Urinary Excretion of Ephedrine in Man without pH Control following Oral Administration of Three Commercial Ephedrine Sulfate Preparations

PETER G. WELLING, KWANG P. LEE, JAYANT A. PATEL, JONATHAN E. WALKER*, and JOHN G. WAGNER†

Abstract □ In a feasibility study, 25-mg. single oral doses of ephedrine sulfate, in the form of a commercial syrup and two commercial capsules, were administered in crossover fashion to three nonsmoking male subjects. Urinary pH was not controlled, but adequate urinary flow rates were maintained by regulated water intake. Urinary excretion of ephedrine was measured over a 48-hr. period by a GC method. There were no significant differences in average amounts of ephedrine excreted during any of the 11 sampling intervals, in average peak excretion rates, nor in average times of occurrence of the peak excretion rate. There were insufficient subjects used in this preliminary study to form definite conclusions about differences between or among dosage forms. The urinary excretion data were adequately described by the two-compartment open model with first-order absorption and lag time. The average elimination half-life of ephedrine was 5.99 hr. estimated from the β-values obtained by exponential fitting. Average urinary pH was 6.30 (95 of the 99 samples fell within 5.32 to 7.28). The data provided little, if any, evidence of significant relationships between deviations of observed from theoretical excretion rates of ephedrine and urinary pH, urinary excretion rates of ephedrine and urinary pH, or excretion rates of ephedrine and urinary flow rates.

Keyphrases □ Excretion rates, urinary—ephedrine sulfate, without pH control □ Ephedrine sulfate—oral commercial preparations, urinary excretion rates without pH control, man □ GLC—determination, ephedrine sulfate in urine

It was suggested that the study of the kinetics of basic drugs in humans could give rise to invalid conclusions unless reabsorption of drug and/or metabolite in the kidney tubules is absent or negligible (1). Acidification of the urine by oral administration of ammonium chloride during such a study is used to obtain a urinary pH of 4–5. Theoretically, the greater concentration of ionized species of drug at this pH would reduce reabsorption compared to that at higher urinary pH values. This argument was applied to such drugs as the ephedrines, amphetamines, and chlorpheniramine (1–4). Controlled acidification of urine, although linearizing logarithmic excretion-rate values with respect to time, may bring about a situation somewhat removed from that obtained under normal clinical conditions not only in the GI tract but also in the kidneys. Furthermore, kinetic constants derived from such a study could be quite different from those obtained under more normal clinical conditions.

This problem was investigated during a recent preliminary study on the relative bioavailability of some commercial preparations of ephedrine sulfate. This feasibility study was to determine whether absorption of ephedrine sulfate from commercial capsules is different than from a solution (syrup) and whether the absorption of the drug from some commercial capsules is dissolution rate controlled. During this study, urinary pH was not controlled. High urinary flow rates were obtained, however, by providing subjects with controlled amounts of water. Unchanged drug in urine was measured by a GC assay, different from that of Beckett and Wilkinson (2).

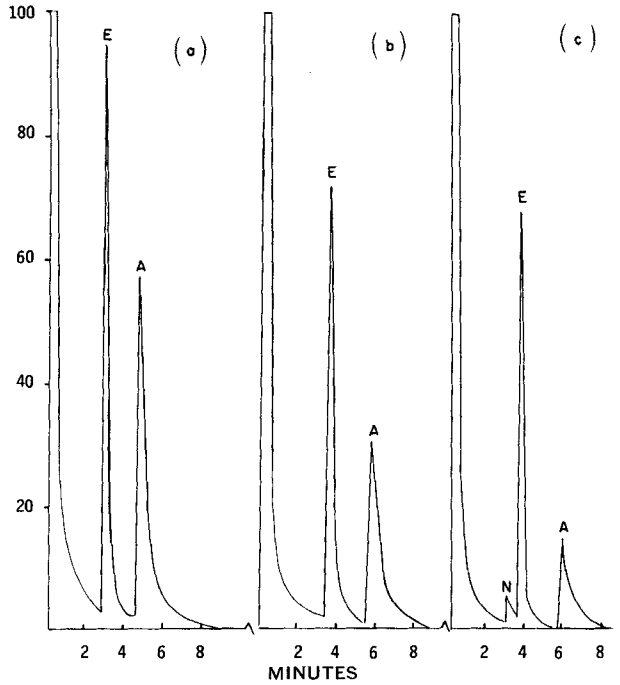


Figure 1—*Examples of gas chromatograms. (a) Gas chromatogram obtained from an extract of blank urine spiked with ephedrine sulfate (12 mcg./ml.) and 4-aminoacetophenone (5 mcg./ml.). (b) Gas chromatogram obtained by assay of a 0.5–1.0-hr. urine sample of Subject 1. (c) Gas chromatogram obtained by assay of a 6–9-hr. urine sample of Subject 1. Key: E, ephedrine peak; A, 4-aminoacetophenone peak; and N, norephedrine peak.*

EXPERIMENTAL

Reagents—The following were used: standard—ephedrine sulfate USP[1]; internal standard—4′-aminoacetophenone[2]; ether for anesthesia[3]; 5 *N* NaOH solution; sodium hydroxide T.S. USP; 2 *N* HCl solution; anhydrous Na_2SO_4, granular[1]; and Silyl-8[4].

Dosage Forms—One solution of ephedrine sulfate as the syrup and two commercial brands of capsules, hereafter called Treatments A, B, and C[5], were employed.

[1] Mallinckrodt Chemical Works.
[2] Eastman Organic Chemicals, No. 631.
[3] E. R. Squibb & Sons, No. 4084.
[4] Pierce Chemical Co.
[5] A = ephedrine sulfate syrup NF XII, Eli Lilly & Co., Control No. 3JC75A; B = ephedrine sulfate capsules USP XVII, 25 mg. (3/8 gr.), Eli Lilly & Co., Control No. 3DA76A; and C = ephedrine sulfate capsules, 3/8 gr. (25 mg.), American Pharmaceutical Co., Control No. 9R12876.

Table I—Amounts of Ephedrine[a] Excreted in Urine during Each Sampling Interval (A) and Urinary pH (B)

Time Interval, hr.	Treatment A, Subject 1	Treatment A, Subject 2	Treatment A, Subject 3	Treatment B, Subject 1	Treatment B, Subject 2	Treatment B, Subject 3	Treatment C, Subject 1	Treatment C, Subject 2	Treatment C, Subject 3
0–0.5									
A	0.172	0.562	1.94	0.144	0.021	0.056	0.085	0.074	0.188
B	6.73	5.20	5.65	5.56	6.52	6.61	6.25	5.21	5.71
0.5–1.0									
A	1.47	2.02	2.70	1.40	0.483	1.12	1.17	3.06	1.87
B	6.82	6.23	6.41	6.46	6.72	6.53	6.17	5.89	6.32
1.0–1.5									
A	0.653	1.60	1.44	1.04	0.917	1.54	1.48	2.69	1.79
B	7.08	6.40	6.49	6.51	6.83	6.52	6.54	6.12	6.15
1.5–2.0									
A	0.793	1.36	1.46	0.849	1.12	1.07	1.14	1.49	1.31
B	7.08	6.33	6.50	6.48	6.79	6.10	6.59	6.38	6.13
2.0–2.5									
A	0.556	1.33	1.03	0.773	0.503	0.746	0.990	1.06	1.24
B	7.82	6.00	6.46	6.48	6.82	6.96	6.64	6.60	6.43
2.5–3.0									
A	0.557	0.918	1.27	0.455	0.642	0.759	0.731	1.15	1.50
B	7.06	6.43	6.43	6.48	6.75	6.75	6.65	6.65	5.89
3–6									
A	2.57	3.22	3.17	4.22	3.52	4.24	4.68	4.50	2.40
B	6.92	6.04	5.68	6.42	6.82	5.45	6.04	6.68	5.86
6–9									
A	1.30	2.52	2.37	0.557	3.38	2.70	2.97	1.97	4.17
B	5.60	6.87	6.22	5.26	6.57	6.41	5.50	7.01	5.81
9–12									
A	2.49	1.76	2.07	1.94	2.88	3.01	2.62	1.97	1.44
B	6.27	5.73	5.43	6.17	5.51	6.42	5.73	5.70	6.67
12–24									
A	2.94	2.22	2.29	3.75	3.44	3.27	2.50	2.91	3.61
B	6.77	6.22	6.34	6.01	6.60	7.02	5.57	5.71	5.85
24–48									
A	0.950	0.780	0.492	0.599	0.766	0.898	0.627	0.492	0.595
B	5.86	5.77	6.13	6.38	5.77	6.98	5.88	6.34	6.55

[a] Expressed as milligrams of ephedrine sulfate.

Apparatus—All final estimations were done on a gas chromatograph[6] with a flame-ionization detector and a recorder (Mosely) with a chart integrator (disc).

The 1.83-m. (6-ft.) GC column had an internal diameter of 4 mm. and was packed with 3% OV-1 on Gas-Chrom Q, 100–200 mesh. The column was conditioned overnight between runs at 180° with minimum nitrogen flow. It was silanized with 10 μl. of Silyl-8 after each run. Operating conditions were: oven temperature, 140°; injection block temperature, 140°; and detector temperature, 230°. Gas flow rates were: air, 300 ml./min. at 20 lb./sq. in.; hydrogen, 35 ml./min. at 10 lb./sq. in.; and nitrogen, 60 ml./min. at 20 lb./sq. in.

Extractions were done on a vertical rotary mixer, 31 r.p.m., adapted to hold 15-ml. centrifuge tubes. All centrifugations were done in a centrifuge (Adams Dynac) at maximum speed (2460 r.p.m., 1280 r.c.f.).

Assay Procedure—Urine (5 ml.), water (1 ml.), and 2 *N* HCl (0.5 ml.) were combined in a 15-ml. centrifuge tube, fitted with a Teflon-lined screw cap, and mixed by circular rotation for 5 min. The mixture was extracted with 2 × 2 ml. of ether for 5 min. and centrifuged for 10 min.; then the ether layers were discarded. To the urine were then added 5 *N* NaOH (0.5 ml.) and exactly 0.5 ml. of a 0.05-mg./ml. solution of internal standard in water. The mixture was extracted with 3 × 2 ml. of ether. The extracts were combined in a 15-ml. centrifuge tube.

Anhydrous sodium sulfate (600 mg. or 0.4 ml. soft packed in a finely tapered centrifuge tube) was added to the ether extracts, and the tube was agitated vigorously on a vortex mixer for 1 min. After centrifugation, the ether layer was decanted off into a finely tapered centrifuge tube and the volume was reduced to approximately 50 μl. under a gentle stream of nitrogen at room temperature. Approximately 4–5 μl. was injected onto the column.

Although some peak tailing was observed with this system, it was not sufficient to interfere with peak area measurement. Ephedrine, norephedrine (the principal urinary metabolite of ephedrine), and internal standard had retention times of approximately 3.9, 3.6, and 5.8 min., respectively. The norephedrine peak could be clearly seen at the later urinary collections. No attempt was made in this study, however, to quantitate it. This peak did not interfere with peak area measurement of ephedrine. Typical chromatograms are shown in Fig. 1.

Quantitation was achieved by use of the relationship:

$$\text{concentration of ephedrine sulfate} = \frac{E/A}{0.128} \qquad \text{(Eq. 1)}$$

where E = peak area due to ephedrine, A = peak area due to the internal standard, and 0.128 = a constant obtained by dividing E/A values by urinary concentration of ephedrine sulfate in spiked urine during assay standardization.

Two spiked samples were carried through with each assay batch as a check on assay reproducibility.

Assay Standardization—Five-milliliter lots of blank urine from a nonsmoking male were spiked with 0.1, 0.2, 0.4, and 0.6 ml. of a 0.1-mg./ml. solution of ephedrine sulfate in water to give urine concentrations of 2, 4, 8, and 12 mcg./ml. The water volume thus added was deducted from the 1.0 ml. of water added at the start of the assay. The assay of the spiked urine was carried out exactly as already described, and the required constants were obtained.

Assay of Dosage Forms—The two lots of capsules and the syrup were assayed for ephedrine sulfate content by official assay procedures (5, 6). To determine capsule-to-capsule variation within a batch, individual capsules were also assayed by a modification of the USP method for ephedrine sulfate capsules. The contents of one capsule were carried through the USP assay with suitable adjustments of solvent volumes. The 0.1 *N* NaOH and H_2SO_4 solutions used in the original assay were each diluted 10 times to enable similar titration volumes to be measured for the smaller amount of ephedrine sulfate present.

Study Conditions—Three nonsmoking male subjects, age 27–32 years and weighing between 68.1 and 77.2 kg. (150 and 170 lb.), with normal vital signs, were used. All subjects had received no known enzyme-inducing agents for 30 days and no medication at

[6] Hewlett-Packard F&M model 402.

all for 7 days preceding the study, and they received none during the study.

Treatments consisted of one 25-mg. or $^3/_8$-gr. capsule or a volume of syrup containing 25 mg. of ephedrine sulfate (6.25 ml., 8.14 gr.). Capsules were swallowed whole with 6 fl. oz. of water. Syrup was weighed into a suitable container, and total dosage was ensured by rinsing all syrup from the container with water during administration.

Subjects were fasted overnight (from 10 p.m.) and for 4 hr. postdosing. The dose was administered at about 8 a.m., and urine was collected quantitatively in polyethylene bottles containing 0.5 ml. of toluene in the intervals 0–0.5, 0.5–1, 1–1.5, 1.5–2, 2–2.5, 2.5–3, 3–6, 6–9, 9–12, 12–24, and 24–48 hr. following treatment. To ensure adequate urine flow, 6 fl. oz. of water was taken orally at −1.5, −1, −0.5, 0, 0.5, 1, 1.5, 2, 2.5, 3, 6, and 9 hr. on each treatment day.

The volume and pH of each urine sample at room temperature were recorded. A small aliquot was then retained and quick frozen until just prior to the assay. Treatments were separated by a 7-day period. The following three-phase crossover dosage schedule was used:

	Treatment—Week		
Subject	I	II	III
1	A	B	C
2	B	C	A
3	C	A	B

RESULTS

Assay Standardization—The $E/(A \times$ concentration) ratio obtained from nine sets of urine spiked with ephedrine sulfate at 2.0–12.0 mcg./ml. on 9 different days (35 measurements) averaged 0.128 ± 0.0196 (one standard deviation). The ratios obtained from the different concentrations, 2.0, 4.0, 8.0, and 12.0 mcg./ml., were 0.129 ± 0.024, 0.123 ± 0.015, 0.124 ± 0.018, and 0.136 ± 0.021, respectively.

The ratios obtained from urines spiked at a concentration of 8.0 mcg./ml. and assayed on the same days as the subjects' urines (16 measurements) averaged 0.137 ± 0.017. This mean did not differ significantly from 0.128 ± 0.0196 ($t = 1.63$, $df = 49$, $0.2 > p > 0.1$); therefore, the figure 0.128 was used as the denominator in the equation to obtain the urinary concentration of ephedrine.

Dosage Form Assay—Four official assays of ephedrine sulfate syrup (Treatment A) yielded a concentration of 25.1 ± 0.43 mg. of drug/6.25 ml. of syrup. Capsules C yielded 25.1 and 25.4 ± 1.5 mg./capsule in two official and five single-capsule assays, respectively. Capsules B yielded 24.1 ± 0.15 and 24.1 ± 0.37 mg./capsule in three official and four single-capsule assays, respectively. Potencies obtained from the official assays were used as the basis of all absorption and recovery estimations. The very small standard deviations obtained in the single-capsule assays enabled the doses to be stated accurately.

Urinary Excretion of Ephedrine—The amounts of ephedrine sulfate excreted in urine and the urinary pH values are given in Table I.

A typical excretion rate, urinary pH, and urinary output profile are shown in Fig. 2. Only small deviations from this pattern were observed throughout the study by subjects receiving all three dosage forms. The average excretion rates of ephedrine sulfate are summarized in Fig. 3. Although lower excretion rates are apparent after Treatment B compared to those after Treatments A and C during the 1–3-hr. period, analysis of variance for crossover design showed no significant differences among treatment average amounts of ephedrine sulfate excreted in any time interval or in the total 48-hr. period, either expressed as milligrams or as percent of the administered dose.

There were also no significant differences among either peak ephedrine excretion rates or among times of occurrence of the peak excretion rate. There were no significant differences among elimination half-lives of ephedrine, obtained either by plotting terminal logarithmic excretion rates *versus* time or from exponential fitting. These data are summarized in Table II.

The individual subject and treatment average cumulative amounts of ephedrine sulfate excreted as a function of time were fit by the two-compartment model with first-order absorption and lag time.

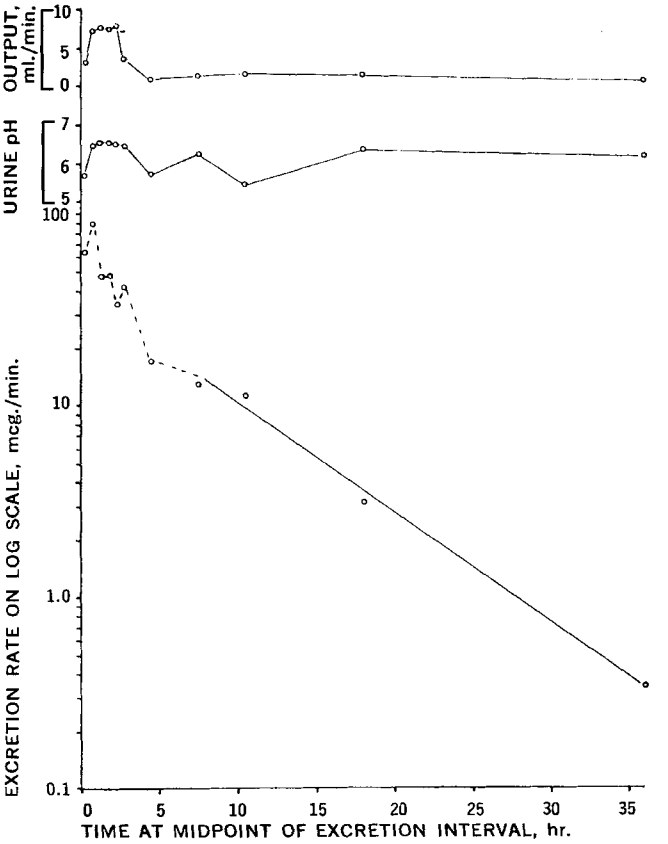
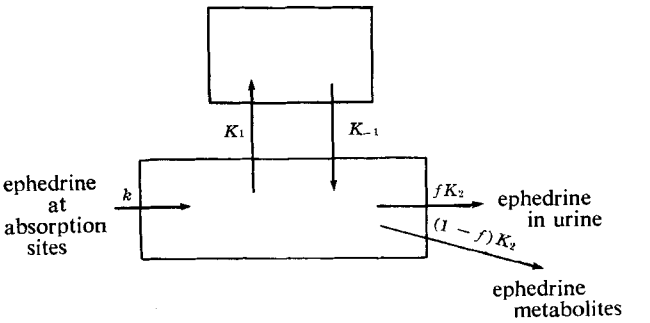


Figure 2—*Semilogarithmic plot of urinary excretion rate of ephedrine and plots of urinary pH and urinary output against midpoints of the excretion intervals for Subject 3 following Treatment A.*

By using Eqs. 2 and 3 (7), in conformity with the model shown in Scheme I, the data were fitted with the program NONLIN[7] on an IBM 360/67 computer.



Scheme I

$$A_u = A_u^{\infty}\left[1 - kK_2\left\{\left(\frac{K_{-1}-\alpha}{\alpha(k-\alpha)(\beta-\alpha)}\right)e^{-\alpha(t-t_0)} + \left(\frac{K_{-1}-\beta}{\beta(k-\beta)(\alpha-\beta)}\right)e^{-\beta(t-t_0)} + \left(\frac{K_{-1}-k}{k(\alpha-k)(\beta-k)}\right)e^{-k(t-t_0)}\right\}\right] \quad \text{(Eq. 2)}$$

where $\alpha > \beta$ and:

$$\alpha,\beta = {}^1/_2[(K_1 + K_{-1} + K_2) \pm \sqrt{(K_1 + K_{-1} + K_2)^2 - 4K_{-1}K_2}] \quad \text{(Eq. 3)}$$

$K_2 = fK_2 + (1 - f)K_2$ = overall rate constant for elimination of ephedrine

[7] The program NONLIN was supplied by Dr. C. M. Metzler, The Upjohn Co., Kalamazoo, Mich.

Table II—Summary of Average Cumulative Milligrams of Ephedrine Sulfate Excreted, Peak Excretion Rates, Times of Occurrence of Peak Excretion Rates, Half-Lives Obtained from Terminal Data and from Exponential Fitting, and Results of Tests of Significance by Analysis of Variance for Crossover Design

Parameter	Treatment Averages A	B	C	Statistics[a]
Milligrams of ephedrine sulfate excreted at				
0–0.5 hr.	0.890	0.074	0.116	a
0.5–1 hr.	2.06	1.00	2.03	b
1–1.5 hr.	1.23	1.17	2.00	b
1.5–2 hr.	1.20	1.01	1.32	b
2–2.5 hr.	0.972	0.674	1.10	a
2.5–3 hr.	0.915	0.619	1.13	c
3–6 hr.	2.99	3.99	3.86	b
6–9 hr.	2.06	2.21	3.03	a
9–12 hr.	2.11	2.61	2.01	a
12–24 hr.	2.49	3.49	3.00	b
24–48 hr.	0.717	0.754	0.550	a
Total (0–48 hr.)	17.6	17.6	20.1	b
Percent of assay dose excreted in 48 hr.	70.1	72.2	80.2	c
Peak excretion rate, mcg./min.	68.7	45.2	71.2	b
Time of occurrence of peak excretion rate	0.75	1.25	0.92	a
Half-life, hr. (terminal rate)	6.12	5.51	5.33	a
Half-life, hr. (exponential fit)	5.99	6.71	5.28	a

[a] a = not significant ($p > 0.25$), b = not significant ($0.25 > p > 0.10$), and c = not significant ($0.10 > p > 0.05$), according to analysis of variance for crossover design.

f = fraction of ephedrine reaching Compartment 1 that is excreted in the urine

In the initial computer fitting, six parameters (k, K_1, K_{-1}, K_2, A_u^∞, and t_0) were estimated. Results of these initial fits indicated very small lag times, t_0, for data obtained after Treatments A and C, and estimates of A_u^∞ were the same or only slightly larger (range 0–0.5 mg.) than the corresponding observed cumulative amounts excreted in 48 hr. For data observed after Treatment B, the estimated t_0 values were large enough that they should not be ignored and, again, the estimates of A_u^∞ were nearly the same as the corresponding observed cumulative amounts excreted in 48 hr. (range from −0.7 to 0.4 mg.). The goodness of fit of observed data to the theoretical cumulative excretion curve derived from the model is demonstrated by the closeness of r^2 and Corr. to 1.00 and the lack of areas of poor fit. In these initial fittings, although values of r^2 and Corr. ranged from 0.997 to 1.00, the standard deviations of the estimated parameters were quite large since so many parameters were being estimated.

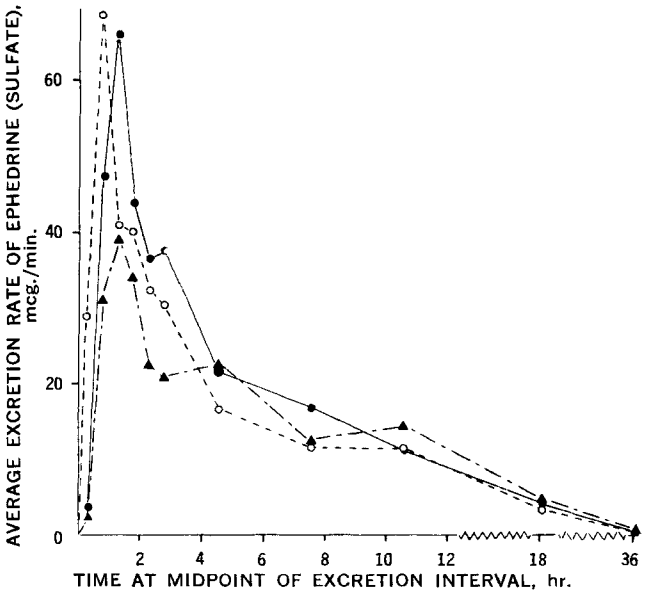


Figure 3—*Average excretion rate profiles for Treatments A, B, and C. Key:* O- - -O, *Treatment A;* ▲— - —▲, *treatment B; and* ●——●, *Treatment C.*

In the final computer fitting, four parameters (k, K_1, K_{-1}, and K_2) were estimated from data obtained after Treatments A and C. For these fittings, the lag time, t_0, was made equal to zero, and the A_u^∞ value obtained from the initial fitting was used as a constant in each case. For data obtained after Treatment B, five parameters (k, K_1, K_{-1}, K_2, and t_0) were estimated; again, the A_u^∞ value obtained from the initial fitting was used as a constant in each case. The parameters estimated and their standard deviations are given in Table III. The reduction in the number of parameters estimated significantly reduced the magnitude of the standard deviations. However, the standard deviations derived from urinary data with this model are still larger than those usually seen when plasma concentration data are fit to the same model. Table III also lists the values of A_u^∞ employed, the measures of fit, r^2, and Corr., the calculated values of α and β, and the $T_{1/2}$ calculated from β (*i.e.*, $t_{1/2} = 0.693/\beta$). The parameters and standard deviations shown in Table III were derived from individual subject data and would not be improved by utilizing a larger panel of subjects. Figure 4

Table III—Estimated Parameters (Their Standard Deviations) and Some Secondary Parameters Resulting from Nonlinear Least-Squares Fitting of Cumulative Amounts of Ephedrine Sulfate Excreted as a Function of Time (Two-Compartment Open Model with First-Order Absorption and Lag Time)

Parameters	Treatment A 1	2	3	Treatment B 1	2	3	Treatment C 1	2	3
k (hr.$^{-1}$)	2.75 (4.10)[a]	1.21 (6.66)	4.11 (1.70)	0.643 (1.09)	1.81 (7.31)	2.18 (17.5)	1.31 (1.57)	0.926 (1.15)	4.13 (9.28)
K_1 (hr.$^{-1}$)	0.370 (0.410)	0.796 (0.268)	0.594 (0.149)	1.41 (1.75)	0.178 (0.834)	0.238 (1.84)	0.285 (0.434)	1.51 (1.00)	0.609 (0.916)
K_{-1} (hr.$^{-1}$)	0.514 (0.134)	0.406 (0.101)	0.421 (0.021)	0.308 (0.357)	0.576 (0.529)	0.473 (0.703)	0.794 (0.918)	0.398 (0.332)	0.648 (0.181)
K_2 (hr.$^{-1}$)	0.175 (0.106)	0.503 (0.226)	0.414 (0.064)	0.714 (1.52)	0.142 (0.189)	0.190 (0.482)	0.193 (0.157)	0.943 (1.32)	0.263 (0.215)
t_0 (hr.)	0.0[b]	0.0[b]	0.0[b]	0.119 (0.125)	0.480 (0.858)	0.452 (1.53)	0.0[b]	0.0[b]	0.0[b]
A_u^∞ (milligrams)	14.7[b]	18.3[b]	20.3[b]	16.0[b]	18.1[b]	18.7[b]	19.5[b]	21.3[b]	20.3[b]
r^2[c]	0.999	1.00	1.00	0.998	0.998	0.996	0.999	0.999	0.997
Corr.[d]	0.998	1.00	1.00	0.997	0.998	0.999	0.999	0.998	0.997
Secondary parameters									
α (hr.$^{-1}$)	0.996	1.57	1.29	2.34	0.792	0.786	1.14	2.72	1.40
β (hr.$^{-1}$)	0.0929	0.130	0.135	0.0942	0.103	0.114	0.135	0.138	0.122
$T_{1/2}$ (hr.)	7.46	5.35	5.15	7.35	6.71	6.08	5.14	5.01	5.69

[a] Standard deviation. [b] Held constant during fitting. [c] $r^2 = (\Sigma obs.^2 - \Sigma dev.^2)/\Sigma obs.^2$. [d] Corr. is the correlation coefficient for the regression of predicted and observed cumulative amounts of ephedrine excreted.

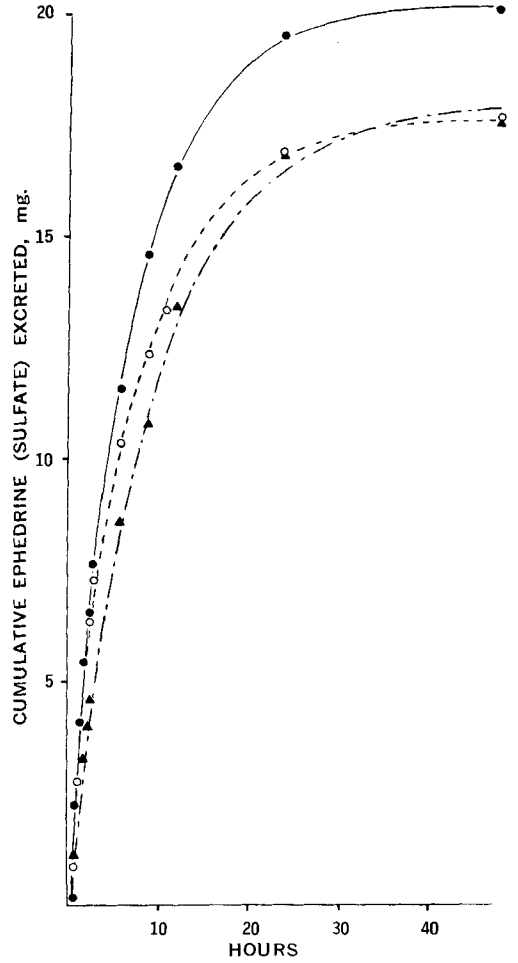


Figure 4—*Average cumulative amounts of ephedrine (as sulfate) excreted in the urine as a function of time. The lines drawn through the points are the theoretical curves based on nonlinear least-squares fitting to Eqs. 2 and 3. Key: O- - - -O, Treatment A; ▲— - —▲, Treatment B; and ●——●, Treatment C.*

shows the fits to the average data points for each treatment; these fits are similar to those obtained with the individual subject's data.

The overall average elimination half-life of ephedrine was 5.65 hr. (range 4.94–7.17 hr.), obtained by plotting terminal values of the logarithm of the excretion rate *versus* time, and 5.99 hr. (range 5.01–7.46 hr.), obtained by exponential fitting to the two-compartment open model. These half-lives appear more representative of those applicable to the clinical use of the drug than the lower values reported by Wilkinson and Beckett (1) obtained under conditions of controlled acidic urinary pH.

Correlation coefficients between deviations of actual from theoretical urinary excretion rates of ephedrine and urinary pH are given in Table IV. Three sets of correlation coefficients based upon two different mathematical models were calculated. The coefficients in Columns 1 and 2 of Table IV are based upon theoretical excretion rates calculated with Eq. 2; coefficients were calculated for the β-phase only and also using the entire data. The correlation coefficients in Column 3 of Table IV are based on deviations of observed and theoretical rates where the latter were obtained with Eq. 4 using terminal excretion rate data only:

$$\ln Y = \ln Y_0 - k_{el} \cdot t \qquad \text{(Eq. 4)}$$

In Eq. 4, Y is the theoretical excretion rate at time t, Y_0 is the rate obtained by extrapolating the least-squares excretion rate line back to time zero, and k_{el} is the slope of the straight line.

By employing Eq. 2, four negative and five positive correlations, all nonsignificant, were obtained over the whole excretion time. From the β-phase data, four positive and five negative correlations, all nonsignificant, were obtained. From Eq. 4, five positive and two negative nonsignificant correlations and two significant negative correlations were obtained. Significant correlations also could not be obtained between excretion rates of ephedrine sulfate and urinary flow rates, nor between urinary excretion rates of ephedrine *per se* and urinary pH.

Table IV—Correlation Coefficients between Deviations[a] of Observed and Theoretical Urinary Excretion Rates of Ephedrine and Urinary pH

		Correlation Coefficients		
		Equation 2		
Subject	Treatment	β-Phase	Whole Period	Equation 4
1	A	+0.292	−0.023	+0.302
1	B	+0.830	+0.452	−0.310
1	C	+0.543	−0.095	+0.325
2	A	+0.003	+0.562	+0.383
2	B	−0.563	−0.324	−0.883
2	C	−0.589	+0.303	+0.188
3	A	−0.163	+0.018	−0.927
3	B	−0.283	−0.223	+0.124
3	C	−0.082	+0.296	−0.244

[a] Deviations above theoretical lines were considered positive, and those below were considered negative.

DISCUSSION

In such a small preliminary study of the type performed, there are really insufficient data to make conclusions about differences between or among dosage forms. A larger panel of subjects would be required. However, the data collected were analyzed by appropriate analyses of variance for crossover design, and no significant differences were found. Previous pharmacokinetic studies were done with ephedrine solutions and, to the authors' knowledge, the present study is the first to be performed in man using capsule dosage forms.

Wilkinson and Beckett (4) evaluated their data according to the one-compartment open model and obtained excellent fits. The data in the present study were evaluated with the two-compartment open model with first-order absorption, and excellent fits were also obtained. Such equivocal results are not surprising in light of the report of Wagner and Metzler (8). In fitting to the two-compartment open model with first-order absorption, the standard deviations of the estimated parameters (Table III) are quite large, as might be expected from urinary excretion data.

The ratio β/K_2 gives the fraction of the total amount of drug in the body that is in the inner compartment during the β-phase; this value averaged 0.431 with a standard deviation of 0.226.

There is no doubt at all that alteration in urinary pH affects the urinary excretion of ephedrine. In the studies of Wilkinson and Beckett (1), under conditions of controlled acidic urine (pH about 5), a mean overall elimination $t_{1/2}$ value of 3.03 hr. was reported. In these studies with uncontrolled urinary pH, the mean urinary pH value was 6.30 with a standard deviation of 0.49; 95 out of the 99 urine samples fell within the pH range 5.32–7.28. The average overall elimination $t_{1/2}$ value was 5.66 hr. when estimated by a procedure similar to the one used by Wilkinson and Beckett (1). However, the lack of significant correlations between ephedrine excretion rates and urinary pH and the deviations of actual from theoretical urinary excretion rates and urinary pH obtained in the authors' studies are of interest. The pKa of ephedrine is 9.58 (9). In the urinary pH range encountered in these studies, the percent of the ionized drug in the urine varied from 99.99 to 99.51%. In such a narrow range of urinary pH and percent ionized drug, it is not too surprising that no significant correlations were obtained, particularly in light of analytical error, possible differences in recorded pH of urine and actual tubular pH, and the method of correlation, which employed small differences in predicted and observed rates (both of which are subject to error) with the observed pH (which was relatively constant).

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* Department of Pharmacology (Program in Investigative Clinical Pharmacology, Upjohn Center for Clinical Pharmacology), Medical School, University of Michigan, Ann Arbor, MI 48104

† To whom reprint requests should be sent: Room W3535, Pharmacy Laboratory, University Hospital, Ann Arbor, MI 48104

Total Synthesis of (±)-Bisnorargemonine

CHUNG-HSIUNG CHEN*, T. O. SOINE†, and KUO-HSIUNG LEE‡

Abstract □ (±)-Bisnorargemonine (I) was synthesized by two alternate but similar routes, differing only in the relative positions of the methoxy and benzyloxy groups on the aromatic rings of the 1-benzylisoquinoline-derived moieties. Both the standard Bischler–Napieralski procedure and the Pictet–Gams modification were employed in the construction of the needed isoquinoline intermediates. Conversion of the latter to the methiodide followed by partial reduction to the *N*-methyl-1,2-dihydro form and, finally, acid-catalyzed cyclization provided (±)-I, which was identical with natural (−)-I except for melting point and optical rotatory power.

Keyphrases □ (±)-Bisnorargemonine—synthesis, structure identification □ Alkaloids, from benzylisoquinolines—synthesis, structure identification, (±)-bisnorargemonine □ UV spectrophotometry—structure, identification, (±)-bisnorargemonine □ NMR spectroscopy—structure, identification, (±)-bisnorargemonine

Bisnorargemonine, an alkaloid, was first isolated by Kier and Soine (1) from *Argemone munita* subsp. *rotundata* (Rydb) G. B. Ownb. It was shown to have a pavine-type ring system by diazomethylation to argemonine (II) (2) which had earlier been established (3, 4) as the (−)-rotatory form of known (±)-*N*-methylpavine (5). Furthermore, based on its unique NMR spectrum, Structure I had been postulated for natural bisnorargemonine (6).

The purposes of this study were to substantiate the suggested structure by an unequivocal synthesis and to provide a practical synthetic method for preparing the alkaloid for other related studies.

The successful synthesis of (±)-norargemonine (III) by Lee and Soine (7) provided a model procedure which could, presumably, be followed in the synthesis of (±)-I reported here. Scheme I[1] shows that the final step in the synthesis of III is by way of the acid-catalyzed ring closure of IV*c* through a C_4-protonated immonium intermediate (5) to provide the pavine-type skeleton. It is apparent that, following the same scheme, two alternate routes lead to the same racemic target compound, *i.e.*, IV*a* → I*a* and IV*b* → I*b*, I*a* and I*b* being identical. The first route, IV*a* → (±)-I, has a methoxyl group directing cyclization and was the subject of a previous communication (8). The alternate route, IV*b* → (±)-I, with a benzyloxy group directing cyclization, also was achieved but, considering the strong acid medium used in cyclization, it is very likely that ether cleavage occurred prior to cyclization and that the ensuing phenolic hydroxyl group was the actual directing group. Since the original communication (8) was of necessity brief, both synthetic schemes are described in detail here.

[1] Ph = phenyl in this and subsequent schemes.

DISCUSSION

The construction of the isoquinolines, XI*a* and XI*b*, was the key in the synthetic scheme to the target compound, (±)-I (Scheme II). One common way of achieving this objective has been catalytic dehydrogenation of the corresponding 1,2,3,4-tetrahydroisoquinolines as employed in the synthesis of III (7). Unfortunately, dehydrogenation was always accompanied by cleavage of the benzyloxy groups, and subsequent benzylation was necessary to resynthesize the benzyl ether. Exploration of the Pictet–Gams modification of the Bischler–Napieralski procedure (9) for the synthesis of XI*a* and XI*b* provided a more convenient route since the dehydrogenation step was eliminated.

As outlined in Scheme II, the β-methoxy-β-phenylethylamines (VII*a* and VII*b*) were prepared from the corresponding β-nitrostyrenes (V*a* and V*b*) by addition of sodium methoxide and subsequent reduction of the adducts (VI*a* and VI*b*) with lithium aluminum hydride, as described by Rosenmund *et al.* (10). The acids (VIII*a* and VIII*b*) were synthesized from *O*-benzylvanillin and *O*-benzylisovanillin according to the procedures of Douglas and Gulland (11) and Robinson and Sugasawa (12), respectively. Prior to condensation with the amines, VIII*a* and VIII*b* were converted to their acid chloride forms, IX*a* and IX*b*.

The amides, X*a* and X*b*, were readily obtained by interaction of VII*a* with IX*a* and of VII*b* with IX*b*, respectively. Compounds X*a* and X*b* were subsequently cyclized in one step with phosphorus oxychloride to XI*a* and XI*b*, respectively. The NMR spectral