Human Urinary Excretion of the Quaternary Ammonium Compounds Anisotropine Methylbromide and Propantheline Bromide

By MORRIS PFEFFER, JOSEPH M. SCHOR, SANFORD BOLTON, and RALPH JACOBSEN

Urinary excretion was studied after the administration of anisotropine methylbromide p.o. and i.v. and propantheline bromide p.o. to a group of volunteers. Orally administered material was excreted in the urine for periods of 2–5 days while i.v. administered material was excreted for 16 days. In all cases, periodic excretion peaks were observed. The urinary excretion of anisotropine methylbromide and propantheline bromide seems similar.

A PRELIMINARY STUDY of the excretion of a tablet formulation of the quaternary ammonium antispasmodic anisotropine methylbromide[1] (8-methyltropinium bromide 2-propylpentanoate), in six subjects was the subject of a previous publication (1). The observation of prolonged excretion with discrete periods of peak excretion of the compound has been confirmed and extended with a larger group of subjects. Several oral formulations and an intravenous administration are included. Similar results have been obtained for propantheline bromide[2] (β-diisopropylaminoethyl 9-xanthene carboxylate methobromide) tablets, indicating that the observed pattern of excretion might be a general property of quaternary ammonium compounds (QAC's).

EXPERIMENTAL

Reagents—Anisotropine methylbromide originated and was compounded at Endo Laboratories, Inc. Propantheline bromide was obtained as a commercially available 15-mg. tablet and 30-mg. ampul. Tropaeolin 00 (orange IV) was purchased from Matheson, Coleman and Bell. The grades and sources of supply of all other reagents were indicated previously (1).

Analytical Procedures—All spectrophotometric analyses were performed with a Hitachi Perkin-Elmer 139 spectrophotometer.

The analytical methods employed for both anisotropine methylbromide and propantheline bromide are similar in that they depend upon the formation of a salt between the cationic agent and an anionic dye. The dye salt is extracted from an aqueous medium into an immiscible nonpolar solvent. The analytical procedure for anisotropine methylbromide, employing picric acid as the anionic dye, has been reported previously (1).

Propantheline bromide did not yield an extractable dye salt in the picric acid assay system, presumably due to the presence of a hydrophilic hydroxyl group on the quaternary hydrolysis product of the ester. Hydrolysis was due to the highly alkaline aqueous medium necessary for this assay system. Since Biles *et al.* (2) had successfully analyzed for propantheline bromide with tropaeolin 00, this dye was used here, and a suitable assay was developed for the estimation of propantheline cation in human urine. Absorption spectrum for the propantheline-tropaeolin 00 dye salt in chloroform following extraction from an aqueous solution is given in Fig. 1. Five milliliters of urine or neutral aqueous solution containing propantheline cation was mixed with 0.5 ml. of a saturated aqueous solution of tropaeolin 00 and this mixture was extracted with 5.0 ml. of chloroform by agitation on a Vortex Genie mixer for 15 sec. Longer agitation on a wrist-action shaker for periods up to 30 min. did not improve extraction. A plot of the absorbance at 409 mμ (A_{409}) of the chloroform-extracted dye salt *versus* the original aqueous concentration of propantheline bromide was linear over the range of 0–114 nmoles/ml. The molar absorptivity was 1.76×10^7. The sensitivity

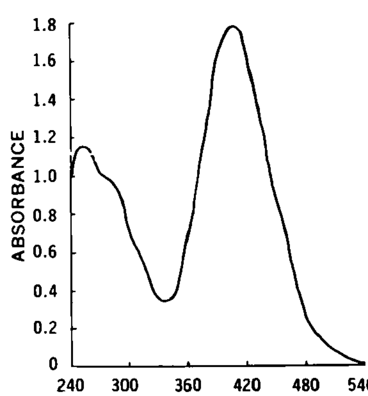

Fig. 1—Absorption spectrum of the propantheline-tropaeolin 00 dye salt in chloroform after extraction from aqueous solution. Propantheline bromide concentration 100 nmoles/ml. H_2O.

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[1] Marketed as Valpin by Endo Laboratories, Inc., Garden City, N. Y.

[2] Marketed as Pro-Banthine by G. D. Searle & Co., Chicago, Ill.

of the tropaeolin 00 analytical method for propantheline cation is almost identical to the sensitivity of the picric acid method for methylanisotropinium cation (1).

The naturally occurring QAC's choline chloride, acetylcholine chloride, phosphocholine chloride, and thiamine did not yield discernible dye salt absorption spectra in chloroform extracts when tested in the tropaeolin 00 assay at concentrations up to 0.3 μmoles/ml. H_2O. These compounds had previously been found not to interfere in the picric acid assay (1).

Divatia and Biles (3) reported that the response of QAC's to tropaeolin 00 decreases as the molecular weight of the QAC decreases.

It has been reported that in the picric acid assay for methylanisotropinium ion in human urine there is a component of blank absorption as well as a systematic analytical loss (1). It was demonstrated that both these factors remained constant for any given individual, although they varied from one to another. It developed that this was equally true for the tropaeolin 00 assay for propantheline cation in urine. The blank absorptions and analytical recoveries for both assay systems in the 12 subjects used in this study were calculated by comparing analyses of 0, 5, 10, and 15 nmoles/ml. of each QAC when in deionized water and when added to their normal urines. These control urines were collected for consecutive 8-hr. periods, starting 30 min. before breakfast, over three 24-hr. periods—a collection schedule corresponding to that used during the QAC excretion studies. The resultant correction factors are listed in Table I. The magnitude of their variations are indicated by the standard deviations (*SD*).

Previously (1) the correction values for methyl anisotropinium cation in urine had been determined for 24-hr. collections. In the present series of experiments, correction values were calculated separately for each of the three 8-hr. periods to determine if there were any diurnal variations in blanks and recovery values. Analysis of variance of the blank and recovery values did not indicate significant variation, over the three 8-hr. periods, of the values for any given individual. However, there were significant differences between individuals for blanks and recoveries. There was no apparent correlation between the blank and recovery values.

The values for methylanisotropinium and propantheline cations were determined by correcting the respective A_{365} and A_{409} values obtained from experimental urine samples, for corresponding time intervals, according to the equation

$$B = (A - D)100/C$$

where B = corrected absorbance, A = observed absorbance, D = blank absorbance values for that time period, and C = calculated percentage analytical recovery for that time period. Concentrations were calculated in terms of nanomoles that were then converted back to their equivalents in mcg. of the bromide salts of the QAC's.

TABLE I—ANALYTICAL CORRECTION VALUES FOR HUMAN SUBJECTS

Subject No.	Time Interval, Hr.	Anisotropine Methylbromide: Mean Blank $A_{365} \pm SD$	Anisotropine Methylbromide: Mean Recovery % ± *SD*	Propantheline Bromide: Mean Blank $A_{409} \pm SD$	Propantheline Bromide: Mean Recovery % ± *SD*
1	0–8	0.024 ± 0.004	83.5 ± 11.2	0.037 ± 0.005	81.0 ± 1.6
	8–16	0.033 ± 0.002	80.5 ± 7.1	0.024 ± 0.002	77.4 ± 0.3
	16–24	0.023 ± 0.005	81.0 ± 5.7	0.029 ± 0.000	84.2 ± 1.0
2	0–8	0.061 ± 0.004	80.0 ± 2.3	0.073 ± 0.004	48.8 ± 0.3
	8–16	0.035 ± 0.004	86.0 ± 0.6	0.035 ± 0.003	70.7 ± 0.5
	16–24	0.025 ± 0.003	91.2 ± 4.0	0.026 ± 0.001	73.1 ± 0.4
3	0–8	0.029 ± 0.006	58.4 ± 5.7	0.033 ± 0.000	37.8 ± 1.6
	8–16	0.023 ± 0.004	60.1 ± 1.7	0.034 ± 0.003	58.8 ± 4.1
	16–24	0.018 ± 0.007	64.7 ± 7.7	0.028 ± 0.001	24.3 ± 1.4
4	0–8	0.030 ± 0.009	59.6 ± 5.4	0.042 ± 0.019	22.6 ± 4.7
	8–16	0.026 ± 0.007	71.6 ± 4.9	0.040 ± 0.007	64.1 ± 0.2
	16–24	0.027 ± 0.002	63.7 ± 3.8	0.026 ± 0.002	31.2 ± 4.7
5	0–8	0.026 ± 0.001	57.5 ± 1.6	0.056 ± 0.001	58.9 ± 0.3
	8–16	0.024 ± 0.003	63.0 ± 4.4	0.017 ± 0.004	72.6 ± 1.2
	16–24	0.027 ± 0.003	62.2 ± 5.2	0.022 ± 0.003	57.4 ± 0.3
6	0–8	0.013 ± 0.002	71.7 ± 1.5	0.021 ± 0.003	58.5 ± 2.7
	8–16	0.014 ± 0.000	68.6 ± 3.6	0.010 ± 0.003	62.8 ± 7.1
	16–24	0.016 ± 0.004	78.1 ± 4.6	0.013 ± 0.002	70.7 ± 4.5
7	0–8	0.026 ± 0.006	72.4 ± 2.2	0.017 ± 0.000	49.4 ± 4.9
	8–16	0.027 ± 0.004	73.5 ± 1.4	0.029 ± 0.005	56.7 ± 3.2
	16–24	0.020 ± 0.001	79.0 ± 2.3	0.022 ± 0.003	64.2 ± 6.0
8	0–8	0.020 ± 0.002	73.9 ± 2.5	0.018 ± 0.002	49.3 ± 1.7
	8–16	0.011 ± 0.003	79.0 ± 5.3	0.029 ± 0.000	51.3 ± 2.0
	16–24	0.013 ± 0.000	79.3 ± 3.8	0.022 ± 0.000	66.4 ± 0.9
9	0–8	0.024 ± 0.002	90.3 ± 0.4	0.019 ± 0.002	64.1 ± 0.8
	8–16	0.022 ± 0.002	88.9 ± 0.4	0.021 ± 0.004	60.9 ± 0.9
	16–24	0.032 ± 0.005	91.3 ± 2.6	0.021 ± 0.004	59.6 ± 2.0
10	0–8	0.018 ± 0.002	81.3 ± 0.5	0.031 ± 0.001	69.9 ± 2.5
	8–16	0.023 ± 0.001	71.0 ± 8.2	0.049 ± 0.006	74.7 ± 0.7
	16–24	0.023 ± 0.002	82.0 ± 5.6	0.055 ± 0.001	72.7 ± 0.7
11	0–8	0.038 ± 0.006	80.0 ± 6.5	0.094 ± 0.001	86.7 ± 0.4
	8–16	0.032 ± 0.004	75.6 ± 1.7	0.044 ± 0.006	77.6 ± 1.7
	16–24	0.022 ± 0.004	87.7 ± 5.0	0.038 ± 0.003	85.9 ± 1.6
12	0–8	0.018 ± 0.005	44.1 ± 1.3	0.014 ± 0.002	34.3 ± 1.9
	8–16	0.044 ± 0.005	46.8 ± 5.8	0.011 ± 0.000	39.9 ± 4.1
	16–24	0.036 ± 0.001	35.4 ± 5.0	0.023 ± 0.006	17.0 ± 1.5

Table II—Complete Three-Way Analysis of Variance

Source	df^a	SS^b	MS^c	F
People	11	3.97		
People × formulations	31	19.6	0.632	
Times	20	71.0		
Times × formulations	60	15.0	0.251	$F(60,260) = 1.27$
Times × people	220	57.8		
Times × people × formulations	620	123	0.198	
Formulations	3	3.10	1.03	$F(3,31) = 1.63$

[a] df = degrees of freedom (see no. of experimental subjects for each formulation in Table III). [b] SS = sum of squares. [c] MS = mean square.

Creatinine concentrations in urine specimens were determined by the method of Taussky (4). Neither anisotropine methylbromide nor propantheline bromide interfered in this determination.

Excretion Study—Twelve males were employed. None took any other medication during these experiments. None showed any abnormalities of urine specific gravity,[3] blood,[4] protein,[4] glucose,[4] or pH.[4]

The formulations administered are listed in Table III. These were taken 30 min. before breakfast, except the intravenous injection which was given at 11 a.m. Complete dosage of the liquid formulations was ensured by also administering washings of the containers.

Consecutive 8-hr. collections of urine were started immediately upon dosing and were not terminated until nine consecutive 8-hr. samples gave negative results.

The urines were preserved by the addition of SFT tablets[5] and refrigerated until analyzed. All analyses were performed within 48 hr. of sample collection. The SFT tablets did not interfere with determinations of either QAC in water or urine or with the creatinine assay.

RESULTS

The term fractional excretion represents that portion of a QAC dose excreted during any single 8-hr. interval. The mean fractional excretions reported here have been averaged for the 12 subjects for each of the different formulations for each corresponding 8-hr. period and are expressed as percent of total dose. Excretion after intravenous administration is considered separately from oral administration.

After intravenous administration of anisotropine methylbromide, approximately 50% of the dose is excreted in the first 8-hr. period (Fig. 2). Excretion continues for 16 days with a pattern of fluctuating excretion rates until an average of 108% of the administered drug is excreted.

The mean fractional excretions of the orally administered QAC's are presented in Fig. 3. The same pattern of periodic excretion peaks is seen. Although excretion from these formulations is not as prolonged

[3] Adams Midget Urinometer, Clay-Adams Inc., New York, N. Y.
[4] Labstix, Ames Co., Elkhart, Ind.
[5] Sodium fluoride-thymol tablets, Cambridge Chemical Products, Detroit, Mich.

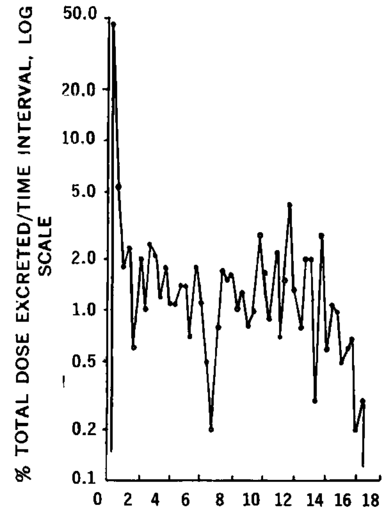

Fig. 2—Fractional excretion of anisotropine methylbromide by human subjects, after i.v. administration Total dose, 5 mg.

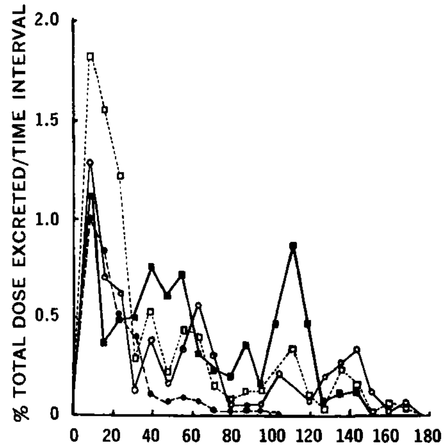

Fig. 3—Fractional excretion of QAC's by human subjects after oral administration. Dosages: anisotropine methylbromide, 25 mg.; propantheline bromide, 30 mg. Key: ●, anisotropine methylbromide tablets; ○, anisotropine methylbromide elixir; □, anisotropine methylbromide kaolin-pectin suspension; ■, propantheline bromide tablets.

as excretion from the intravenous dose, duration of excretion is still appreciable. An analysis of variance (Anova) of this data was performed (Table II). Neither F value obtained in the complete 3-way Anova is significant. Overall QAC excretion levels do not differ. The times × formulations interaction shows no evidence for lack of parallelism between the various fractional excretion plots. It would seem, therefore, that the correspondence in time of occurrence of the excretion peaks in Fig. 3 is real and is indicative of a fundamental similarity in excretion behavior of the two QAC's under study. Superimposition of the corresponding section of the plot for fractional excretion of i.v. administered anisotropine

Table III—Total Excretion of QAC's

QAC	Formulation[a]	n	Dose, mg.	Mean Total Excretion, mg.	Mean Total Excretion, as % of Dose	Mean Total Excretional Time, hr.
Anisotropine methylbromide	Tablets	12	25	0.76	3.1	55
	Tablets[b]	6	20	1.42	7.1	91
	Elixir	12	25	1.42	5.7	125
	Elixir	3	20	0.99	4.9	51
	Kaolin-pectin suspension	11	25	1.49	6.0	64
	i.v. Injection	12	5	5.4	108	400
Propantheline bromide	Tablets	11	30[c]	2.20[d]	5.7	95
Anova[e]	F_1				1.63	7.48[f]
	F_2				0.47	2.96

[a] Formulation potencies: anisotropine methylbromide, 10-mg. tablet, 2 mg./ml. elixir, 0.33 mg./ml. kaolin-pectin suspension, 5 mg./ml. for i.v. injection, propantheline bromide, 15 mg./tablet. [b] Data from Pfeffer *et al.* (1) included for purposes for comparison. [c] Thirty mg. of anisotropine methylbromide is the molar equivalent of 24.2 mg. of anisotropine methylbromide. [d] Equivalent to 1.73 mg. of anisotropine methylbromide. [e] Anova excludes data from i.v. injection and the anisotropine methylbromide tablets and elixir administered at dosages of 20 mg. $F_1 = F$ for differences among all four oral dose forms. $F_2 = F$ for differences between means of the three oral anisotropine methylbromide dose forms and the propantheline bromide tablets. Significance at $p = 0.05$; $F_1 = 2.91$; $F_2 = 4.16$; at $p = 0.01$; $F_1 = 4.48$, $F_2 = 7.53$. [f] $p = 0.01$.

methylbromide (Fig. 2) onto Fig. 3 would reveal that a parallelism in excretion peaks is maintained between oral and intravenous dosage although the levels excreted differ. There was no obvious relationship between urine volume, weight or age of the subjects, and the variables reported upon here.

The mean values for total excretion time and total excretion are presented in Table III. The statistical analysis indicated that there are no significant differences between the observed QAC levels excreted after administration of the various oral formulations, either when they are compared to each other or when propantheline bromide is compared to the pooled mean excretion level of all the oral anisotropine methylbromide formulations.

The observed total excretion times of the anisotropine methylbromide in its various oral formulations also are not significantly different from the total excretion times for propantheline bromide administered as tablets.

In previous studies QAC excretion has been found to be highly variable (5–11). Despite these normally accepted variations, it is apparent that the several formulations of anisotropine methylbromide are equivalent. It is also of deep interest that the observed excretions of these two antispasmodics are very similar.

DISCUSSION

In a previous paper (1) it was indicated that, due to the nature of the picric acid assay, metabolized anisotropine methylbromide was not likely to be detected. The total mean recovery value of 108% after intravenous administration of anisotropine methylbromide has, therefore, three interesting implications: (*a*) all anisotropine methylbromide in the bloodstream may be eventually excreted through the kidneys, (*b*) this drug is apparently not metabolized to polar compounds in the human body, (*c*) if this QAC is undergoing enterohepatic recycling, the efficiency of intestinal reabsorption of the material excreted in the bile must approach 100%.

Rowland and Beckett (12) have observed successive peaks during the excretion, by humans, of amphetamine and methylamphetamine. This they attributed to the diurnal urinary pH rhythm. The degree of ionization of these weak bases shifts as the urinary pH shifts, altering the degree of reabsorption from the distal tubule to the kidney. This would not, however, appear to serve as an explanation for the periodic excretion peaks seen with QAC's. These strong quaternary bases, for which pKa's are not determinable, will not alter their degree of ionization at any physiologically attainable pH. Furthermore, the timing of the excretion peaks for these QAC's does not correspond to the timing of the diurnal pH changes reported by Rowland and Beckett.

Retention of QAC's in an intracellular tissue reservoir would not seem a likely explanation either. Although the QAC might be slowly released it should not be released in "pulses." If there is an upper limit to the amount of methyl anisotropinium cation the kidney can excrete in a given time period, it has not been approximated in these experiments. After intravenous injection of 5 mg. of anisotropine methylbromide, approximately 2.5 mg. can be excreted in an 8-hr. period and the average amount excreted in any succeeding peak is appreciably larger than the amount excreted in any peak after oral administration. Thus, in these studies, the amount excreted through the kidney would seem to be a function of the amount available to the kidney.

Enterohepatic cycling of the QAC seems to offer the best explanation of the observed phenomenon. Bile, after its formation, is stored in the gall bladder and is periodically released into the intestinal tract (13). This periodicity of bile release might correspond to the periodicity of QAC excretion. QAC excreted into the bile would therefore be held in the gall bladder to eventually be released with the bile. The QAC would be reabsorbed and a portion excreted in the kidney while the balance would be reexcreted into the bile.

By inference, the total excretions after oral dosage represent the total gastrointestinal absorption of anisotropine methylbromide. The amounts absorbed are retained *in vivo* for a prolonged period of time.

The excretion pattern and amount excreted for orally administered propantheline bromide is almost exactly the same as that of anisotropine methylbromide. The question of whether this could be a common excretion pattern for QAC's is raised.

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Keyphrases

Urinary excretion—quaternary ammonium compounds
Anisotropine methylbromide—human urinary excretion
Propantheline bromide—human urinary excretion
IV, oral administration—excretion times
Colorimetric analysis, urine—spectrophotometer

Significance of Kinetic Aspects in the Simultaneous Administration of Drugs

By MILDRED J. TARASZKA and ARLINGTON A. FORIST

Kinetic aspects, such as half-lives for absorption and elimination, as well as limiting solubilities, which should be considered in the simultaneous administration of drugs are discussed. Two simple hypothetical cases are presented: the selection of the ratio of two drugs with different rate constants for absorption and elimination to obtain similar average asymptotic serum levels of each drug on multiple-dose administration, and the selection of the ratio of two drugs with different rate constants for absorption and elimination and different solubilities to minimize the risk of crystalluria. Extension of the latter to the triple sulfas, on the basis of solubility and human blood level data in the literature, has given a "best" ratio of 1:3:4 for sulfadiazine:sulfamerazine:sulfamethazine, respectively, rather than the 1:1:1 now used.

When two or more drugs are combined in a single formulation or are given in separate dosage forms at the same time, several factors should be considered in choosing the individual drugs and the amounts of each drug to be used: (*a*) biological spectra, (*b*) minimum effective circulating concentrations for biological activity, (*c*) possibilities of reactions between the combined drugs, (*d*) possibility of one drug affecting the biological response to the other drug(s), (*e*) rate constants for absorption or absorption half-lives, (*f*) rate constants for elimination or biological half-lives, (*g*) rates of production and elimination of metabolites (especially if the metabolites may be active or toxic), (*h*) intrinsic solubilities of the individual drugs and/or metabolites if crystalluria is a potential side reaction.

It is the authors' opinion that drug combinations could be more beneficial if all of the above factors are considered. However, only kinetic aspects that affect drug combinations are considered in this paper; that is, all the calculations are based on the assumption that the individual drugs have the correct spectra of biological activity, similar minimum effective circulating concentrations, and that they are compatible in the *in vitro* and *in vivo* systems. The importance of the kinetic aspects can be seen if the selection of the optimum dosage schedule is considered for multiple-dose therapy with a combination of two drugs which have biological half-lives of largely different magnitudes.

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DISCUSSION

Example 1—When two or more drugs are combined in a single formulation, or the drugs are given in separate dosage forms at the same time, the multiple-dose serum levels attained with each drug will depend on each biological half-life plus *other* factors. The ratio of two drugs given in combination, which should be used to attain similar serum levels on multiple dosing, can be estimated in the following manner, assuming that the intrinsic drug solubilities, biological activities, and production of metabolites do not have to be considered. Furthermore, it is assumed that the single-dose serum level and urinary data for each drug fit the following model:

$$A \xrightarrow{k_1} C \xrightarrow{k_2} D \qquad \text{(Model 1)}$$