Urinary Excretion of Chlorpheniramine and Its *N*-Demethylated Metabolites in Man

By PETER KABASAKALIAN, MARILYN TAGGART, and EDWARD TOWNLEY

The *N*-demethylated metabolites of chlorpheniramine, *N*-desmonomethylchlorpheniramine, and *N*-desdimethylchlorpheniramine were found in the urine of males using a repetitive dosage schedule.

CAVALLITO, CHAFETZ, AND MILLER (1) studied the urinary excretion of chlorpheniramine[1] on four humans who orally took 10.2-mg. chlorpheniramine maleate in gelatin capsules. The total 24-hr. urinary excretions fluctuated from 12 to 57% of the ingested dose among the four subjects. Their analytical method, however, was nonspecific, and their high results were above the upper limits found by Beckett and Wilkinson (2).

Beckett and Wilkinson (2) studied the influence of urine pH and flow rate on the renal excretion of chlorpheniramine. They found that renal excretion rates vary with both urinary pH and the rate of urine flow. When an aqueous solution of 14.2 mg. of chlorpheniramine maleate was orally administered to male subjects, the excretion rates fluctuated from 4.5 to 11.5% in 24 hr. When the urine was maintained acid (pH 5.0 ± 0.5) by the administration of ammonium chloride, 20.0 to 26.5% was excreted, whereas when the urine was maintained alkaline (pH 8.0 ± 0.5) by the administration of sodium bicarbonate, 0.3 to 0.4% was excreted. They also found that under constant acid urine conditions a high urine flow rate increased the excretion rate. The volume-dependent fluctuations became negligible when (by water loading the subjects) the urine rate was maintained above 150 ml./hr.

Since the data of Beckett and Wilkinson (2) suggested that chlorpheniramine is extensively metabolized, its probable *N*-demethylated metabolites were sought in this work. The multiple-dosage regimen appeared to be ideally suited to the problem of locating these metabolites. All one had to do was to find urinary excretion products which increased, leveled off, and declined with the dosage regimen.

EXPERIMENTAL[2]

Protocol—A male subject under a normal diet with no urine pH or fluid intake control was used in this study.

Single-Dose Regimen—The subject orally took a single dose of 12 mg. of chlorpheniramine maleate in tablet form (three 4-mg. chlorpheniramine maleate tablets). Urine was collected normally for 5.5 days and combined into 12-hr. samples.

Multiple-Dose Regimen—The same subject orally took 4 mg. of chlorpheniramine maleate in tablet form daily for 29 days. Urine was collected normally for 39 days and combined into 12-hr. samples.

Analytical Methods—The urine was analyzed by the general method of Beckett and Wilkinson (2) which involved extraction with ether at pH 10, concentration, and subsequent gas chromatography on a 5% potassium hydroxide coated Anakrom ABS column[3] containing 3% polyethylene glycol 20M[4] as liquid phase and operating at 180°C. A 76.2-cm. (2.5-ft.) column exhibited 1,500 theoretical plates for chlorpheniramine.

Free chlorpheniramine had a retention time of 6.0 min. Quantitative determinations were made using a chlorpheniramine reference standard. Isolation by thin-layer chromatography on alumina (3) and subsequent mass spectroscopy (4) showed an *m/e* value of 58 for $CH_2{=}N(CH_3)_2$ indicative of a tertiary amine and *m/e* values of 203, 202, and 167 which are characteristic of the *p*-chlorobenzyl-2-pyridyl group. It did not form derivatives either with cyclopentanone or acetone using the procedure of Capella and Horning (5) or with acetic anhydride (6).

Metabolite X had a retention time of 10.8 min., identical to that of an authentic sample of *N*-desmonomethylchlorpheniramine. Quantitative determinations were made using the authentic sample as a calibration standard. Isolation by thin-layer chromatography on alumina (3) and mass spectroscopy (4) showed *X* to be a secondary amine with an *m/e* value of 44 for $CH_2{=}NH(CH_3)$ and the *m/e* values of 203, 202, and 167 of the *p*-chlorobenzyl-2-pyridyl group. It formed a derivative (retention time >30 min.) with cyclopentanone (indicative of primary and secondary amines), but none with acetone (indicative of the absence of a primary amine) using the procedure of Capella and Horning (5). It formed a derivative with acetic anhydride (6) (retention time >30 min.).

Metabolite Y had a retention time of 14.8 min., identical to that of an authentic sample of *N*-desdimethylchlorpheniramine. Quantitative determinations were made using an authentic sample as a calibration standard. Isolation by thin-layer chromatography on alumina (3) and subsequent mass spectroscopy (4) showed *Y* to be a primary amine with an *m/e* value of 30 for $CH_2{=}NH_2$ and the *m/e* values of 203, 202, and 167 of the *p*-chlorobenzyl-2-

Received April 10, 1967, from the Research and Development Division, Schering Corporation, Bloomfield, NJ 07003
Accepted for publication October 27, 1967.

[1] Chlorpheniramine is *dl*-2-{*p*-chloro-α-[2-(dimethylamino)-ethyl]benzyl} pyridine. It is marketed as Chlor-Trimeton by Schering Corporation, Bloomfield, N. J.

[2] All gas chromatograms were obtained on a F and M model 400 gas chromatograph with a flame-ionization detector and a Minneapolis Honeywell recorder. The mass spectra were obtained on a Consolidated Electrodynamics Corporation model CEC-103C mass spectrometer.

[3] Available from Analabs, Incorporated, Hamden, Conn.

[4] Marketed as Carbowax by Union Carbide Corporation, New York, N. Y.

TABLE I—URINARY EXCRETION OF FREE CHLORPHENIRAMINE BY SUBJECT WHO RECEIVED ONE DOSE OF THREE 4-mg. CHLORPHENIRAMINE MALEATE TABLETS

Time After Drug[a] Taken, Days	Combined 12-hr. Urine Volume, ml.	Chlorpheniramine[c] in 12-hr. Urine mg.
0.0	...[b]	...[d]
0.5	550	0.215
1.0	530	0.404
1.5	800	0.151
2.0	630	0.111
2.5	530	...
3.0	420	0.082
3.5	585	0.059
4.0	540	0.033
4.5	540	0.062
5.0	520	0.030
5.5	390	0.048

[a] Experiment started at 7:30 p.m. [b] Sample not collected. [c] Calculated as chlorpheniramine maleate. [d] No assay results obtained because of sample loss, *etc.*

pyridyl group. It formed derivatives, indicative of a primary amine, with cyclopentanone (retention time = 26 min.) and with acetone (retention time = 17 min.) using the procedure of Capella and Horning (5). It formed a derivative with acetic anhydride (6) (retention time >30 min.).

RESULTS AND DISCUSSION

Single-Dose Regimen—The urinary excretion of free chlorpheniramine by the subject on the single-dose regimen is shown in Table I.

The amount of unchanged chlorpheniramine excreted by the single subject in the first 24 hr. was 5.2% of the ingested dose. This is well within the range (4.5 to 11.5%) found by Beckett and Wilkinson (2) for several subjects who were not under pH or fluid intake control. The total estimated excretion of free chlorpheniramine was about 12% of the ingested dose.

Multiple-Dose Regimen—The urinary excretion of free chlorpheniramine by the subject in a multiple-dose regimen is listed in Table II.

The 12-hr. urinary excretion rate varies with time in a "saw-tooth" manner. The peaks and valleys increased with time until a "steady state" (repetitive 24-hr. excretion pattern) was reached. The minimum values or valleys occur prior to the repetitive drug ingestion and the peaks following ingestion. The gradually increasing pattern indicates that chlorpheniramine was building up in the body.

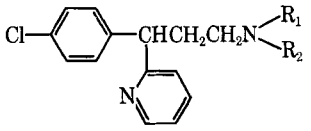
In the steady state, the input of the drug to the body should equal the output from the body. Large fluctuations were observed during the steady-state condition and were probably caused by either the variance in the daily fluid intake, urine pH, or the

TABLE II—URINARY EXCRETION OF FREE CHLORPHENIRAMINE AND ITS METABOLITES BY SUBJECT WHO DAILY RECEIVED ONE 4-mg. CHLORPHENIRAMINE MALEATE TABLET FROM DAY 0 TO DAY 28

Time,[a] Days	Combined Vol.[b] of 12-hr. Urine, ml.	Chlorpheniramine[c] Excreted in 12 hr. Urine, mg.	Metabolite *X*[d] Excreted in 12-hr. Urine, mg.	Metabolite *Y*[e] Excreted in 12-hr. Urine, mg.[f]	Time,[a] Days	Combined Vol.[b] of 12-hr. Urine, ml.	Chlorpheniramine[c] Excreted in 12-hr. Urine, mg.	Metabolite *X*[d] Excreted in 12-hr. Urine, mg.	Metabolite *Y*[e] Excreted in 12-hr. Urine, mg.[f]
0.0	...	...	...	...	19.5	655	0.268	0.365	0.182
0.5	595	0.211	0.036	...	20.0	290	0.136	0.141	0.070
1.0	340	0.085	0.049	...	20.5	420	0.345	0.304	0.189
1.5	570	0.173	0.087	...	21.0	305	0.213	0.257	0.080
2.0	450	0.066	0.057	...	21.5	475	0.309	0.366	0.120
2.5	610	0.215	0.091	0.019	22.0	400	0.061	0.099	0.041
3.0	395	0.143	0.134	0.017	22.5	485	0.274	0.311	0.126
3.5	380	0.304	0.258	...	23.0	450	0.198	0.330	0.120
4.0	480	0.144	0.271	...	23.5	570	0.295	0.333	0.098
4.5	570	0.317	0.564	...	24.0	420	0.162	0.276	0.107
5.0	420	0.187	0.134	...	24.5	370	0.574	0.262	0.076
5.5	665	0.260	0.293	0.055	25.0	385	0.291	0.216	0.070
6.0	430	0.158	0.211	0.016	25.5	405	0.478	0.336	0.182
6.5	530	0.292	0.212	...	26.0	...	...	...	...
7.0	...	...	...	...	26.5	500	0.574	0.279	0.096
7.5	310	0.364	0.325	0.101	27.0	590	0.148	0.185	0.068
8.0	420	0.139	0.250	0.035	27.5	345	0.387	0.192	0.108
8.5	515	0.264	0.266	...	28.0	490	0.194	0.218	0.093
9.0	400	0.281	0.220	...	28.5	365	0.366	0.236	0.117
9.5	620	0.274	0.210	...	29.0	400	0.241	0.208	0.102
10.0	375	0.187	0.260	...	29.5	375	0.203	0.199	0.142
10.5	540	0.357	0.147	...	30.0	355	0.123	0.085	0.069
11.0	315	0.141	0.081	...	30.5	325	0.057	0.152	0.103
11.5	485	0.412	0.316	...	31.0	395	0.032	0.092	0.036
12.0	260	0.159	0.214	...	31.5	420	0.035	0.099	0.073
12.5	510	0.378	0.417	...	32.0	305	0.020	0.070	0.059
13.0	425	0.165	0.253	...	32.5	550	0.017	0.071	0.062
13.5	605	0.330	0.370	...	33.0	455	0.013	0.043	0.032
14.0	...	...	...	...	33.5	670	0.011	0.038	0.043
14.5	405	0.207	0.285	0.113	34.0	820	0.006	0.017	0.014
15.0	405	0.120	0.228	0.091	34.5	590	0.005	0.029	0.032
15.5	390	0.215	0.273	0.109	35.0	...	...	...	...
16.0	510	0.066	0.177	0.062	35.5	260	0.003	0.014	0.038
16.5	455	0.196	0.377	0.188	36.0	340	0.002	0.009	0.022
17.0	305	0.139	0.257	0.117	36.5	610	0.003	0.007	0.021
17.5	460	0.286	0.322	0.154	37.0	380	0.008	0.004	0.006
18.0	515	0.201	0.210	0.056	37.5	550	0.004	0.003	0.007
18.5	490	0.346	0.304	0.151	38.0	350	0.003	0.002	0.004
19.0	295	0.182	0.232	0.121	38.5	745	0.006	0.002	0.006
					39.0	520	0.003	0.001	0.002

[a] Underscored days 4-mg. chlorpheniramine maleate tablet ingested; experiment started at 7:30 p.m. [b] ...sample not collected. [c] Reported as chlorpheniramine maleate. [d] Reported as *N*-desmonomethylchlorpheniramine maleate. [e] Reported as *N*-desdimethylchlorpheniramine maleate. [f] ... assay result not obtained.

TABLE III—STRUCTURAL FORMULA OF CHLORPHENIRAMINE AND ITS METABOLITES



Compd.	R_1	R_2
Chlorpheniramine	CH_3	CH_3
Metabolite *X*	CH_3	H
Metabolite *Y*	H	H

diurnal excretion pattern. The calculated *average* daily excretion rate was 0.502 mg., or 12.6% of the repeated 4-mg. chlorpheniramine dose. This compares favorably with the 12% that was estimated from the single-dose experiment.

On termination of the drug-intake regimen, the chlorpheniramine excretion rate decreased. From the total amount of free chlorpheniramine excreted after the last dose is taken on day 28.0, and using the average 0.502 mg. of chlorpheniramine excreted per 4-mg. dose, one can calculate the equivalent amount of chlorpheniramine maleate present in the subject immediately after the last dose. This calculation yields a figure of 9.3 mg. of chlorpheniramine maleate (equivalent to 2.4 tablets, each containing 4 mg. of chlorpheniramine maleate).

Metabolites X and Y—The *N*-dealkylation reaction, especially of monomethyl and dimethyl-substituted primary amines is a major pathway of drug metabolism (7). If these metabolites were present, the gas chromatographic method of Beckett and Wilkinson (2) should have revealed them, albeit with much higher retention times (6, 8) than the parent compounds. Unfortunately, in the single dose regimen, it is difficult to differentiate the desired metabolites from other constituents of urine. The metabolites, *X* and *Y*, which were found to be *N*-desmonomethylchlorpheniramine and *N*-desdimethylchlorpheniramine, respectively (Table III), built up, leveled off, and declined like chlorpheniramine but more slowly.

During the steady-state condition, an *average* of 0.510 mg. of *N*-desmonomethylchlorpheniramine and 0.216 mg. of *N*-desdimethylchlorpheniramine were excreted daily (13.2% and 5.8% of the repeated 4-mg. oral dose). Together with the free chlorpheniramine excreted daily, this accounts for 32% of the dose, leaving the fate of the remaining 68% still unaccounted for.

SUMMARY AND CONCLUSIONS

A repetitive dosage regimen using a commercially available pharmaceutical dosage of chlorpheniramine has been successfully used to identify two metabolites of chlorpheniramine, *N*-desmonomethylchlorpheniramine and *N*-desdimethylchlorpheniramine, in the urine of a single human subject.

ADDENDUM

Kamm and Van Loon (9) have recently reported the presence of the metabolites, *N*-desmonomethylchlorpheniramine and *N*-desdimethylchlorpheniramine, in the urine of the rat and the dog.

REFERENCES

(1) Cavallito, C. J., Chafetz, L., and Miller, L. D., *J. Pharm. Sci.*, **52**, 259(1963).
(2) Beckett, A. H., and Wilkinson, G. R., *J. Pharm. Pharmacol.*, **17**, 256(1965).
(3) Kuntzman, R., Klutch, A., Tsai, I., and Burns, J. J., *J. Pharmacol. Exptl. Therap.*, **149**, 29(1965).
(4) Budzikiewicz, H., Djerassi, C., and Williams, D. H., "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964.
(5) Capella, P., and Horning, E. C., *Anal. Chem.*, **38**, 316(1966).
(6) Johnson, D. E., Rodriguez, C. F., and Burchfield, H. P., *Biochem. Pharmacol.*, **14**, 1453(1965).
(7) McMahon, R. E., *J. Pharm. Sci.*, **55**, 457(1966).
(8) Beckett, A. H., and Rowland, M., *J. Pharm. Pharmacol.*, **17**, 59(1965).
(9) Kamm, J. J., and Van Loon, E. J., *Fed. Proc.*, Abstr. No. 548, 1967.

Keyphrases

Chlorpheniramine—urinary excretion
Metabolites, chlorpheniramine—isolated, identified
GLC—analysis, urine
TLC—separation
Mass spectroscopy—identity

A Micro-Extraction Technique with Compounds Isolated from Thin-Layer Chromatograms

By ALMA L. HAYDEN, WILSON L. BRANNON, and NEUMAN R. CRAIG

A micro-apparatus is described for the extraction of drugs and related compounds from thin-layer or paper chromatograms by continuous elution with 1 ml. solvent. The sample, after extraction, is ground with KBr, mounted on a micro potassium halide plate, and placed in the center of a paper frame with a window area for infrared analysis. Spectra can be determined on 5- or 10-mcg. samples without beam condensers, ordinate expanders, or micro-dies.

RECENT INVESTIGATIONS in this laboratory have involved structure characterization and identification of microgram quantities of drugs and related materials by infrared spectroscopy. Most often, these have been polar compounds which have been isolated from thin-layer and paper chromatograms where impurities from the matrix and eluting solvent may interfere. To minimize these effects while

Received June 30, 1967, from the U. S. Department of Health, Education, and Welfare, Food and Drug Administration, Division of Pharmaceutical Chemistry, Spectrophotometric Research Branch, Washington, DC 20204
Accepted for publication October 5, 1967.