Bioavailability and Dissolution Behavior of Trisulfapyrimidine Suspensions

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Abstract □ The bioavailability of seven commercial trisulfapyrimidine suspensions was studied in 14 adult male volunteers. Fifteen blood samples were collected over a 48-hr period following administration of a 1-g dose of each suspension. Serum was assayed for each component (sulfadiazine, sulfamerazine, and sulfamethazine) by high-pressure liquid chromatography. Analysis of variance indicated several significant differences among the seven commercial preparations with respect to C_{max}, T_{max}, and *AUC* for sulfadiazine, sulfamerazine, and sulfamethazine. The *in vitro* behavior of each suspension was then studied by the paddle method of the Food and Drug Administration. A 0.5-ml sample was introduced into 900 ml of hydrochloric acid (2.2×10^{-4} *M*) at 37° and dissolved using a paddle speed of 25 rpm. Samples withdrawn at 15 and 30 min were analyzed by high-pressure liquid chromatography, and the percent of sulfadiazine, sulfamerazine, and sulfamethazine was calculated. Significant correlation was obtained between an *in vivo* parameter (C_{max} for sulfadiazine) and an *in vitro* parameter (percent sulfadiazine dissolved in 30 min). Results indicate that this method is suitable for the *in vitro* screening of trisulfapyrimidine suspensions.

Keyphrases □ Bioavailability—trisulfapyrimidine suspensions, compared to *in vitro* dissolution behavior, various commercial preparations, screening methods □ Trisulfapyrimidine suspensions—bioavailability, compared to *in vitro* dissolution behavior, various commercial preparations, screening methods □ Dissolution, *in vitro*—trisulfapyrimidine suspensions, compared to bioavailability, various commercial preparations, screening methods □ Screening methods—trisulfapyrimidine suspensions, bioavailability compared to *in vitro* dissolution behavior, various commercial preparations

In vitro dissolution testing offers a convenient and inexpensive means of predicting bioavailability differences among different tablet and capsule formulations of the same drug. However, *in vitro* studies for suspension dosage forms have not been reported. Because suspensions exposed to aqueous fluids are similar to the disintegrated form of tablets and capsules, these dosage forms also may show dissolution rate-limited absorption. Thus, *in vitro* dissolution rate studies are needed on drugs commonly available on a multisource basis as suspensions. Drugs of this type include various sulfonamide derivatives.

Such studies also require *in vivo* testing of various brands of the same drug to establish comparative bioavailability. The results of *in vitro* testing can then be correlated with *in vivo* results and also can be used as a test of lot-to-lot variability of suspensions produced by the same manufacturer. With this type of information, *in vivo* testing can be eliminated, lot-to-lot specifications can be developed, and rapid screening procedures for new sulfa drug suspensions could become available.

Several reports (1–3) dealt with the dissolution behavior of drugs in suspensions. The bioavailability and dissolution rates of sulfa drugs also were investigated (4–11). Although bioavailability differences among various sulfonamide dosage forms were reported, attempts to correlate *in vitro* dissolution data with *in vivo* bioavailability data were unsuccessful (7–11).

The objective of this research was to establish a suitable dissolution test method for correlating *in vivo* comparative bioavailability data of different commercially available oral trisulfapyrimidine suspensions. This objective required investigation of both the *in vitro* and *in vivo* parameters. By using a specific high-pressure liquid chromatographic (HPLC) method, the behavior of each trisulfapyrimidine component was studied separately.

EXPERIMENTAL

Bioavailability Study—Prior to admission to the study, 14 healthy adult males were given a complete medical examination; a medical history was taken, and laboratory tests were performed, including complete blood and urine tests. Subjects were accepted for the study only if all laboratory test results were within the normal range accepted by the examining physician. The average age of the subjects was 23.4 years (range of 21.2–29.3 years), the mean weight was 77.6 kg (range of 61.2–86.2 kg), and the mean height was 182.5 cm (range of 175.3–193.0 cm). They could not take chronic medications of any kind for a week prior to the study and any drugs during the entire test period.

Subjects were informed of all study details, the potential hazards of participation, and the benefits. They were also informed that they could withdraw from the study at any time for any reason. Toward the latter stages of the study, one subject developed a rash. It probably appeared gradually and was noticed only after administration of the fifth dose of the drug. This subject was not allowed to receive the remaining two scheduled treatments (Weeks 6 and 7), and the missing values were estimated statistically by the method of Li (12).

The seven (A–G) commercial trisulfapyrimidine suspensions[1] were supplied by the Food and Drug Administration (FDA). To eliminate any possible residual effects, each subject received the seven different brands (A–G) in a complete crossover design. A 10-ml dose of each suspension (providing a total of 1.0 g of sulfa drug) was administered in the morning to the subjects after an overnight fast. The suspensions were shaken well, and 10-ml doses were withdrawn into glass syringes. The doses were then administered by emptying the contents of the syringe into the mouth. The syringe was rinsed once with 10 ml of water, which was also emptied into the subject's mouth. Drug dose delivery was accurate to within 0.1% of the stated volume.

Each subject was then instructed to drink an additional 110 ml of water. Subjects were allowed to eat lunch after abstaining from all food for 3 hr after drug administration. Doses were administered every alternate week, the time interval of 2 weeks being considered adequate for virtually complete drug elimination.

Preliminary pharmacokinetic data indicated that 15 blood samples were adequate to obtain a complete blood level profile. Samples were collected at 0, 0.167, 0.332, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 30.0, and 48.0 hr. All blood samples were obtained by venipuncture with

[1] A, Trisem, lot C53747, Beecham-Massengill Pharmaceutics, Bristol, Tenn; B, Terfonyl, lot 60080, E. R. Squibb and Sons, Princeton, N.J.; C, Sulfaloid, lot 17612, Westerfield Laboratories, Cincinnati, Ohio; D, Trisureid, lot 5M249, Reid Provident Laboratories, Atlanta, Ga.; E, Quad-Ramoid, lot 28431, Paul B. Elder Co., Bryan, Ohio; F, Sulfose, lot 1951619, Wyeth Laboratories, Philadelphia, Pa.; and G, Neotrizine, lot 9ND79A, Eli Lilly and Co., Indianapolis, Ind.

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Table I—Mean (±*SD*) Values of *In Vivo* Parameters of Sulfadiazine (I), Sulfamerazine (II), and Sulfamethazine (III) Obtained for Seven Commercial Trisulfapyrimidine Suspensions (A–G) after Administration to 14 Subjects

In Vivo Parameter	Product						
	A	B	C	D	E	F	G
C_{max}, μg/ml							
I	12.1 ± 2.0	11.9 ± 1.7	11.2 ± 1.6	10.4 ± 2.4	9.3 ± 2.1	9.1 ± 2.5	6.0 ± 1.6
II	27.3 ± 3.5	27.4 ± 4.1	25.3 ± 3.7	24.1 ± 4.5	23.0 ± 3.4	24.3 ± 4.4	18.0 ± 4.9
III	19.6 ± 4.3	19.6 ± 5.3	18.5 ± 5.0	15.8 ± 4.1	16.3 ± 5.0	16.3 ± 5.2	18.0 ± 5.7
T_{max}, hr							
I	3.9 ± 1.3	3.3 ± 1.1	4.4 ± 1.6	4.3 ± 1.4	4.9 ± 1.3	5.1 ± 1.5	4.6 ± 1.4
II	2.6 ± 1.4	2.8 ± 1.3	3.9 ± 1.8	4.1 ± 2.0	4.8 ± 1.7	4.7 ± 1.8	5.6 ± 2.6
III	1.1 ± 0.9	1.2 ± 0.7	1.3 ± 0.7	2.1 ± 1.1	2.7 ± 1.0	2.1 ± 0.9	1.5 ± 0.8
$AUC_0^\infty \times K_e$, μg/ml							
I	13.9 ± 2.3	12.6 ± 2.2	12.1 ± 3.0	11.0 ± 2.7	10.8 ± 2.5	11.1 ± 3.4	7.8 ± 2.6
II	29.0 ± 2.8	28.6 ± 4.1	28.7 ± 6.0	27.6 ± 4.4	27.6 ± 3.2	28.5 ± 3.9	25.6 ± 6.6
III	19.4 ± 3.9	20.4 ± 4.5	20.4 ± 4.6	18.5 ± 5.2	20.0 ± 4.7	29.6 ± 4.2	20.6 ± 5.4

Table II—Mean (±*SD*) Percent of Sulfadiazine, Sulfamerazine, and Sulfamethazine Dissolved in 15 and 30 min by the Paddle Method

Product	Sulfadiazine		Sulfamerazine		Sulfamethazine	
	15 min	30 min	15 min	30 min	15 min	30 min
A	64.9 ± 3.4	84.2 ± 3.7	71.4 ± 2.4	91.1 ± 2.3	63.3 ± 2.4	84.5 ± 2.3
B	79.5 ± 1.8	92.5 ± 2.3	84.8 ± 2.6	98.3 ± 2.2	84.2 ± 2.7	97.8 ± 2.7
C	44.3 ± 3.9	67.3 ± 4.4	39.0 ± 3.5	58.9 ± 4.1	44.6 ± 4.0	65.0 ± 4.2
D	84.5 ± 2.4	99.1 ± 1.2	83.8 ± 2.6	98.7 ± 1.4	83.9 ± 2.6	98.9 ± 1.4
E	46.4 ± 4.7	53.5 ± 4.9	28.3 ± 3.0	32.7 ± 3.3	19.5 ± 1.8	25.7 ± 1.5
F	21.6 ± 2.9	41.7 ± 4.4	41.7 ± 2.9	42.5 ± 5.1	23.0 ± 3.5	42.1 ± 6.6
G	8.3 ± 1.7	23.5 ± 3.0	10.2 ± 2.4	26.6 ± 3.5	11.9 ± 2.5	28.9 ± 3.5

6-ml silicone-coated vacuum tubes[2]. Samples were refrigerated and allowed to clot before being centrifuged at 3500 rpm. Serum was separated from the clot with the aid of a Pasteur pipet, transferred to a 4-ml glass vial, and frozen until assayed. Samples were assayed for sulfadiazine, sulfamerazine, and sulfamethazine by the HPLC procedure described here.

Dissolution Methodology—The dissolution of seven trisulfapyrimidine suspensions (A–G) was determined at 37° in 900 ml of a dissolution medium (Medium M) consisting of 899 ml of distilled water to which 1.0 ml of 0.2 *M* HCl was added. The medium pH was checked at the start and at the end of each experiment and was fairly constant at 3.4. A low stirring rate of 25 rpm was chosen since other work had demonstrated very rapid dissolution of some trisulfapyrimidine suspensions with higher stirring rates.

The apparatus consisted of a cylindrical 1000-ml round-bottom flask[3], which was secured in a multiple-spindle dissolution drive apparatus[4] and immersed in a controlled-temperature bath[5] maintained at 37°. Each spindle was provided with an independent clutch so that any spindle could be stopped without disturbing the others. The apparatus was capable of achieving speeds from 25 to 250 rpm ± 5% of the preset speed. The flask was equipped with a Plexiglas cover with a center port for entry of the paddle shaft and three side ports for sample introduction and removal. The paddle[6], consisting of a 7.6-cm polytef[7] blade attached to a polytef-coated, 0.95-cm stainless steel shaft, was positioned exactly 2.5 cm above the flask bottom.

The suspension (0.5 ml) was introduced carefully into the bottom of the flask using a 1-ml syringe with an attached 17.8-cm stainless steel needle[8]. Paddle rotation was engaged and controlled at a constant 25 rpm. Previous dissolution trials indicated that Products A–D dissolved at a much greater rate than did Products E and F, and that the dissolution of Product G was very slow. On the basis of the preliminary data, 15- and 30-min sampling times were selected.

Samples were withdrawn with 5-ml glass syringes through an attached 7.6-cm, 20-gauge stainless steel needle[9] secured to the flask cover and positioned to extend 6.8 cm into the dissolution medium. Thirty seconds prior to sample removal, the needle was cleared of any remaining fluid by withdrawing 3 ml of the medium and replacing it into the flask immediately. A 3-ml sample was then removed, filtered through a 13-mm, 0.2-μm membrane filter[10] using a Swinny adaptor, and analyzed by HPLC for sulfadiazine, sulfamerazine, and sulfamethazine. An equal volume of drug-free Medium M was replaced in the flask immediately after sample withdrawal.

After removal of the 30-min sample, the stirring rate was increased 10-fold to ensure complete dissolution of the suspension; a final 90-min sample was removed for analysis. Several additional samples were withdrawn after the 90-min sample in a few cases and were found to contain amounts of drug identical to the 90-min sample. The amount of drug dissolved at 15 and 30 min was expressed as a percentage of this last sample.

Analytical—All samples were analyzed for sulfadiazine, sulfamerazine, and sulfamethazine by an HPLC method developed in this laboratory (13). Samples of sulfadiazine[11], sulfamerazine[12], sulfamethazine[13], and sulfamethizole[14] were used as received. All other materials were reagent grade.

For the *in vivo* study, a stock solution of trisulfapyrimidines was prepared by dissolving 100 mg each of sulfadiazine, sulfamerazine, and sulfamethazine in 0.1 *M* NaOH and adjusting the volume to 50 ml in a volumetric flask. The internal standard stock solution was prepared by dissolving 50 mg of sulfamethizole in 1 *M* NaOH and adjusting to volume in a 100-ml volumetric flask. Serum standards were prepared by taking appropriate small volumes of the stock triple-sulfa solutions and adding these to control serum[15]. Ten microliters of internal standard solution was added to 0.2 ml of sample (standard or unknown) in a 1-ml vial[16] followed by 0.1 ml of a 14% trichloroacetic acid solution. The samples were mixed and centrifuged[17] at 2500 rpm for 15 min.

Five microliters of the clear supernate was injected onto the column[18]. Peak height ratios were calculated by dividing the peak height of the drug by the peak height of the internal standard. Calibration curves were constructed daily from results obtained from spiked control serum samples containing equal amounts of each trisulfapyrimidine in the 1–30-μg/ml range by plotting peak height ratios *versus* concentration of the individual sulfa drug.

For the *in vitro* study, a stock trisulfapyrimidine solution was prepared by dissolving 100 mg each of sulfadiazine, sulfamerazine, and sulfamethazine in 0.1 *N* NaOH and adjusting the volume to 100 ml in a volu-

[2] Vacutainers, silicone-coated interior, 7-ml capacity, 13 × 100 mm, Becton Dickinson and Co., Rutherford, N.J.
[3] No. 333710-51, Kimble Products, Owens, Ill.
[4] Spec. 72B, Hanson Research Corp., Northridge, Calif.
[5] Blue M Electric Co., Blue Island, Ill.
[6] Paddle shaft (⅜ in.) 65-700-001 with 3-in. Teflon blade 65-700-300, Hansen Research Corp., Northridge, Calif.
[7] Teflon, du Pont.
[8] Transfer set needle, Travenol Laboratories, Morton Grove, Ill.
[9] Pitkin spinal needle 1162, Becton Dickinson and Co., Rutherford, N.J.
[10] Gelman Instrument Co., Ann Arbor, Ill.
[11] Lot R02114, Eli Lilly and Co., Indianapolis, Ind.
[12] Lot M07059, Eli Lilly and Co., Indianapolis, Ind.
[13] Lot R02011, Eli Lilly and Co., Indianapolis, Ind.
[14] Lot R159178, Ayerst Laboratories, New York, N.Y.
[15] Obtained from the Central Blood Bank, Pittsburgh, Pa.
[16] Reacti-Vials 13261, Pierce, Inc., Rockford, Ill.
[17] IEC EXD centrifuge 460G, Damon/IEC Division, Needham Heights, Mass.
[18] Waters Associates prepacked μBondapak C-18 column.

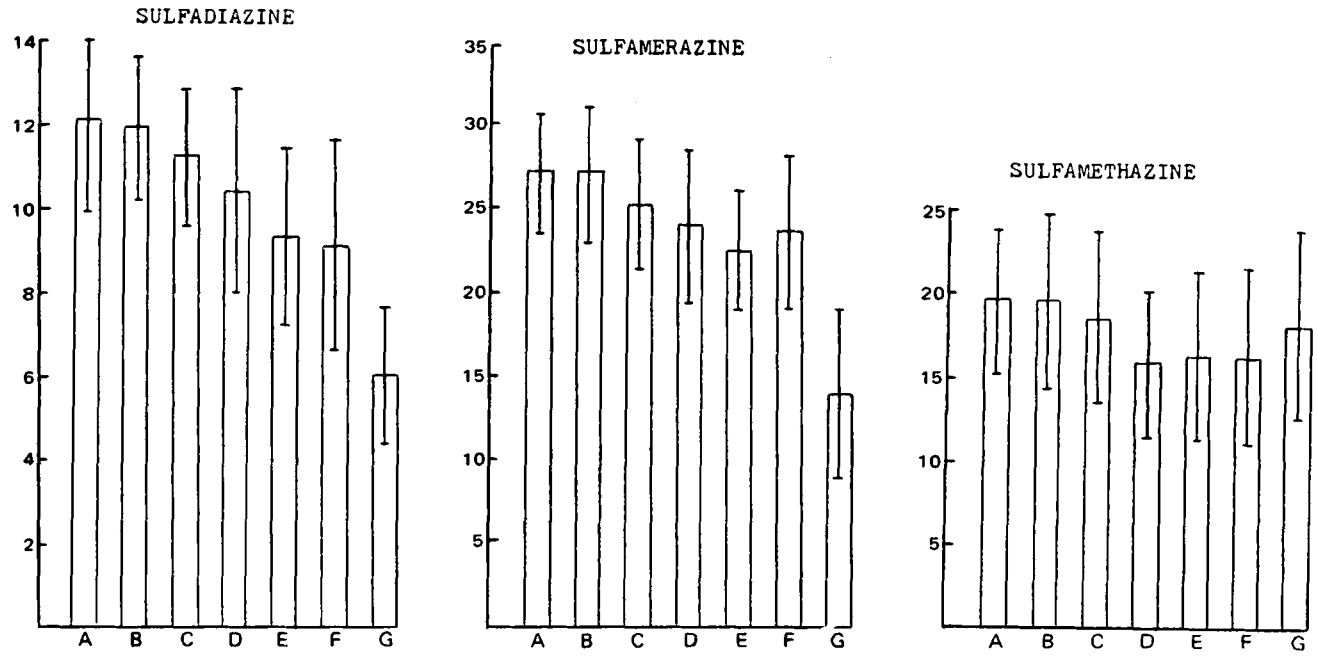

Figure 1—*Mean* (±SD) *maximum serum concentrations (micrograms per milliliter) of sulfadiazine, sulfamerazine, and sulfamethazine observed in 14 subjects after oral administration of seven commercial trisulfapyrimidine suspensions.*

metric flask. Standards (2, 4, 10, 20, and 30 μg/ml) were prepared by diluting appropriate small volumes of the stock solution with 0.1 *M* NaOH. The internal standard solution was prepared by dissolving 100 mg of acetaminophen[19] in hot water and adjusting the volume to 100 ml in a volumetric flask when cold. The solution prepared in this manner corresponded to a concentration of 1 mg/ml. Ten microliters of internal standard solution was added to 0.2 ml of filtered dissolution medium sample in a 5-ml culture tube. The sample was mixed, and 5 μl was injected onto the column[18]. Standards were treated similarly, calibration plots being constructed daily by plotting individual sulfa drug peak height ratios *versus* concentration.

Data Analysis—The peak serum concentration (C_{max}) and the time of the peak concentration (T_{max}) were obtained from the individual serum level *versus* time curves. The areas under the curve from 0 to 48 hr (AUC_0^{48}) were calculated by the trapezoidal rule and then corrected for the area beyond the last data point to obtain the total area (AUC_0^∞). The individual AUC_0^∞ values were then "normalized" (14) by multiplying the calculated AUC_0^∞ by the elimination rate constant (K_E) obtained from the terminal linear portion of the serum level *versus* time curves. These normalized areas were obtained for each of the three sulfa drugs in the suspensions: sulfadiazine (I), sulfamerazine (II), and sulfamethazine (III).

For each parameter, one-way analysis of variance was carried out by the computerized statistical program BMD 08V (15). A repeated-measures design permitted tests in the same subject of treatment interactions that would not be seen if each treatment were given to a different subject. The *F* ratios were compared with tabulated *F* ratios to determine the level of statistical significance (with seven treatments and 14 subjects, df = 6/76, taking into account two missing values, the minimum *F* value is 2.13 for $p < 0.05$ and 2.89 for $p < 0.01$). Any statistical differences found among treatments were compared further to determine differences between treatment pairs by Tukey's HSD tests (16).

Dissolution data were based on the amount of drug added to the dissolution medium. Thus, the concentration of the last sample (after complete dissolution of the suspension) represented 100%. Concentrations at all other sampling periods were expressed as a percentage of this last sample to obtain the percentage of drug dissolved in 15 and 30 min. For each sulfa drug, the means of 12 determinations for each trisulfapyrimidine suspension were used for calculation of the linear correlation coefficients (r) between these *in vitro* measurements and the *in vivo* data obtained from the subjects. Tables of r values were used for determining the statistical significance of the correlations (with df = 5 for the preparations, the minimum r values are 0.75 for $p < 0.05$ and 0.83 for $p < 0.02$).

[19] Lot X1667, Ruger Chemical Co., Irvington, N.J.

RESULTS

For the 14 subjects, mean values of the *in vivo* parameters are summarized in Table I for sulfadiazine (I), sulfamerazine (II), and sulfamethazine (III), respectively, for each of five parameters.

Observed Maximum Serum Concentration (C_{max})—The analysis of variance indicated statistically significant ($p < 0.01$) differences among the seven treatments administered with respect to I–III. The *F* ratios were 21.68 for I, 17.62 for II, and 5.85 for III. Tukey's HSD tests indicated that Product G was significantly ($p < 0.01$) different from all the other products and produced a much lower C_{max} value for I and II but not for III (Fig. 1). With respect to III, significantly low C_{max} values were observed with Product D.

Time for Maximum Serum Concentration (T_{max})—The analysis of variance indicated statistically significant ($p < 0.01$) differences among the seven treatments administered with respect to I–III. The *F* ratios were 3.85 for I, 6.80 for II, and 8.06 for III. Tukey's HSD tests indicated that the T_{max} values were significantly higher with Products E and F for I, with Product G for II, and with Product E for III.

Areas under the Curve—The analysis of variance of normalized areas indicated a significant ($p < 0.01$) difference among the seven treatments only with respect to I (F = 12.53). Tukey's HSD tests applied to I indicated significantly low values for Products G, D, E, and F.

***In Vitro* Study**—The mean (±*SD*) percentage of I–III dissolved in 15 and 30 min is shown in Table II for the seven trisulfapyrimidine suspensions. Analysis of variance of the data indicated large differences among the seven products with respect to I–III. Tukey's HSD tests indicated statistically significant ($p < 0.01$) differences between most pairs of suspensions.

***In Vivo–In Vitro* Correlation**—For the three drugs, each *in vivo* parameter was correlated with the percent of drug dissolved in 15 and 30 min for the seven suspensions. Significant values of the linear correlation coefficient were observed between: (*a*) percent of I dissolved in 30 min and C_{max} ($r = 0.86, p < 0.02$), (*b*) percent of I dissolved in 15 min and C_{max} ($r = 0.80, p < 0.05$), (*c*) percent of II dissolved in 30 min and C_{max} ($r = 0.76, p < 0.05$), (*d*) percent of II dissolved in 30 min and T_{max} ($r = -0.85, p < 0.02$), and (*e*) percent of II dissolved in 15 min and T_{max} ($r = -0.83, p < 0.05$).

DISCUSSION

Significant bioavailability differences among commercial trisulfapyrimidine suspensions were revealed by differences in the rate and extent of absorption of the individual suspension components. A number of differences were observed for C_{max} and T_{max}, but only a few were found for *AUC*. Thus, while the relative trisulfapyrimidine suspension bio-

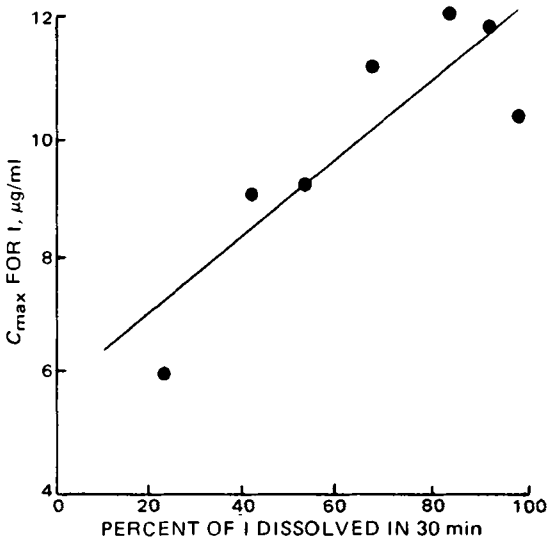

Figure 2—*Relationship between the* in vivo *measure of* C_{max} *and the* in vitro *measure of percentage of sulfadiazine (I) for the seven commercial trisulfapyrimidine suspensions, showing the regression line for the correlation coefficient of* +0.86 ($p < 0.02$).

availability was fairly uniform in terms of the extent of absorption, the absorption rate as shown by C_{max} and T_{max} was more variable.

Of all the statistically significant differences observed for the comparative bioavailability (bioequivalency) of the trisulfapyrimidine suspensions, the largest F ratio was in the C_{max} parameter for I. Product G showed the lowest mean C_{max} value for I. Since significant differences in the AUC values also were observed only for I, the dissolution characteristics of I were the most closely monitored and the variables in the dissolution study were controlled in such a way as to correspond to these *in vivo* study results.

With the FDA paddle method, the dissolution medium (pH 3.4) that was most suitable for the trisulfapyrimidine suspensions contained 2.2×10^{-4} M HCl with no added salts at a 37° temperature and a 25-rpm stirring rate. The dissolution data indicated large significant variations among the products. However, the absence of statistically significant ($p < 0.05$) differences among the 12 determinations on any one product indicated that the dissolution method was very reproducible.

Of all the significant correlations for C_{max} or T_{max} with *in vitro* parameters, the greatest r value was between C_{max} for I and the percent of I dissolved in 30 min (Fig. 2). The greatest bioavailability differences among the products also were observed for the C_{max} parameter for I. The paddle dissolution method thus appeared to be a very strong indicator of the *in vivo* situation and suitable to set standards for the I and II components of trisulfapyrimidine suspensions in terms of the percent of drug dissolved in 30 min.

A suitable guideline for determination of a minimal dissolution standard might be the mean percentage of drug (I or II) dissolved in 30 min for Products A–D. These values were 85% for I and 87% for II. While dissolution standards could be set for I and II, it was not possible to set a dissolution standard for III since a suitable *in vivo–in vitro* correlation was not obtained for this component. However, the establishment of actual standards that require minimal performance of only one or two components of a multicomponent product would be a matter to be decided upon by the appropriate regulatory agency.

Additional work carried out in these laboratories suggests that simple UV measurements for total trisulfapyrimidines could be used by determining dissolution rate profiles of the suspensions. The possibility of correlating *in vitro* data with *in vivo* parameters is being explored.

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