cation of this framework will help remove some of the mystery surrounding the role of cholesterol in biological membranes.

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Bioavailability Determination of Two Crystal Forms of Sulfameter in Humans from Urinary Excretion Data

NAWAL KHALAFALLAH, SAID A. KHALIL[x], and MAMDOUH A. MOUSTAFA

Abstract □ Urinary excretion data were used to determine the bioavailability of crystal Forms II and III of sulfameter in humans. Agreement was observed between the ratio of absorption parameters of the two forms determined in the present study and those previously obtained from blood level data. Although the urine data revealed a significant difference in the rate of absorption of the two forms, no significant difference was observed in the extent of absorption of both forms as indicated by the 72-hr urinary excretion data. Urinary excretion rates during the absorption phase, without further mathematical treatment, were statistically shown to be adequate means for comparing the bioavailability of sulfameter crystal forms. The use of urinary excretion data of sulfameter as an alternative to the use of blood level data is discussed.

Keyphrases □ Sulfameter—bioavailability of two crystal forms, human urinary excretion data □ Sulfonamides—bioavailability of two crystal forms of sulfameter, human urinary excretion data □ Bioavailability—two crystal forms of sulfameter, human urinary excretion data □ Polymorphism—bioavailability of two crystal forms of sulfameter, human urinary excretion data

The polymorphism of sulfameter (1), as well as the GI absorption of its crystal Forms II and III (2), was recently reported. The *in vitro* dissolution behavior of the two crystal forms was shown to be reflected in their bioavailability as determined from blood level data (2). The use of urinary excretion data, however, was thought of as a simpler and perhaps more accurate (3) alternative to the use of blood level data in bioavailability determination. Reduction of costs and the elimination of venipunctures are also obvious advantages. Urinary excretion data have been successfully used to evaluate the bioavailability of various drugs including aspirin (4), riboflavin (5), chloramphenicol (6), tetracycline products (7), and sulfamethizole (8).

Several mathematical treatments have been developed for bioavailability determination using urinary excretion data. The direct proportionality between excretion rate and blood level of free unchanged drug, measured at the mean time of the urine collection period (4, 9), is a common prerequisite to such treatments. Nelson (10) developed an equation for determining the amount of drug absorbed at a certain time. Later, Wagner and Nelson (9), using a one-compartment open model, simplified the Nelson equation for calculating the percentage of drug absorbed from urinary excretion data. A two-compartment open model for data treatment was proposed by Loo and Riegelman (11), who introduced terms describing the tissue distribution phase into the Wagner–Nelson equation. Recently, Perrier and Gibaldi (12) pointed out the possibility of overestimating the absorption rate constant, using either of the previous treatments, in cases of drugs with incomplete availability.

The mechanism of drug excretion may inflict certain complications on the linear relationship between excretion rate and blood level of free unchanged drug and, consequently, upset the fundamental assumption on which the application of urine data is based. A previous report (13) showed that the above-mentioned relationship could be affected by active tubular secretion, passive reabsorption, and protein binding. It was also shown (13) that when the extent of drug protein binding is constant and the passive reabsorption is not affected by urine pH, the urinary

Table I—Cumulative Urinary Excretion Data of Sulfameter Forms II and III

Subject	Crystal Form	Cumulative Amount of Sulfameter Excreted, mg										
		2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr
M.A.	II	9.2	24.2	44.1	65.5	87.8	110.8	230	375	457	528	590
	III	4.8	14.8	30	49	70	91	200	321	402	475	537
S.K.[a]	II	14.4	40	85	142	196	242	426	583	668	736	790
	III	8	22	46	81	123	163	357	520	618	695	757
S.K.	II	16	38	71	118	162	208	413	479	572	649	712
	III	8	26	49	81	120	160	381	520	617	694	756
O.A.	II	16	35	60	89	117	145	299	422	521	606	674
	III	10	23	39	60	85	112	262	380	474	557	627
E.S.	II	2.5	11	27	47	70	98	235	353	447	—[b]	—[b]
	III	3.5	11	25	42	61	84	209	318	406	485	551
N.K.	II	4	15	36	68	100	128	253	344	422	492	551
	III	3.5	12	28	48	73	99	238	344	434	516	577
M.M.	II	11	24	42	62	84	108	212	324	425	511	583
	III	11	24	42	64	90	117	287	411	513	594	665
A.R.	II	12	30	54	90	132	169	353	448	518	585	642
	III	12	25	42	63	87	114	257	359	448	525	591
H.B.[c]	II	—	—	—	—	—	—	—	—	—	—	—
	III	9	22	41	72	108	146	314	427	523	602	667

[a] Subject S.K. in another trial, 2 months earlier. [b] Uncollected urine samples. [c] Subject H.B. did not take Form II.

excretion rate should directly reflect the blood level. Sulfameter, similar to other sulfonamides, was reported (14) to be excreted by glomerular filtration and passive tubular reabsorption. The extent of protein binding of sulfonamides was reported (14, 15) to be constant in the dosage range adopted (1 g). Furthermore, the pKa value of sulfameter (6.8) (16) is high enough to minimize the effect of urine pH on passive reabsorption (17). Therefore, urinary excretion data of sulfameter were thought of as a possible substitute to blood level data in availability determination.

The present study is concerned with the determination of the absorption parameters of sulfameter Forms II and III from urinary excretion data. Comparison of such data with previously determined (through blood level data) absorption parameters (2) and *in vitro* dissolution data (1) is also considered.

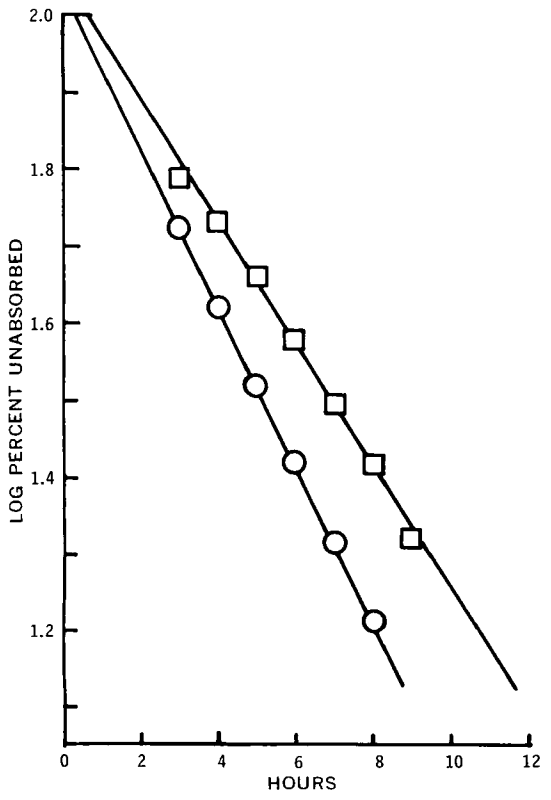

Figure 1—*Log percent sulfameter unabsorbed* versus *time. Mean of Subjects M.A., O.A., E.S., M.M., and A.R. Key:* ○, *Form II; and* □, *Form III.*

EXPERIMENTAL

Materials and Apparatus—Sulfameter[1] crystal Forms II and III were prepared (1), screened to a particle size of 80–90 μm, and identified by IR spectrophotometry[2] immediately before use.

Absorption Study—One gram of either crystal form was suspended in a mixture of 25 ml of 20% mucilage of acacia and 25 ml of simple syrup, a mixture previously shown to inhibit polymorphic transformation (18). This mixture was immediately administered, after an overnight fast, to eight healthy volunteers (six males and two females; age, 25–40 years with an average of 32 years; weight, 50–90 kg with an average of 68 kg). This administration was followed by 50 ml of water used to rinse the containing vessel. No food was permitted for 4 hr after drug administration. Urine samples were collected at 0, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 hr after administration. Each urine sample was refrigerated for not more than 24 hr, appropriately diluted with 0.1 *N* HCl, and acid hydrolyzed, and the total (drug and metabolites) sulfameter content was determined colorimetrically[3] according to Bratton and Marshall (19).

A crossover study was performed, with the same volunteers, 1 month later.

RESULTS AND DISCUSSION

Urinary excretion data of sulfameter crystal Forms II and III are shown in Table I. Application of the Wagner–Nelson (9) treatment to the amounts excreted during absorption resulted in the absorption parameters[4] given in Table II and Fig. 1. Both the availability rate constant, K_A (for subjects showing first-order ab-

[1] Supplied through the courtesy of Alexandria Co. for Pharmaceutical and Chemical Industries, Egypt.
[2] Perkin-Elmer model 237-B spectrophotometer.
[3] Unicam SP 500 spectrophotometer.
[4] The elimination rate constant, K_E, required for the application of the Wagner–Nelson treatment was calculated for each subject from the slope of the semilog urinary excretion rate–time plot from 24 to 70 hr; K_E was found to vary among subjects from 0.014 to 0.019 hr^{-1}.

Table II—Absorption Parameters of Sulfameter Forms II and III

Subject	Crystal Form	K_A[a], hr^{-1}	$t_{50\%}$, hr	Lag Time, hr	$\frac{K_{AII}}{K_{AIII}}$
M.A.	II	0.262	2.63	—	1.34
	III	0.195	3.58	—	
S.K.[b]	II	152[c]	3.35	—	1.35
	III	113	4.49	—	
S.K.	II	140[c]	3.53	—	1.46
	III	96	5.15	—	
O.A.	II	0.295	2.38	—	1.77
	III	0.167	4.12	—	
E.S.	II	0.201	4.50	1.10	1.35
	III	0.149	5.65	1.10	
N.K.	II	165[c]	4.02	1.00	1.51
	III	109	4.62	—	
M.M.	II	0.193	4.00	0.35	1.00
	III	0.193	4.00	0.35	
A.R.	II	0.237	3.00	—	1.40
	III	0.169	4.30	—	
H.B.	II	—	—	—	—
	III	126[c]	4.75	0.80	
Mean	II	0.237[d]	3.44	—	1.40[f]
	III	0.175	4.49[e]	—	
Paired t test, significance level greater than		97.5%	99.5%		
Blood level data[g]	II	0.595	—	—	1.42
	III	0.422	—	—	

[a] Availability rate constant. [b] Subject S.K. in another trial, 2 months earlier. The values given are not included in the ratio and mean calculations. [c] Zero-order availability rate constant, milligrams per hour. [d] Mean of Subjects M.A., O.A., E.S., M.M., and A.R. [e] Excluding Subject H.B. [f] Mean of ratios for different volunteers. [g] See Ref. 2.

sorption), and the $t_{50\%}$ absorption (time required for absorption of 50% of the drug) of sulfameter Form II were significantly different from those of Form III (Table II). The ratio of the absorption rate constant (K_A) for Forms II and III is in agreement with that previously obtained from blood level data (2). The ratio of the *in vitro* apparent solubilities of Forms II and III (1) (1.6) is also in fair agreement with the absorption rate constants. Although the rate of absorption differed between the two forms, the extent of absorption, as indicated by the 72-hr urinary excretion data (Table I), did not differ significantly. Since the biological half-life of sulfameter ($t_{1/2}$) is more than 48 hr (20), determination of the total amount excreted would involve urine collection for at least 2 weeks (9). The 72-hr urinary excretion data were considered a good indication of the amount eventually excreted, since K_E (which may be used to calculate total amount excreted[4]) did not differ, for each subject, between the two forms.

The absorption of sulfameter from suspension was observed to follow zero-order kinetics with some volunteers; with others, a

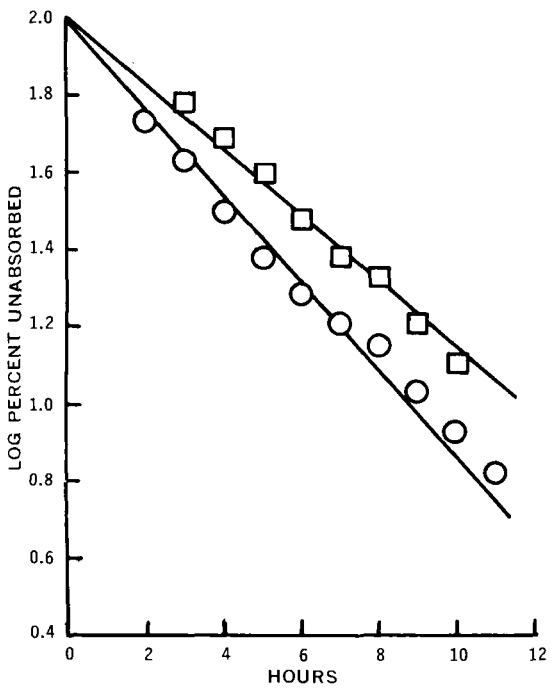

Figure 2—*Log percent sulfameter unabsorbed* versus *time for Subject M.A. showing first-order absorption. Key:* ○, *Form II; and* □, *Form III.*

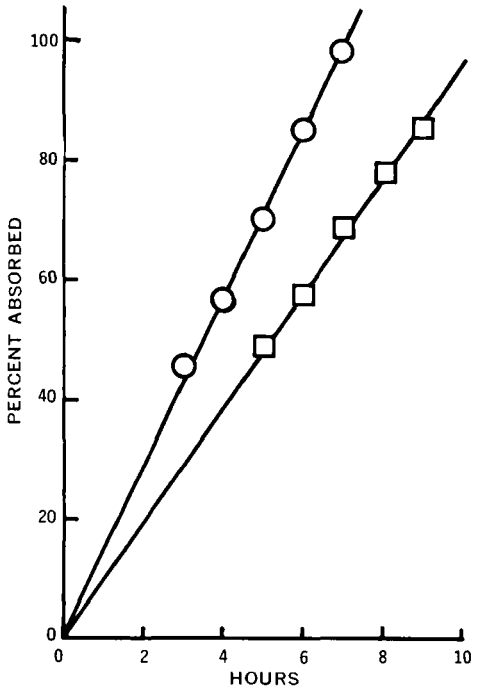

Figure 3—*Percent sulfameter absorbed* versus *time for Subject S.K. showing zero-order absorption. Key:* ○, *Form II; and* □, *Form III.*

Table III—Mean Urinary Excretion Rates of Sulfameter Forms II and III

	Hours								
	2	3	4	6	8	12	24	36	60
					II				
Mean urinary excretion rate[a], mg/hr (standard deviation)	6.33 (2.48)	7.96 (2.50)	9.40 (2.53)	12.94 (3.85)	14.95 (4.77)	11.88 (3.09)	10.58 (1.13)	8.14 (1.23)	5.82 (0.54)
					III				
	4.85 (1.63)	6.07 (1.48)	7.22 (1.58)	9.70 (1.93)	11.80 (2.81)	10.92 (2.53)	11.16 (1.93)	8.70 (0.64)	6.00 (0.27)
Paired t test, significance level greater than	97.5%	97.5%	99.0%	97.5%	95.0%				
Ratio of mean excretion rates of Forms II and III	1.31	1.31	1.30	1.34	1.27				

[a] Excluding data for Subjects H.B. and S.K. (2-month earlier trial).

better fit to first-order kinetics was observed (Table II and Figs. 2 and 3). The zero-order absorption pattern is not uncommon with oral dosage forms (tablets and suspensions), which are limited in their availability by a dissolution step, and has been attributed in some cases to such factors as low drug solubility, limited gastric fluid volume, and high viscosity at the site of absorption (15, 21), all of which prevailed to some extent in the present study. The lag time observed with some volunteers in the Fig. 1-type plot (Table II) resulted from fitting a straight line to a slightly curvilinear relationship. This phenomenon has been reported to be due to dissolution, gastric emptying, *etc.* (12, 22).

The results given in Table II show that urinary excretion data are a useful alternative to blood level data in providing information of a comparative nature concerning the absorption of sulfameter crystal Forms II and III. However, absolute absorption rate constants obtained in the present study differed from those obtained from blood level data (2). The discrepancy might be attributed to between-study variability, bearing in mind that two different groups of subjects participated in both studies.

Excretion rates also could be used as an additional absorption parameter (4) without having to resort to any further mathematical treatment. Table III shows that the mean excretion rates of sulfameter Form II during the absorption phase are significantly different from those of Form III. Furthermore, the ratios of the mean excretion rates of Forms II and III during absorption (Table III) are very close to the ratios reported in Table II and Ref. 2.

The present study revealed some intersubject variation in excretion rates. The extent of variation, as indicated by the standard deviation (Table III), was observed to be greater during the absorption phase (up to 8 hr). Excretion rates in the postabsorptive phase were close to each other. This is perhaps an indication that excretion rates of different volunteers are independent of urinary pH, which was not controlled in the present study. Only slight intrasubject variations were observed (Subject S.K., Table I).

In conclusion, it is suggested that different crystal forms (or dosage forms) of sulfameter may be compared as to their bioavailability rates by comparing their excretion rates during the absorption phase. Such a procedure would save considerable time and effort. Urinary excretion data are also suggested as a simpler alternative to blood level data in studying the kinetics of absorption and deriving absorption parameters that enable the comparison of different formulations of sulfameter.

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[x] To whom inquiries should be directed.