Human Urinary Excretion of Orally Administered Anisotropine Methylbromide

By MORRIS PFEFFER, JOSEPH M. SCHOR, NORMAN GLUCK, MARTIN G. SEMMEL, and SOLOMON GRIBOFF

A procedure is described for the quantitative determination of anisotropine methylbromide in undiluted human urine. It has been used to follow the urinary excretion of this compound after oral administration of 20 mg. to six humans. A sustained excretion has been found in human volunteer subjects, in one case lasting up to 104 hr.

AN INVESTIGATION of anisotropine methylbromide[1] (8-methyltropinium bromide 2-propylpentanoate), a quaternary antispasmodic, required the development of a method sufficiently sensitive for its estimation in materials of biological origin.

A modification of the picric acid assay of Chin and Lach (1), itself adapted from a method reported by Slonecker *et al.* (2), was found to have definite advantages over the previously reported method of Mitchell and Clark (3) in ease and speed of operation, with a resultant gain in accuracy and precision, as well as permitting the analysis of human excretion of the quaternary ammonium compound (QAC) in undiluted urine.

EXPERIMENTAL

Reagents—Anisotropine methylbromide, methyltropinium bromide, and homatropine methylbromide originated at Endo Laboratories. Atropine methylbromide was of Mann assayed grade. Choline chloride, acetylcholine chloride, and phosphocholine chloride were obtained from Calbiochem, the first two of commercial grade and the last of A grade. Thiamine hydrochloride was a USP supplied standard. Chloroform (J. T. Baker Chemical Co.) was of spectrophotometric reagent grade. All other chemicals were commercial products of reagent grade.

Analytical Procedures—The analytical method is an ion-pair extraction of methyl anisotropinium picrate from an alkaline aqueous solution into a nonpolar medium. A 5-ml. aqueous QAC-containing solution or 5 ml. of urine was made alkaline (pH >11) with 0.05 ml. of concentrated ammonia and 1 ml. of 0.1% aqueous picric acid solution added. After addition of 5 ml. of chloroform, the mixture was extracted by agitation on a Vortex Genie mixer for 15 sec. The upper aqueous layer was removed by aspiration, and the absorbance at 365 mμ (A_{365}) of the chloroform layer was determined in a Hitachi Perkin-Elmer 139 spectrophotometer using 10-mm. silica cells. The spectrophotometer was set to zero absorbance with the chloroform layer derived from a reagent blank. Chloroform–urine emulsions were broken by centrifuging 10 min. at 1000 r.p.m. at ambient temperature.

The sensitivity of the method was increased roughly threefold by limiting it to a single extraction, thereby avoiding dilution of the absorbing material. Replicate alkaline solutions were extracted, in duplicate, with, respectively, one, two, or three 5-ml. portions of chloroform, and it was determined that 92% of all the methyl anisotropinium picrate extractable in three successive extractions came out in the first extraction.

The characteristics of the picric acid assay system are similar to those reported by Chin and Lach (1). Absorption spectra of the chloroform extracts of the alkaline QAC–picric acid solutions were determined. The QAC's used are listed above in the reagent section. Standard curves were determined by plotting A_{365} of the chloroform extracts *versus* the concentrations of the aqueous QAC solutions which were subjected to analysis. The absorption spectrum of the chloroform extract derived from the anisotropine methylbromide–picric acid reaction mixture is essentially identical to the absorption spectra reported for benzalkonium picrate and cetylpyridinium picrate by Chin and Lach (1). Chloroform extracts of alkaline aqueous or urine solutions of choline chloride, acetylcholine chloride, phosphocholine chloride, thiamine hydrochloride, methyltropinium bromide, atropine methylbromide, or homatropine methylbromide mixed with picric acid yielded negligible absorption spectra; the naturally occurring quaternaries, choline, its deriva-

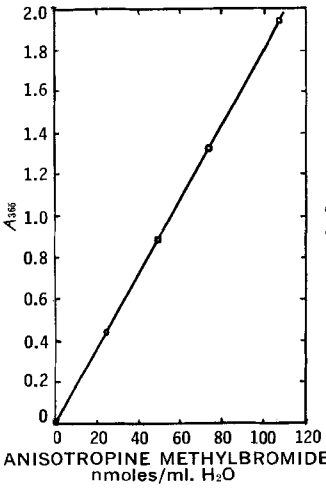

Fig. 1—Standard curve, methyl anisotropinium picrate in chloroform.

Received July 7, 1967, from the Department of Biochemistry and Department of Medicine, Endo Laboratories, Inc., Garden City, NY 11530
Accepted for publication August 21, 1967.
[1] Valpin, Endo Laboratories, Inc.

tives, or thiamine, will not interfere with the methyl anisotropinium assay when they are present in urine. Atropine methylbromide and homatropine methylbromide are several orders of magnitude more susceptible to alkaline hydrolysis than anisotropine methylbromide (4), both forming the unreactive quaternary alcohol, methyltropinium bromide. Presumably, thiamine hydrochloride produced no interaction due to prior destruction in the highly alkaline medium (5). At concentrations up to 110 nanomoles/ml. (nmoles/ml.), the only QAC which reacted to produce a standard curve was anisotropine methylbromide (Fig. 1).

When control, human urines from untreated individuals were compared to water blanks assayed at the same time, there was always a component of blank absorption. In addition, when human urines containing added anisotropine methylbromide were compared to water standards of the same concentrations, there was always an analytical loss. This loss could be due to the presence in urine of anions which have a greater affinity for methyl anisotropinium cation than does picrate. The blank absorptions and analytical losses were calculated by comparing quintuplicate samples of 0, 5, 10, and 15 nmoles/ml. anisotropine methylbromide in deionized water and in control urine, collected daily, over a period of 5 days. It was determined that the quantities recovered varied from one individual to another but were quite constant for the individual (Table I). The values for methyl anisotropinium ion in urine samples were determined by correcting the experimental A_{365} values for these factors.

Creatinine concentrations of urine specimens were determined by the method of Taussky (6) wherein ether removes reactive impurities while the creatinine is reacting with picric acid. The color of the picric acid–creatinine reaction product is developed with alkali. It was found that QAC's did not interfere with this assay in either aqueous solution or urine.

TABLE I—REPRODUCIBILITY OF BLANK VALUES AND ANALYTICAL RECOVERIES IN ANALYSIS OF URINE FOR ANISOTROPINE METHYLBROMIDE

Donor No.	Mean Blank A_{365} ± S.D.[a]	Mean Analytic Recovery,[b] % ± S.D.
1	0.019 ± 0.001	82.9 ± 5.5
2	0.024 ± 0.001	81.4 ± 3.5
3	0.021 ± 0.005	44.1 ± 7.2
4	0.018 ± 0.002	73.9 ± 7.4
5	0.041 ± 0.002	97.2 ± 1.7
6	0.014 ± 0.003	...
7	0.020 ± 0.002	...
8	0.043 ± 0.008	71.2 ± 5.8
9	0.038 ± 0.017	72.1 ± 5.2
10	0.018 ± 0.009	92.2 ± 5.3

[a] S.D. = standard deviation. [b] Losses are due to components normally present in urine. Expressed as percent of compound recovered after addition to urine from that donor.

TABLE II—CORRECTION VALUES AND DOSAGE FOR HUMANS

Subject	Mean Blank A_{365} ± S.D.	Mean Analytical Recovery, % ± S.D.	Anisotropine Methylbromide, Dose, mg./Kg.
A	0.015 ± 0.003	91.9 ± 6.7	0.264
B	0.016 ± 0.002	93.9 ± 3.5	0.304
C	0.020 ± 0.003	93.1 ± 4.6	0.284
D	0.026 ± 0.006	90.2 ± 6.4	0.244
E	0.021 ± 0.004	78.1 ± 10.3	0.200
F	0.021 ± 0.002	43.4 ± 6.6	0.310

TABLE III—ANISOTROPINE METHYLBROMIDE EXCRETION AS CUMULATIVE PERCENTAGE OF TOTAL DOSE (2 TABLETS, 20 mg.)

Subject	Total Excretion, %	Time, hr.	Time for ¾ Excretion, hr.
A	5.4	80[a]	48
B	10.7	96[a]	48
C	3.5	72[a]	60
D	7.6	104	72
E	4.1	96	24
F	11.4	96	64

[a] Excretion still continuing at termination of urine collection.

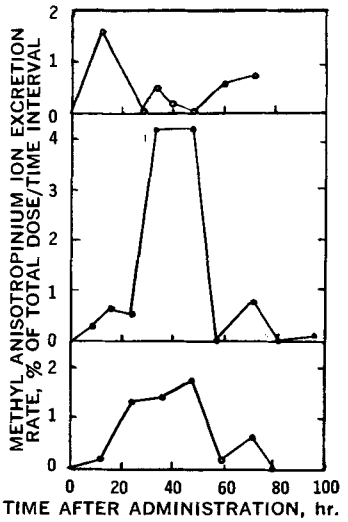

Fig. 2—Fractional urinary excretion of 20 mg. of anisotropine methylbromide administered as two 10-mg. tablets. Top, subject C; *middle, subject* B; *bottom, subject* A.

Excretion Study—After determining the blank value and the analytical loss in four separate 24-hr. urine samples per subject (Table II), six male subjects each took 20 mg. of anisotropine methylbromide (as two 10-mg. tablets) 0.5 hr. before breakfast. None of the subjects were taking any other medication at the time and none showed specific gravity abnormalities or any urine pathology; negative for blood, protein, and glucose and normal for pH as determined with Clinistix.[2] The mg./Kg. dosages of anisotropine methylbromide are listed in Table II. Urine collections from subjects *A*, *B*, and *C* were carried out for 80, 96, and 72 hr., respectively. Subjects *D*, *E*, and *F* were followed for 144 hr. The results for methyl anisotropinium ion excretion are presented in Table III and Figs. 2 and 3.

[2] Ames Co., Inc., Elkhart, Ind.

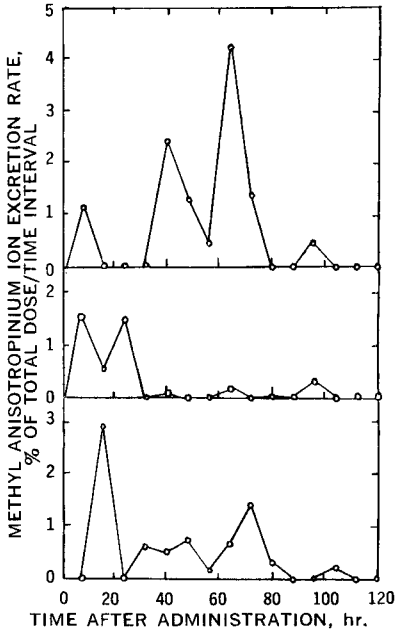

Fig. 3—Fractional urinary excretion of 20 mg. of anisotropine methylbromide administered as two 10-mg. tablets. Top, subject F; middle, subject E; bottom, subject D.

RESULTS AND DISCUSSION

Only methyl anisotropinium ion or nonhydrolyzed metabolic products would be likely to be detected by the picric acid assay method. If any such metabolites are present in urine, they are being included in the results reported as methyl anisotropinium ion excretion. Conversely, any drug broken down and being excreted as methyltropinium ion is being excluded.

The pattern indicated for methyl anisotropinium excreted by humans is most interesting. The data for fractional excretion (Figs. 2 and 3) indicate the presence of several excretion peaks over a period of 4 days. The magnitude of each of these peaks, and of the total cumulative excretion (Table III), varies with the subject. Previous studies of urinary excretion of QAC's after oral administration do not seem to have been carried out past 24 hr. (7–12). It is, as yet, unclear whether this sustained excretion is a common phenomenon in QAC excretion. The total excretions reported in Table III for anisotropine methylbromide show that anisotropine methylbromide, like previously studied quaternary compounds, is incompletely absorbed (7, 9, 11).

These results indicate not only absorption of anisotropine methylbromide, but also retention in the body of a portion of the absorbed material for an appreciable length of time. The compound may be stored for varying lengths of time in tissue depots, or perhaps recurring reabsorption of anisotropine methylbromide excreted in the bile has this effect. The periodicity of excretion would then be a function of the release of bile from the gall bladder. There are other explanations possible, but the present data do not permit definite conclusions to be drawn.

These results refer to only a limited number of human volunteers, and the variability which has previously been reported in QAC excretion (7–11) is also present here.

REFERENCES

(1) Chin, T. F., and Lach, J. L., *J. Pharm. Sci.*, **54**, 1550 (1965).
(2) Slonecker, J. H., Morrbery, J. B., Schmidt, P. R., Pittsley, J. E., Watson, R. R., and Jeanes, A., *Anal. Chem.*, **37**, 243(1965).
(3) Mitchell, R., and Clark, B. B., *Proc. Soc. Exptl. Biol. Med.*, **81**, 105(1952).
(4) Weiner, N., and Gordon, S. M., U. S. pat. 2,962,499 (November 29, 1960).
(5) Maier, G. D., and Metzler, D. E., *J. Am. Chem. Soc.*, **79**, 4386(1957).
(6) Taussky, H. H., *Clin. Chem. Acta*, **1**, 210(1956).
(7) Rennick, B. R., Moe, G. K., Lyons, R. H., Hoobler, S. W., and Neligh, R., *J. Pharmacol. Exptl. Therap.*, **91**, 210 (1947).
(8) Mahfouz, M., *Brit. J. Pharmacol.*, **4**, 295(1949).
(9) Dollery, C. T., Emslie-Smith, D., and McMichael, J., *Lancet*, **I**, 296(1960).
(10) Levine, R. M., and Clark, B. B., *J. Pharmacol. Exptl. Therap.*, **114**, 63(1955).
(11) Harington, M., *Clin. Sci.*, **12**, 185(1953).
(12) Levine, R. M., and Clark, B. B., *J. Pharmacol. Exptl. Therap.*, **121**, 63(1957).

Keyphrases

Anisotropine methylbromide
Urinary excretion
Ion-pair extraction
Picric acid reagent
Colorimetric analysis