING FROM 100 TO 1400 mg. PER DAY OF CHLORPROMAZINE

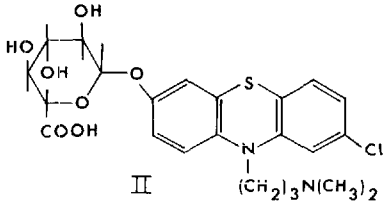
Patient	Dose, mg./24 hr.	Metabolites Excreted,[a] %			Conjugated/ Unconjugated
		Conjugated	Unconjugated	Total	
A	1400	25	3.6	28.6	7.0
		24	6.0	30.0	4.0
B	1200	37	8.2	45.2	4.7
		30	12.0	42.0	2.5
C	1200	28	4.3	32.3	6.6
		21	5.0	26.0	4.2
D	1200	32	3.6	35.6	8.9
		31	2.8	33.8	11.0
E	900	27	5.1	32.1	4.9
		26	4.1	30.1	6.3
F	900	26	3.2	29.2	8.1
		23	2.9	25.9	7.9
G	800	22	4.5	26.5	4.9
		21	5.5	26.5	3.8
H	750	29	5.5	34.5	5.3
		19	2.1	21.1	9.1
I	600	16	7.5	23.5	2.1
		22	6.4	28.4	3.4
J	600	44	10.1	54.1	4.4
		37	4.3	41.3	7.4
K	600	35	4.1	39.1	8.5
		34	4.6	38.6	7.5
L	300	51	19.0	70.0	2.7
		43	10.0	53.0	4.3
M	300	31	7.7	38.7	4.0
		31	9.3	40.3	3.2
N	300	33	3.8	36.8	8.7
		32	3.9	35.9	8.2
O	100	38	7.0	45.0	5.4
		38	8.0	46.0	4.8

[a] Average of two determinations calculated as percentage of the dose per 24-hr. as chlorpromazine hydrochloride.



I

7-Methoxychlorpromazine



II

Chlorpromazine (7)-*O*-Glucuronic Acid

urine. The authors have estimated that deaminated chlorpromazine metabolites represent less than 1% of the daily drug dose, and Goldenberg and Fishman (12) conclude that the unconjugated 7-hydroxy derivatives represent about 0.1% of the daily drug dose. However, the 7-hydroxy group of drug metabolites occur in somewhat larger amounts in patients (21) with chlorpromazine-induced skin pigmentation (20).

The procedures used to separate the chlorpromazine metabolites from urine do not exclude all the urinary constituents that give colored products in 50% sulfuric acid. However, the nonspecific background absorption of these interfering constituents was eliminated by the use of the background cancellation method previously described.

Other drugs derived from phenothiazine or imipramine produce colored radical ions in 50% sulfuric acid, and the biotransformation products derived from these drugs could presumably be measured by an assay procedure similar to the one reported here provided that appropriate reference compounds are available.

Results.—The excretion of chlorpromazine and its conjugated and unconjugated metabolites was not related to drug dose (100–1400 mg. per day). Thus, it was shown (Table I) that a patient receiving 100 mg. and another 1200 mg. chlorpromazine per day, both excreted 45% of the administered dose. These results differ markedly from those of Nadeau and Sobolewski (9), who reported earlier that the limits of urinary drug excretion were reached at a dose of 200 mg. per day.

In the present study total drug excretion varied from 21.1 to 70% of the administered dose, with an average excretion calculated at 37%. Previous data of Posner *et al.* (4), who had used methods similar to those of Nadeau and Sobolewski (9), showed recoveries of 7.2 to 24.7%. These authors also stated that there was a trend toward decreasing urinary excretion with increasing drug dose. Using an assay procedure employing biologically derived reference compounds, Huang *et al.* (10) recently studied seven patients receiving 300 to 1200 mg. of chlorpromazine per day, and recovered an average of 58% of the administered dose in urine. These recoveries were higher than those obtained in the present study, and the authors also observed a tendency toward decreased urinary excretion of

conjugated metabolites with increasing drug dose. The present study differs from earlier ones (4, 9, 10) by not showing any dose-excretion correlation, and by yielding per cent excretion values substantially higher than those of Posner *et al.*, considered less than comprehensive, but lower than those of Huang *et al.*, believed to include normal urinary contaminants.

The reported results (Table I) could not be expected to show a definite correlation between drug excretion and such clinical parameters as diagnosis, drug response, or mental status of the patients; this group of patients was selected only to establish the spread of urinary excretion over a large range of chronic doses. The population sample, though small, included members of the three major ethnic groups with the most diverse psychiatric diagnoses. However, preliminary data on the correlation between side effects of drug therapy and urinary drug metabolism were recently reported for the initial phases of drug administration (19) as well as for drug-induced skin hyperpigmentation (22, 23).

The method is currently being used to study the time required to reach a steady excretion rate, to determine the effect of simultaneously administered compounds on the biotransformations and urinary excretion of chlorpromazine, and to elucidate species differences in chlorpromazine metabolism.

When studying the urinary drug excretion in mental patients, a number of factors not readily controlled in a normal hospital setting may affect the reproducibility of the chemical results. They are variations in diet, and intake of food and liquids. Most patients were found to increase their urinary output by a factor of 2 or 3 under chronic administration; the volumes eventually became reasonably stable at this higher level. Large fluctuations in consecutive 24-hr. specimens tended to yield erratic results. Therefore, rigorous surveillance of controllable parameters like drug intake (preferably in liquid form) and of 24-hr. urine collections cannot be overemphasized.

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Solubility of Parabens in Syrup Vehicles

By ANTHONY N. PARUTA* and BHOGILAL B. SHETH

The solubilities of methyl, ethyl, propyl, butyl, and benzylparabens have been determined in sucrose vehicles of varying concentration. These syrup vehicles possess dielectric constants less than pure water, their respective dielectric constants decreasing with increasing sucrose concentration. The effect of both the concentration of sucrose added and the dielectric constant upon the solubility of the subject compounds is presented. The solubilities of these materials were seen to change to a relatively small degree with increasing sucrose concentration. Although these changes in solubility are minute, they would appear to be positive in character. The only definitive change noted was for benzylparaben where a relatively large change in solubility was noted.

THE GENERAL use and application of sucrose solutions of varying concentration for liquid pharmaceuticals is still widespread. In an effort to continue (1, 2) to determine the solvency characteristics of these media, the present study was undertaken. It was felt judicious that a study of a set of solutes of varying polar character be undertaken so that the effect of methyl, ethyl, etc., groups upon the solubility in common dissolution media could be studied. In this way, a tendency or characteristic effect of substituent groups could be delineated implying an effect that has been termed "solute polarity."

It has been found that relatively dramatic

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* Present address: College of Pharmacy, University of Rhode Island, Kingston 02881.