Effect of Dissolution Rate of Sulfamethazine from Tablets on Absorption and Excretion of Sulfamethazine

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Abstract □ Three sulfamethazine tablet formulations with *in vitro* drug dissolution rates of fast ($T_{50\%}$ ~ 1.5 min.), medium ($T_{50\%}$ ~ 15–20 min.), and slow ($T_{50\%}$ ~40–50 min.) were prepared to see what correlation, if any, exists between *in vitro* and *in vivo* data. A significant (at the 95% confidence interval) statistical difference existed for areas under the blood level curves and for maximum blood concentration of sulfamethazine when the fast-dissolving formulation was compared to the slow-dissolving formulation. The fast-dissolving formulation gave the largest area and maximum blood concentration of sulfamethazine. The other *in vivo* differences between the three formulations were not significant.

Keyphrases □ Sulfamethazine—absorption □ Absorption rate constants—sulfamethazine □ Dissolution rate—absorption, excretion effect □ Bratton and Marshall reaction—analysis

It is well-known that the dissolution rate of a drug can limit the absorption rate and/or the total absorption of a drug. A few of the recent reviews on this subject are by Morrison and Campbell (1), Wagner (2), and Levy (3). The drug has to dissolve before it can be absorbed, but is it important for a drug to dissolve from its formulation in a few minutes *versus* a couple of hours as measured by an *in vitro* method? It is unlikely that any *in vitro* dissolution method can actually reproduce the *in vivo* dissolution of the drug in the gastrointestinal tract including gastric secretions, stomach emptying times, *etc.* Thus *in vitro* tests represent a more or less empirical approach which must be correlated with quantitative measures of an *in vivo* quantity. The importance of choosing *in vitro* test procedures which will differentiate between drug formulations producing different *in vivo* effects is easily understood. Occasionally for a drug which is well absorbed and has a long biological half-life, an *in vitro* test procedure may differentiate between formulations which do not produce different *in vivo* effects. This will be shown to be the case for the fast- and medium-dissolving formulations of sulfamethazine used in this study. This sensitive *in vitro* procedure gives the formulator a buffer zone in which the product can be controlled to ensure the same *in vivo* parameters. Different drugs will most likely have different ranges of $T_{50\%}$[1] values where they will produce the same *in vivo* effects.

EXPERIMENTAL

A single-dose, three-way crossover study was done on nine adult male subjects in good general health with no history of acute or chronic renal disease or hypersensitivity to any sulfa drug. The subjects weighed between 54.5 and 91 kg. and were randomly assigned subject numbers one through nine and divided into three groups. Each group received each sulfamethazine formulation at 1-week intervals per the usual crossover design. The formulations were administered at 8:00 a.m. to subjects that were fasting since the prior evening meal. Three grams of sulfamethazine (SMZ) was administered as a single dose of six 0.5-g. tablets. Each subject took 240 ml. of water with the tablets, including that used to swallow the tablets. The subjects took nothing by mouth for 2 hr. after treatment; after that time, food and fluids were taken as desired.

Ten-milliliter samples of blood were drawn at specified times after drug treatment. The blood samples were immediately placed in oxalated containers, mixed to prevent coagulation, and kept frozen until assayed. An aliquot of the total 0- to 48-hr. urine collection was preserved under toluene and assayed within 1 week of collection.

The main difference in the three formulations is the *in vitro* dissolution rate as can be seen from Table I. The *in vitro* determinations of drug dissolution were made by the automated dissolution rate apparatus (A.D.R.A.)[2] (4) with modification to ensure adequate mixing (5). The A.D.R.A. results obtained were used to select the SMZ tablet formulations with drug T_{50}'s of approximately 1–5, 15–20, and 40–50 min.

The urine and blood samples were assayed by the Bratton and Marshall method (6) in which naphthylethylenediamine dihydrochloride is used as the coupling agent to produce the color. The

Table I—Tablet Formulations and Dissolution Rates of Sulfamethazine

	Research Number 15,077 (fast)		Research Number 15,078 (medium)		Research Number 15,079 (slow)	
Tablet formula						
Sulfamethazine	500 mg.		500 mg.		500 mg.	
Sucrose	18 mg.		90 mg.		90 mg.	
Starch	48 mg.		20 mg.		—	
Magnesium stearate powder	2 mg.		2 mg.		4 mg.	
Cellulose product[a]	—		20 mg.		20 mg.	
Force	1,000 lb.		1,900 lb.		1,800 lb.	
	Test Results, min.[b]					
	$T_{20\%}$	$T_{50\%}$	$T_{20\%}$	$T_{50\%}$	$T_{20\%}$	$T_{50\%}$
	2.3	4.6	8.1	14.4	23.8	41.8
	2.0	3.0	9.8	17.7	28.3	49.6
	2.0	3.5	8.7	16.8	24.4	45.0
	2.2	3.8	9.4	17.8	24.8	45.2
	2.4	4.5	8.5	16.7	28.0	52.0
Mean	2.2	3.9	8.9	16.7	25.9	46.7
Dissolved,	**Minutes Required for Indicated % Drug to Dissolve**					
10	1.5		5.2		17.0	
20	2.5[c]		8.7		26.4	
30	3.2		11.8		34.0	
40	3.7		14.5		40.7	
50	4.0		16.7[c]		46.6[c]	
60	5.0		18.7		48.5	
70	7.5		20.0		52.0	
80	12.2		21.0		58.0	

[a] Solka Floc BW 40. [b] Conditions of dissolution rate test: A.D.R.A.-continuous cycling with filtration and agitation; flow rate = 1 ml./sec.; 1-mm. cell; $\lambda_{max.}$ = 260 mμ; 1 C.T./1,500 ml. of pH 7.2 THAM buffer, 0.2 *M*, at 37°. [c] Time at which tablet disintegrated.

[1] $T_{50\%}$ is the time required for 50% of the drug to dissolve.

[2] The A.D.R.A. method involves the use of the USP tablet disintegration apparatus.

Table II—Average Sulfamethazine Blood Levels, mg. %

Blood Sampling, hr.	Fast Formulation	Medium Formulation	Slow Formulation
	Free Sulfamethazine Blood Levels		
0	0.04	0.09	0.01
0.5	1.72	2.06	0.72
1	3.70	3.43	2.67
2	5.09	5.31	4.51
4	5.74	5.40	4.78
6	4.63	4.23	3.90
10	3.26	2.51	2.71
15	1.76	1.77	1.87
24	0.80	0.86	0.88
30	0.54	0.58	0.57
36	0.32	0.38	0.41
	Total Sulfamethazine Blood Levels		
0	0	0	0
0.5	1.90	2.37	0.86
1	4.37	4.20	3.07
2	6.41	6.98	5.74
4	8.19	7.96	6.97
6	7.64	7.16	6.20
10	5.95	5.60	5.08
15	4.08	3.89	3.85
24	1.75	1.89	2.01
30	0.96	1.07	1.14
36	0.56	0.66	0.64

term "free" refers simply to the amount of compound which was diazotizable without hydrolysis, *i.e.*, it had a free amino group. Similarly, the term "total" refers to the total amount of compound which was diazotizable after acid hydrolysis and is taken to represent the sum of free plus acetylated SMZ.

RESULTS AND DISCUSSION

The averaged assay results of nine subjects for the free and total levels of SMZ in blood are given in Table II. The data indicate that slightly higher blood levels of SMZ were obtained from the fastest dissolving formulation.

The *in vivo* blood level parameters for each individual and treatment[3] were calculated. The average of these results for the nine subjects for each treatment is tabulated in Table III, along with the coefficient of variation and the level of significant difference. When the average *in vivo* parameters are compared with the treatments, the amounts of SMZ excreted in the urine (and therefore presumably absorbed) are smaller the slower the drug dissolves. The areas under the blood level curves, the maximum blood concentrations, and the 1-hr. blood levels are also smaller the slower the drug dissolves. The 0.5-hr. blood levels do not exactly parallel the measured rates of dissolution in that the blood levels from the fast and medium tablets are reversed, but not at a significant level. This could easily be due to biological variation. Since the solubility of SMZ increases with pH, stomach-emptying times would affect *in vivo* dissolution and absorption and have its greatest effect on early-hour blood levels.

The rate constant of elimination is not expected to be affected by the dissolution rate of active material in the tablet, but when the individual blood level curves are analyzed the average of the nine elimination rate constants is smaller the slower the drug dissolves (Table III). It should be pointed out that on the log plots of the individual blood levels there is a lot of scatter in the experimental points at the later hours. Therefore, points at earlier hours were used to calculate the elimination rate constant and they may still be under the influence of absorption. If absorption is still taking place at the points where the elimination rate constants were estimated, it is expected that the points for the slow-dissolving formulation should be affected the most by absorption. Therefore, the elimination rate constant will have the smallest value for the slow-dissolving formulation. As a result, there is probably a large error in the elimination rate constants.

If the elimination rate constant for each treatment is calculated from the averaged blood level data, the value so obtained is different from that obtained by averaging the nine individual rate constants for each treatment. The values obtained by either procedure parallel the dissolution rate in the same manner and are shown in Table III. The rate constant in parentheses is calculated from the averaged blood level data and the rate constant not in parentheses is the average of the nine rate constants of the individual blood level curves. This difference is due to the fact that the individuals are weighted differently in the two calculations.

The average of absorption rate constants shown in Table III is larger the slower the SMZ dissolves. This probably is a result of error since the calculation of the absorption rate constant by the method of residuals (7) depends on the slope used for the calculation of the elimination rate constant. If the elimination rate constants are estimated from blood levels still under the influence of absorption, they will be ranked according to dissolution rate and the absorption rate constants will have the opposite rank. If dissolution rate is going to affect absorption it is expected that the absorption rate constant would be smaller for the slower-dissolving formulations.

Although most of the *in vivo* parameters parallel the $T_{50\%}$'s of the active material in the different formulations, there is not a significant difference between all the *in vivo* parameters of the different formulations (see last column of Table III). The *in vitro* dissolution method differentiated between the three formulations of sulfamethazine, but the measured *in vivo* parameters did not differentiate between all three formulations. Only three measured *in vivo* parameters between the fast and slow formulations were significantly different on the 95% confidence interval. The *in vivo* parameters

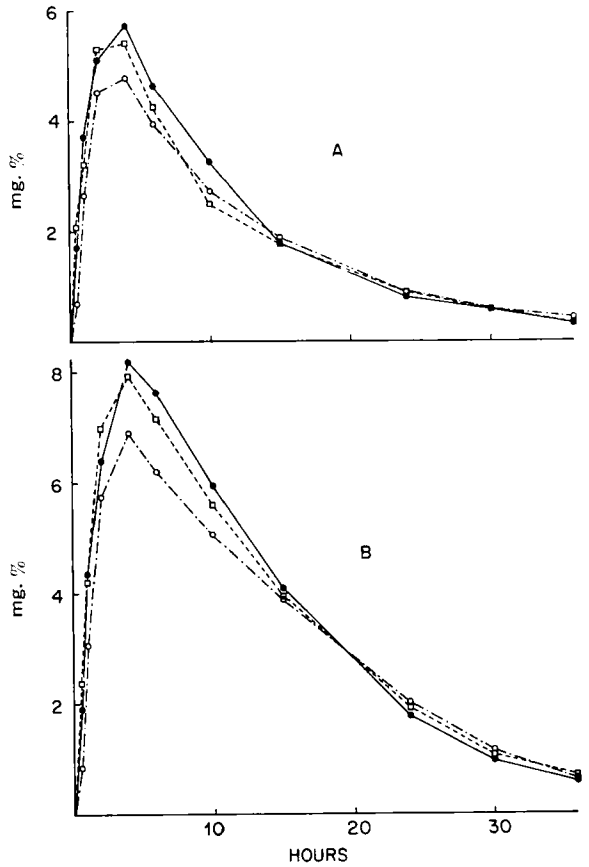

Figure 1—*Average blood levels of free* (A) *and total* (B) *sulfamethazine. Key:* ●, *fast;* □, *medium;* ○, *slow-dissolving formulations.*

were not significantly different when the fast-medium or medium-slow formulations were compared. The test of significance that was applied to the data is Tukey's method (8) for testing all comparisons among means. The analysis of variance of the area under the free SMZ blood-level curves is given in Table IV.[4] The same method of

[3] Treatments refer to the tablet formulations with different $T_{50\%}$ values for the SMZ.

[4] The analysis of variance tables for the other parameters in Table II and/or the individual blood levels of free and total SMZ can be obtained from the authors.

Table III—Average Values for *In Vivo* Parameters of Nine Subjects for a Given Treatment

In Vivo Parameter	Formulation[a]	Coefficient of Variation, %	Average Magnitude of *In Vivo* Parameter	Formulations with Significant Difference on Given p
Total amount of SMZ excreted in 48 hr.	F M S	18	2.675 g. (89%)[b] 2.390 g. (80%)[b] 2.199 g. (73%)[b]	F-S, $p = 0.10$
Area under the free SMZ blood-level curve	F M S	6.2	73.92 mg. % hr. 69.83 mg. % hr. 67.01 mg. % hr.	F-S, $p \leq 0.05$
Area under the total SMZ blood-level curve	F M S	5.8	129.20 mg. % hr. 127.24 mg. % hr. 116.78 mg. % hr.	F-S, $p \leq 0.05$ M-S, $p \leq 0.05$
1-hr. blood levels of free SMZ	F M S	47	3.70 mg. % 3.43 mg. % 2.67 mg. %	
1-hr. blood levels of total SMZ	F M S	46	4.37 mg. % 4.20 mg. % 3.07 mg. %	
0.5-hr. blood levels of free SMZ	F M S	87	1.72 mg. % 2.06 mg. % 0.72 mg. %	M-S, $p = 0.10$
0.5-hr. blood levels of total SMZ	F M S	85	1.90 mg. % 2.37 mg. % 0.86 mg. %	M-S, $p \simeq 0.10$
Maximum concentration of free SMZ in blood	F M S	13	6.25 mg. % 5.87 mg. % 5.07 mg. %	F-S, $p \leq 0.05$ M-S, $p \simeq 0.10$
Maximum concentration of total SMZ in blood	F M S	13	8.52 mg. % 8.03 mg. % 7.06 mg. %	F-S, $0.05 < p < 0.10$
First-order rate constant of absorption of free SMZ	F M S	41	0.663 hr.$^{-1}$ (0.77)[c] 0.811 hr.$^{-1}$ (0.82)[c] 0.869 hr.$^{-1}$ (1.14)[c]	
First-order rate constant of elimination of free SMZ	F M S	19	0.169 hr.$^{-1}$ (0.093)[c] 0.149 hr.$^{-1}$ (0.089)[c] 0.134 hr.$^{-1}$ (0.082)[c]	F-S, $p \leq 0.05$

[a] F has $T_{50\%}$ ~1–5 min.; M has $T_{50\%}$ ~15–20 min.; S has $T_{50\%}$ ~40–60 min. [b] Percent of total dose. [c] The number in parentheses is calculated from the averaged blood-level curve for each treatment. The number not in parentheses is the average of the nine rate constants from individual blood-level curves. The statistics is applied to the averaged rate constant, *i.e.*, the number not in parentheses.

analysis of variance was applied to the other parameters in Table III.

Factors which may have helped to decrease the effect of the different dissolution rates of the drug (in terms of the $T_{50\%}$ value) on the *in vivo* data for SMZ tablets are:

1. SMZ is fairly well absorbed (average of 81%). Rapid dissolution rate is probably more important for a poorly absorbed drug, since with a poorly absorbed drug the absorption sites may be situated in only a small segment of the gastrointestinal tract.

2. SMZ has an average biological half-life of 5 hr. The longer the biological half-life the less the effect of absorption half-life (and in turn the effect of dissolution rate) on the blood level picture.

3. The desired $T_{50\%}$ values for the different formulations were controlled by the disintegration time of the tablets. It can be noted from the dissolution time profile (Table I) that by the time 50% of the drug was released all of the formulations were disintegrated and the release of the drug from then on was more rapid and similar for the three formulations. If the dissolution had continued at the same rate after $T_{50\%}$, as before, a greater effect on the *in vivo* data would be expected.

If a sustained-release formulation of SMZ were made based only on the rate of dissolution of the active material, its rate of dissolution would have to be much less than that for the slow formulation used in this study in order to increase the blood levels at later hours in Fig. 1. Since the area under the blood level curve and the total amount of SMZ excreted for the slow formulation are significantly less than for the fast formulation (Table III), it is indicated that absorption from this type of sustained-release formulation of SMZ would not be as efficient as from a fast-dissolving drug formulation.

Table IV—Analysis of Variance of Areas under the Free SMZ Blood-Level Curves[a]

Source	*df*	*SS*	*MS*
Total	26	69,768.390	
Subjects	8	69,212.650	8,651.581
Periods	2	217.800	108.900
Treatments	2	62.180	31.090
Residual	14	275.760	19.697

[a] Treatment averages: 67.01 (slow); 69.83 (medium); 73.92 (fast). Tukey's allowable difference (*Reference 8*) at 5% level: $D = 5.46$.

CONCLUSIONS

1. Significantly less SMZ was absorbed from the slow formulation, which gave slow *in vitro* release of the drug ($T_{50\%} \approx 40$–60 min.) than from the fast formulation, which gave rapid *in vitro* release of the drug ($T_{50\%} \approx 1$–5 min.).

2. Several *in vivo* parameters paralleled the *in vitro* dissolution rate of SMZ from the fast, medium, and slow formulations, but not all the *in vivo* differences were as great as might be expected from the *in vitro* data. The *in vitro* method of measuring drug dissolution could differentiate between formulations which could not be differentiated by blood levels using nine subjects.

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Percutaneous Absorption Studies of Chloramphenicol Solutions

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Abstract □ An *in vitro* method for measuring the percutaneous absorption of chloramphenicol solutions is described. The apparatus consists essentially of thermostated upper and lower chambers fabricated from methyl methacrylate, with the membrane sandwiched between the chambers. The upper chamber holds the solution containing the drug. The lower chamber allows for a continuous flow of solution which washes away the permeated drug. The two membranes investigated were a filter membrane saturated with peanut oil and a segment of skin obtained from hairless mice. The effect of varying concentrations of surfactants and of propylene glycol on the permeation of chloramphenicol through the barriers is shown. The activation energies for permeation and diffusion of the drug through the filter membrane and for permeation through the skin are evaluated. The partition coefficient is estimated.

Keyphrases □ Chloramphenicol solutions—percutaneous absorption □ Percutaneous absorption—apparatus, *in vitro* determination □ Diagram—*in vitro* percutaneous absorption apparatus □ Sodium lauryl SO_4-effect—chloramphenicol absorption □ UV spectrophotometry—analysis

The vast amount of work carried out in the past on percutaneous absorption has largely dealt with attempts to identify and understand the structure and physicochemical properties of the barrier zone of the skin (1–4), to evaluate factors such as the influence of temperature and hydration on the percutaneous absorption process (5–7), and to study the mechanisms of absorption (8, 9). Other studies (10–12) have been concerned with the relative importance of the transepidermal *versus* the transfollicular routes of diffusion of chemicals through the skin. There have also been studies on the role which topical vehicles play in facilitating or hindering the passage of drugs through the skin (13–15).

There appear to be, however, few studies or techniques which can be used routinely to observe the role which a particular component in a heterogeneous topical vehicle will play on the overall percutaneous absorption of a drug. There are also very few studies dealing with the energies involved in the percutaneous absorption process. The lack of these studies can be partly related to the difficulties of routinely setting up percutaneous absorption studies, and possibly also due to the emphasis given to other factors during the development of a topical vehicle or pharmaceutical product. The emphasis has generally been placed on the compatibility, stability, and appearance of the product, rather than on the influence which the components in the vehicle may have on enhancing or hindering the movement of the drug through the skin.

The present communication is concerned with a relatively easily adaptable dynamic *in vitro* method for measuring percutaneous absorption. Two barriers are evaluated, and the effect of varying the concentrations of surfactants and propylene glycol on the transport of chloramphenicol solutions is demonstrated. The energies involved in the permeation and diffusion processes are measured. A future communication will deal with a correlation of absorption through these barriers with that of excised human skin.

EXPERIMENTAL

Barriers—Whole skin sections stripped from the back and abdomen of male hairless mice[1] were used. The mice with skins clear of lesions and weighing 15–18 g. were sacrificed and the skin removed surgically without injury. The skin was immersed in Ringer's solution and used within 30 min. after removal.

The hairless mice have been used by others in the evaluation of topically applied compounds and have proven to be (16) unusually well adapted for experimentation with topical compounds. It is a healthy animal, and since the skin has no hair, it requires no depilation or shaving process, which often damages the skin. It was also found that the thickness of the skin of animals within a weight range did not vary significantly. There are, however, certain specific

[1] Type HR/HR, Jackson Laboratories, Bar Harbor, Maine.