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RESEARCH ARTICLES

Correlation Between the Bioavailability of Microencapsulated Bacampicillin Hydrochloride in Suspension and *In Vitro* Microcapsule Dissolution

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Abstract □ The relative bioavailability of microencapsulated bacampicillin hydrochloride in suspension was correlated with the *in vitro* dissolution half-lives of the microcapsules. Simultaneously, a sensory evaluation was performed to evaluate the taste acceptability of the suspension. The *in vitro* dissolution half-life is directly related to the coating thickness of the microcapsules. The four suspensions of bacampicillin hydrochloride, containing microcapsules with different coating thickness, were given as single 400-mg oral doses to 12 healthy volunteers after overnight fasting using a crossover design with balanced sequences. Bacampicillin is a prodrug of ampicillin, the concentration of which was determined in plasma and urine by bioassay. There were significant inverse linear relationships between the dissolution half-life and plasma peak concentration, area under the curve, and urinary recovery. The terminal exponential disposition phases of the curves were similar for all four suspensions. There was a significant direct linear relationship between the dissolution half-life and overall taste and bitterness. The results show that the mean bioavailability of bacampicillin hydrochloride from a microcapsule suspension can be predicted from an *in vitro* dissolution half-life. The results also suggest that bacampicillin hydrochloride can be given in a suspension with sufficient microcapsule film thickness to reduce the bitter taste of the drug and still retain adequate bioavailability.

Keyphrases □ Bioavailability—bacampicillin hydrochloride, microencapsulated oral suspensions, correlation with *in vitro* dissolution □ Bacampicillin hydrochloride—encapsulated oral suspensions, *in vivo* bioavailability correlations with *in vitro* dissolution, taste evaluation □ Dissolution—*in vitro*, microencapsulated bacampicillin hydrochloride in oral suspensions, correlation with *in vivo* bioavailability, taste evaluation

Bacampicillin hydrochloride, an oral prodrug of ampicillin, is absorbed more rapidly and to a greater extent than ampicillin (1, 2). The drug cannot be formulated as a simple solution due to its bitter taste. However, using an aqueous suspension of small particles of bacampicillin hydrochloride coated with a thin polymeric film of ethylcellulose (microencapsulated) and, for instance, adjusting the pH of the aqueous phase, the release of bacampicillin can be counteracted and the bitter taste masked[1]. Increased thickness of the microcapsule coating decreases the *in vitro* dissolution rate of bacampicillin in water. This might influence both the rate and extent of absorption of the drug. On the other hand, with too thin a coating it is possible that the microcapsules in the aqueous suspension may release bacampicillin in the mouth prematurely, thereby causing a bitter taste.

The purpose of this investigation was to compare the relative bioavailability of microencapsulated bacampicillin hydrochloride in four aqueous suspensions. The only difference between the suspensions was the coating thickness of the microcapsules. These were characterized by different *in vitro* dissolution half-lives of the bacampicillin. A sensory evaluation of the four suspensions was made simultaneously.

EXPERIMENTAL

Subjects—The study was conducted on three female and nine male nonobese volunteers, aged 23 to 37 years (mean 31 years) with body weights ranging from 56 to 86 kg (mean 74 kg). All were determined to be healthy by routine clinical and laboratory examinations within a 14-d period preceding the study and had no known allergy to penicillins or cephalosporins. The subjects had taken no other drugs for at least 1 week prior to the start of the study.

Pharmaceutical Preparations—Microcapsules of bacampicillin hydrochloride were prepared by fluid bed coating with ethylcellulose. Four different coating thicknesses were used for the microcapsules, with total polymer contents of 17, 23, 29, and 37%, respectively.

[1] R. Sjöqvist, J. Sjövall, H. Nyqvist, and D. Westerlund; unpublished data.

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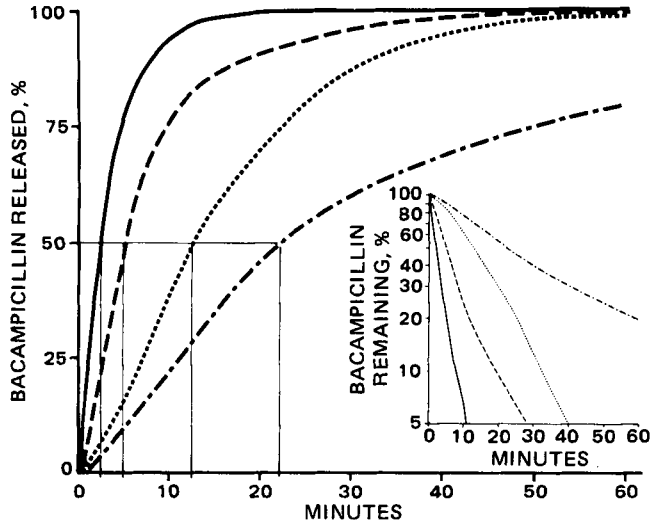

Figure 1—In vitro *dissolution, as a function of time, of four microcapsules of bacampicillin hydrochloride with different coating thicknesses expressed as the total content of polymer in the granules. Vertical lines indicate dissolution half-lives. Key: (—) 17%; (– – –) 23%; (- - -) 29%; (- · - ·) 37%.*

The microcapsules were characterized by different *in vitro* dissolution rates. The *in vitro* release rate of bacampicillin hydrochloride from the microcapsules was determined in the USP XX paddle apparatus with 500 mL of water (37°C) as the dissolution medium. The rotating speed of the shafts was 100 rpm. Six samples of 500 mg of microcapsules were run in each test. The amount of bacampicillin dissolved was monitored continuously in a spectrophotometer at 255 nm.

The microcapsules were administered as a flavored, aqueous suspension[2], of identical composition for the four different microcapsules. The suspension (40 mg/mL) was prepared 0.5 h before administration by adding a standardized volume (4.8 mL) of water to single dose cups containing an exact dose of microcapsules and excipients.

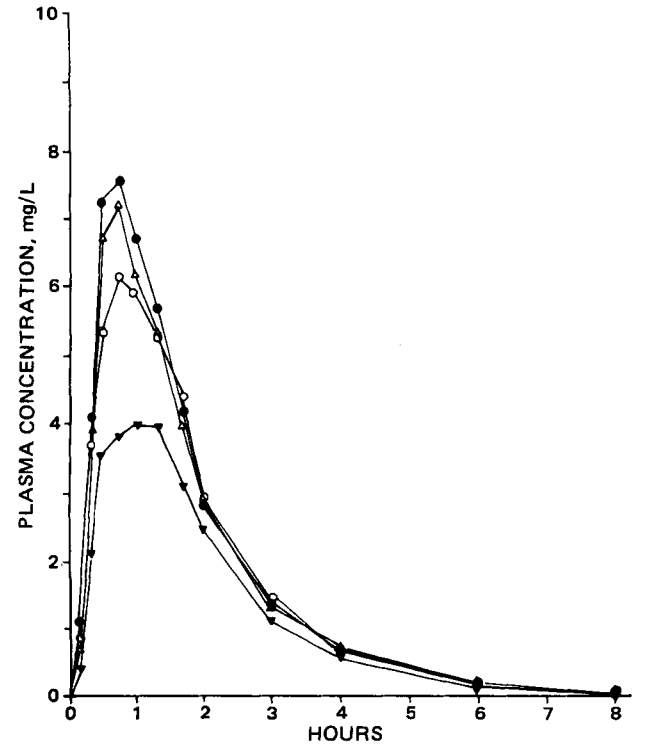

Figure 2—*Mean plasma concentrations after four single 400-mg doses of microencapsulated bacampicillin hydrochloride in suspension with different* in vitro *dissolution half-lives. Crossover study in 12 subjects. Key: (●) 2.5 min; (△) 5 min; (○) 12.5 min; (▼) 22 min.*

[2] PENGLOBE granules for reconstitution; Astra Läkemedel AB, Sweden.

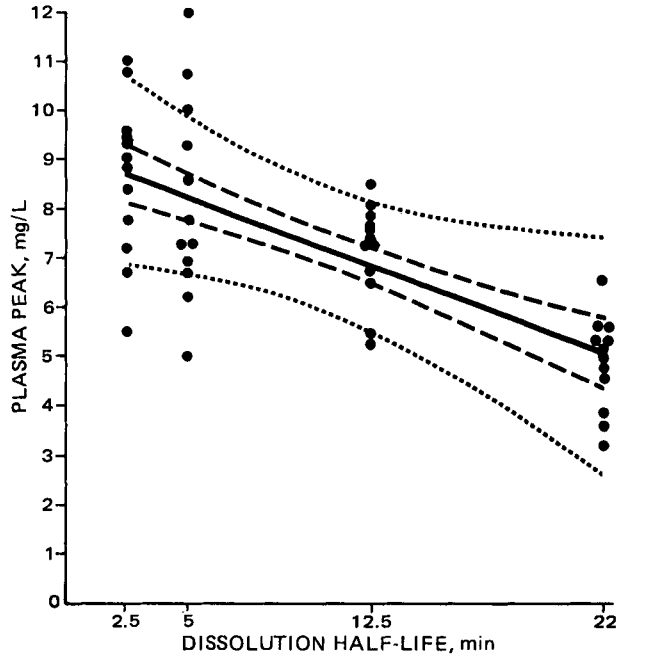

Figure 3—*Relationship between* in vitro *dissolution half-life and individual peak concentration after single 400-mg doses of microencapsulated bacampicillin hydrochloride in suspension. Crossover study in 12 subjects. The 95% confidence limits for the mean regression line (- - -) and for the prediction of a new observation in a random subject (. . .) after dosing with bacampicillin hydrochloride suspension with a given dissolution half-life are indicated.*

Experimental Design—The subjects were given the four suspensions according to a randomized, crossover design with balanced sequences and an interval of 1 week between doses. All subjects fasted for a minimum of 8 h (from midnight to drug administration) with the exception of 250 mL of water taken on rising, at least 1 h before dosing. The suspension was given in single oral doses containing 400 mg of bacampicillin hydrochloride. After swallowing the dose 150 mL of water was immediately ingested, part of which was used to rinse the cup twice. The subjects continued fasting for 3 h after drug ingestion, except for 150 mL of water given after 1 and 2 h and permitted *ad libitum* thereafter. A standardized meal was served 3 h after drug administration. The subjects were instructed not to engage in any strenuous or athletic activities during the days of drug administration. Smoking was permitted, but not snuff. The study protocol was reviewed and approved by a peer committee and written informed consent was obtained.

Sampling and Assay—Blood samples (5 mL) were drawn from the antecubital vein just prior to dosing and at 10, 20, 30, 45, 60, 80, and 100 min and 2, 3, 4, 6, and 8 h after drug administration. All samples were collected by direct puncture using evacuated heparinized blood collecting tubes[3]. Plasma was quickly separated by centrifugation and filtration[4]. The samples were frozen within 20 min of collection and were kept at −70°C in labeled vials until assayed. The total urine output was collected over 2-h intervals during the 8-h study period. The weight of the total volume collected during each interval was recorded and a 5 to 10-mL portion was frozen (−70°C) until assay. The plasma and urine samples were assayed for ampicillin by a microbiological technique using the cylinder plate method with *Micrococcus luteus* ATCC 9341 as the test organism (2).

Pharmacokinetic Calculations—The kinetics of ampicillin after intravenous injection can be adequately characterized by the two-compartment open model (3, 4). The individual plasma concentration *versus* time curves in the present study showed a biexponential decline, which also indicated that the two-compartment open model was valid. To determine the kinetics of absorption, the exponential parameters in the following equation were obtained using the nonlinear regression analysis program NONLIN (5):

$$C_p^t = Ae^{-\alpha t} + Be^{-\beta t} - Ce^{-k_a t} \text{ (mg/L)} \qquad \text{(Eq. 1)}$$

[3] Venoject; Terumo Europe NV, 3030 Leuven, Belgium.
[4] Sera-Clear; Technicon AB, Stockholm, Sweden.

Table I—Pharmacokinetic Variables After Single 400-mg Doses of Microencapsulated Bacampicillin Hydrochloride in Suspension [a]

Dissolution Rate						Urinary
$t_{50\%}$, min	k, h^{-1}	$C_{p,max}$, mg/L	k_a, h^{-1}	β, h^{-1}	AUC, mg·h/L	Recovery, %
2.5(0.13)	16.7(0.87)	8.7(1.6)	4.3(2.1)	0.60(0.24)	14.4(2.1)	76.7(11.3)[b]
5.0(0.23)	8.3(0.38)	8.2(2.0)	3.8(1.2)	0.57(0.13)	13.9(1.6)	74.0(12.3)
12.5(0.56)	3.3(0.15)	7.1(1.0)	3.6(1.9)	0.53(0.11)	13.3(2.2)	73.2(13.4)
22.0(0.72)	1.9(0.06)	4.9(0.94)	3.4(1.8)	0.52(0.14)	10.1(2.1)	53.7(14.2)

[a] Expressed as mean ± *SD* for a crossover study, $n = 12$. [b] $n = 11$.

where A, B, and C are the intercepts at the y-axis (at $t = 0$) obtained from the semilogarithmic plot of C_p^t *versus* t, α and β are first-order disposition rate constants of the biexponential decline of the curve, and k_a is the apparent first-order absorption rate constant. Initial parameter estimates used in NONLIN were calculated using a computer adaptation of the classical residual, peeling-off technique (CSTRIP) (6).

The areas under the plasma concentration–time curves (AUC) were calculated by use of the trapezoidal rule. The estimated area from the last sampling time t_x to infinity was calculated from

$$AUC_{t_x-\infty} = \frac{C_p^{t_x}}{\beta}(\text{mg} \cdot \text{h/L}) \qquad \text{(Eq. 2)}$$

where $C_p^{t_x}$ is the plasma concentration at the last sampling time. An estimation of the relative bioavailability of the four microcapsule suspensions was made by comparing the AUC values as well as the amount of ampicillin excreted in the urine during 8 h. The suspension with the shortest dissolution half-life was used as the standard.

Sensory Evaluation—Within 5–10 min after administration of each dose, the subjects recorded their sensory evaluation on two visual analogue scales. The first scale was related to the overall palatability, with the endpoints "extremely bad" and "excellent." The second was a rating of the bitter aftertaste with the endpoints "strong" and "none."

Statistical Analysis—The relationship between dissolution half-life and each of the response variables [plasma peak (C_{max}), area under the plasma concentration–time curve (AUC), and urinary recovery] was evaluated in a regression analysis. Since a crossover design was used, the observations within a subject are dependent in a manner requiring a modification of the standard regression technique. This modification is described in the *Appendix*.

For the sensory evaluation variables, for which a visual analogue scale was used, a nonparametric approach was taken in the analysis. The direct observations expressed as millimeters on the analogue scale were transformed to rank numbers after an overall ranking of the data. The rank numbers were then used in the regression analysis, a procedure which has been shown to give useful results for data on an ordinal scale (7).

A linear relationship between dissolution half-life and different variables was tested against the hypothesis of no relationship (slope of regression line = 0) using the Student's t test. The criterion for the choice between dissolution half-life ($t_{50\%}$) and dissolution constant [$k = (\ln 2)/t_{50\%}\ h^{-1}$] as the independent variable was the correlation between this variable and the response variable. The highest correlation coefficient was observed consistently for $t_{50\%}$; this measure was chosen, therefore, as the independent variable.

Since β was not expected to respond to differences in the dissolution rate, β was analyzed by using a two-way analysis of variance. The variable time-to-peak is a crude measurement of the absorption rate. Therefore, only descriptive methods were used in the evaluation of this variable. As such, these data give only supplementary information to the results of the nonlinear regression analysis.

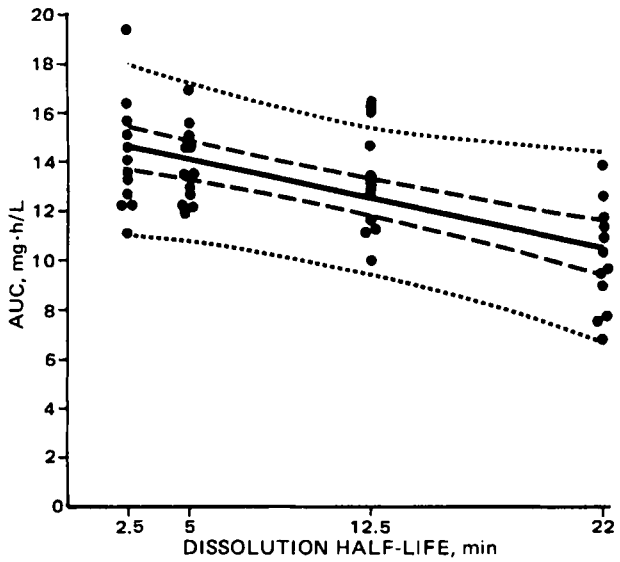

Figure 4—*Relationship between* in vitro *dissolution half-life and area under the plasma concentration–time curve (AUC) after single 400-mg doses of microencapsulated bacampicillin hydrochloride in suspension. Crossover study in 12 subjects. The lines are as defined for Fig. 3.*

RESULTS

***In Vitro* Dissolution**—The amount of polymer in the film coating applied had a strong influence on the *in vitro* dissolution rate of bacampicillin (Fig. 1), which followed first-order kinetics as shown by the linear curves in the inset diagram in Fig. 1. The mean calculated times for 50% of the drug to dissolve (dissolution half-life) were 2.5, 5.0, 12.5, and 22.0 min for the microcapsules with 17, 23, 29, and 37% film coating, respectively. A doubling of the thickness of the film coating thus increased the dissolution half-life by a factor of ~10. A variation in the stirring rate between 50 and 200 rpm did not affect the dissolution rate.

Dissolution Rate *versus* Plasma Levels—The highest mean plasma concentration–time curve was seen after the subjects received the suspension with the shortest dissolution half-life, and the lowest mean curve after receiving the suspension with the slowest dissolution rate (Fig. 2). The suspension with the slowest dissolution rate produced a lower and flatter maximum drug concentration. However, the slopes of the curves for the terminal disposition phase (β) of ampicillin did not differ significantly following administration of the four suspensions (Table I).

After receiving the three suspensions with the more rapid dissolution rates, no subject had a plasma peak below 5 mg/L, as compared with five subjects after receiving the suspension with a dissolution half-life of 22 min. An inverse linear relationship was noted between the plasma peak and dissolution half-life (Fig. 3). The regression lines had a mean (*SD*) slope of −0.19 (0.08), which is significantly different from zero at $p < 0.001$, and a mean correlation coefficient of 0.77 (0.18). There was a major deviation from the expected ranking order of the suspensions in only three subjects, all of whom had the highest peak after receiving the suspension with a dissolution half-life of 5 min. Proceeding from the lower limit of the 95% confidence interval for the regression line, a mean plasma peak of >5 mg/L can be predicted with a microcapsule suspension having a dissolution half-life of ≤19 min.

Dissolution Rate *versus* Absorption Rate—The absorption rate, as estimated from the time of the plasma peak concentrations, tended to increase with a decrease in the dissolution half-life (Table II). There tended to be less variation between individuals after receiving the suspension with the highest dissolution rate. The results of the nonlinear regression analysis show a slight tendency toward an increase in the apparent absorption rate with an increase in the dissolution rate (Table I). The regression lines had a mean (*SD*) slope of −0.04 (0.11), which is not

Table II—Frequency Distribution and Median Time of Plasma Peak Concentrations After Single 400-mg Doses of Microencapsulated Bacampicillin Hydrochloride in Suspension [a]

Dissolution Half-Life,	Time of Plasma Peak, h							
min	0.33	0.5	0.75	1.0	1.33	1.67	2.0	Median
2.5		6	5	1				0.63
5		4	3	1	4			0.75
12.5	1	2	4	2	2	1		0.75
22		4		3	4		1	1.0

[a] Crossover study, $n = 12$.

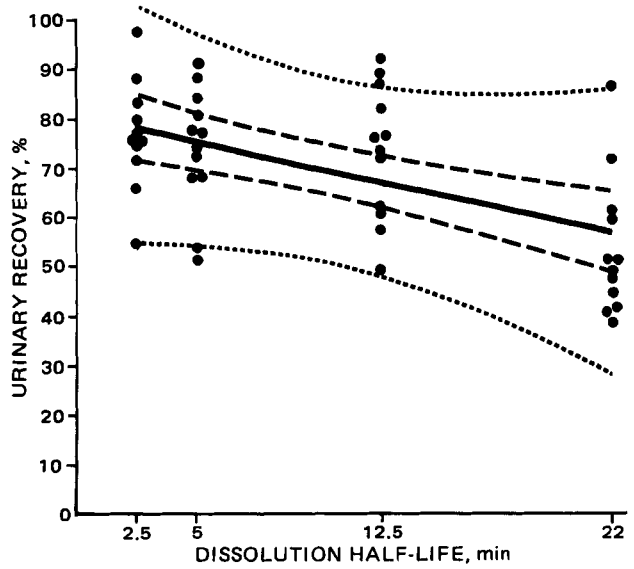

Figure 5—*Relationship between* in vitro *dissolution half-life and urinary recovery of ampicillin during 0–8 h after single 400-mg doses of microencapsulated bacampicillin hydrochloride in suspension. Crossover study in 12 subjects. The lines are as defined for Fig. 3.*

significantly different from zero, and a mean correlation coefficient of 0.61 (0.28).

Dissolution Rate *versus* Bioavailability—The extent of bioavailability was estimated from the AUC and the urinary recovery of ampicillin (Table I). Both of these variables correlated well with the dissolution half-life. The regression lines for the inverse relationship between AUC and dissolution half-life had a mean (*SD*) slope of −0.21 (0.10), which is significantly different from zero at $p < 0.001$, and a mean correlation coefficient of 0.77 (0.19) (Fig. 4). The linear regression lines between dissolution half-life and urinary recovery (Fig. 5) had a mean (*SD*) slope of −1.12 (0.82), significantly different from zero at $p < 0.001$, and a mean correlation coefficient of 0.67 (0.27). There were major deviations from the expected ranking order of the suspensions in only two subjects with regard to AUC and four with respect to urinary recovery. A mean urinary recovery of at least 60% can be predicted with 95% confidence for a suspension having a dissolution half-life of ≤14 min.

There was good agreement between the mean relative bioavailability when calculations were based on total AUC values and the amount of ampicillin recovered in urine (Table III) and a notable reduction in the bioavailability of the suspension with the longest dissolution half-life. The results with the two suspensions with the slowest dissolution rates were more variable when based on urinary excretion than on AUC.

Dissolution Rate *versus* Sensory Evaluation—There was a direct linear relationship between the dissolution half-life and the bitter aftertaste (Fig. 6) as well as the overall taste. The regression lines had mean (*SD*) slopes of 0.68 (0.71) and 0.53 (0.69), respectively, both significantly different from zero at $p < 0.01$ and $p < 0.05$. Their mean coefficients of correlation were 0.65 (0.29) and 0.56 (0.33), respectively. No adverse reactions were reported in any of the subjects.

DISCUSSION

The extent of bioavailability of bacampicillin hydrochloride from the three microcapsule suspensions with an *in vitro* dissolution rate of ≤12.5 min was similar to that previously reported with bacampicillin hydrochloride tablets. After 400-mg doses in tablet form, mean peak serum concentrations of 7.7–8.9 mg/L, a mean AUC of ~15 mg·h/L, and a mean urinary recovery of 71–75% have been reported (1, 2, 8). When given as tablets, 86–87% of the bacampicillin hydrochloride dose is absorbed (3, 4).

The significant correlation between the *in vitro* dissolution half-life of bacampicillin hydrochloride from the microcapsules and pharmacokinetic variables such as plasma peak, AUC, and urinary recovery can be used to predict the *in vivo* behavior of bacampicillin hydrochloride from *in vitro* tests of the microcapsule suspension. The 95% confidence limits for the mean regression lines in our study indicate that we can be fairly confident in predicting the mean of pharmacokinetic variables from *in vitro* data. The prediction of a new observation in a random subject is less accurate, however, due to the variation between individuals and the relatively small sample size in the present study.

Table III—Relative Bioavailability [a] After Single 400-mg Doses of Microencapsulated Bacampicillin Hydrochloride in Suspension

Dissolution Half-Life, min	Bioavailability, % AUC Method Mean	AUC Method *SD*	Urinary Recovery Method Mean [b]	Urinary Recovery Method *SD*
2.5	100.0		100.0	
5	97.9	17.6	97.1	17.1
12.5	93.4	14.9	99.1	24.8
22	70.1	11.9	72.3	22.7

[a] Based on the total AUC or amount of ampicillin recovered in the urine 0–8 h postdose in a crossover study, $n = 12$. [b] $n = 11$.

The bacampicillin hydrochloride microcapsules are coated with ethylcellulose, which is practically insoluble in aqueous solution. Thus, the rate-limiting step with respect to drug dissolution *in vitro* is diffusion through the film. That this process is also a rate limiting factor *in vivo* is supported by the good *in vitro–in vivo* correlation observed. Also, the lack of influence of stirring rate on the *in vitro* dissolution of the microcapsules favors a strong correlation and is similar to the findings of Levy *et al.* (9). The diffusion through the film is highly dependent on wall thickness, which has also been reported with regard to microcapsules containing phenethicillin potassium (10).

The weak correlation between the apparent absorption rate constant (k_a) and the dissolution rate constant (k) indicates that the diffusion through the microcapsule coating is not the rate-limiting step in the absorption process. The results of parameter estimation of a polyexponential model should be interpreted with great caution. This applies also to results obtained from computer programs based on nonlinear regression analysis. Especially when the exponents are close together, as is frequently the case with drugs with a short half-life like the penicillins, this approach may lead to inaccurate estimates of the parameters (11). Furthermore, in such cases the estimates are highly dependent on the choice of starting values in the iterative procedure. This was also obvious from some simulation in the present material, in which the nonlinear regression analysis gave very different estimates after even small changes in the initial values for some subjects.

The suspension with the slowest dissolution rate did not yield more sustained plasma concentrations than the others. A possible reason for this is that the microcapsules had passed the upper duodenum before bacampicillin was released. According to Swahn (12), the upper duodenum is where bacampicillin is most efficiently absorbed. Another possibility is that bacampicillin was released too far in the intestine and then decomposed to ampicillin which was destroyed by the β-lactamases which are abundant in the intestinal flora. The results clearly show that little benefit is to be expected from a slow-release formulation of microencapsulated bacampicillin hydrochloride.

A review of two previous bioavailability studies on volunteers verifies the correlation in the present study and indicates reproducible results. Thus, a similar microcapsule suspension with a dissolution half-life of ~20 min has been used and produced, after a 400-mg dose, a mean plasma peak, AUC, and urinary recovery of 5.2 mg/L, 10.8 mg·h/L, and 55.7%, respectively[5]. The same microcapsule batch (dissolution half-life 22 min) and dose as used in this study, but suspended in water without such excipients as sweeteners, flavoring, and thickening agents, generated a mean plasma peak, AUC, and urinary recovery of 4.3 mg/L, 7.8 mg·h/L, and 45%, respectively, in another group of volunteers[1]. Thus, the excipients do not seem to influence the bioavailability from the microcapsule suspension. A microcapsule formulation essentially similar to that used in the present study has also been found to be well absorbed in infants (13, 14).

Quantitative correlations have been reported between *in vitro* dissolution and *in vivo* bioavailability from other preparations of the present type, *e.g.*, estradiol suspensions with different rates of drug dissolution owing to differences in particle sizes (15). Sjögren and Bogentoft (16) were able to control aspirin absorption from enteric-coated granules with different *in vitro* dissolution profiles. The microcapsules were given in hard gelatin capsules. Wagner (17) and Smolen (18) have noted that *in vitro–in vivo* correlations have often been of little use in predicting the bioavailability of formulations not included in a study. However, the *in vitro–in vivo* correlation may apply to specific products manufactured

[5] J. Sjövall and R. Sjöqvist; unpublished data.

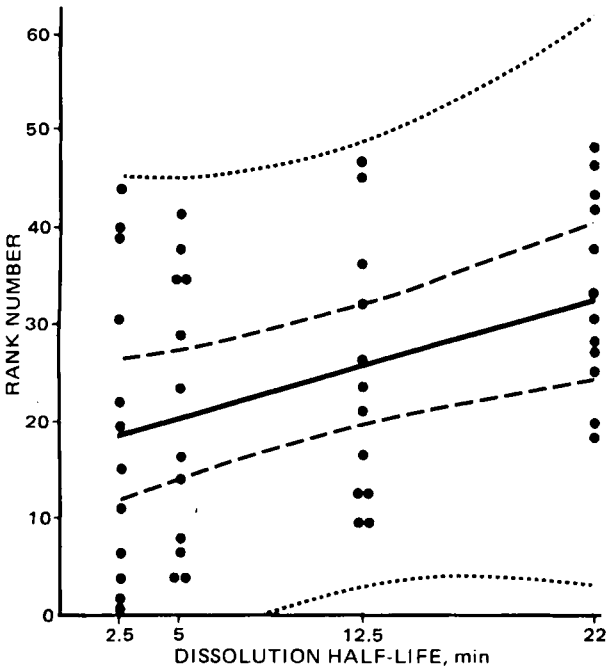

Figure 6—*Relationship between* in vitro *dissolution half-life and bitter aftertaste expressed as the rank number after an overall ranking of sensory recordings on a visual analogue scale. Crossover study in 12 subjects using single 400-mg doses of microencapsulated bacampicillin hydrochloride in suspension. High rank numbers indicate less bitterness. The lines are as defined for Fig. 3.*

by similar methods, *e.g.*, different amounts of coating agents, as shown in the present study. Substances with good solubility in water and lipids, and which show linear pharmacokinetics, offer more favorable conditions for a good *in vitro–in vivo* correlation. These factors are applicable to bacampicillin hydrochloride, which is soluble in both water and lipids. The total amount of bacampicillin hydrochloride absorbed is a linear function of the dose (19).

Although the present study was not optimally designed for a sensory evaluation of the taste of the suspension, a significant correlation between taste and dissolution rate was found. This verifies the results from prediction tests on volunteers in which the microencapsulation of bacampicillin hydrochloride was found to reduce the bitter taste in suspensions to a level similar to that of other aminopenicillins[6].

There is an inverse linear relationship between the bioavailability of bacampicillin hydrochloride from a microcapsule suspension and the microcapsule *in vitro* dissolution half-life. On the other hand, there is a direct linear relationship between masked bitterness and dissolution half-life. The present results suggest that bacampicillin hydrochloride can be administered in a microcapsule suspension with sufficient microcapsule film thickness to reduce the bitter taste and still retain good bioavailability.

APPENDIX

One important objective of this study was to evaluate the relationship between dissolution half-life (x) and each of the response variables (y) plasma peak concentration (C_{max}), area under the plasma concentration–time curve (AUC), urinary recovery, and the results of the sensory recordings. In this evaluation the standard linear regression analysis has been adapted to the crossover design. In principle, this means that individual regression equations have been estimated and then combined in an overall estimate. Formally, the following procedure was used. For each individual ($i = 1, \ldots, n$) the following linear regression model was assumed:

$$y = \alpha_i + \beta_i x + \epsilon, \qquad \text{(Eq. 3)}$$

where α is the intercept and β the slope of the regression line. The random error ϵ is assumed to be normally and independently distributed with mean = 0 and variance = σ_ϵ^2. Each of these models has been estimated separately by using the method of least squares, giving:

[6] R. Sjöqvist; unpublished data.

$$y = a_i + b_i x \qquad \text{(Eq. 4)}$$

An overall linear relationship has been estimated by averaging the individual regression model estimates, which yields:

$$y = a + bx, \qquad \text{(Eq. 5)}$$

where $a = (1/n)\,\Sigma a_i$ and $b = (1/n)\,\Sigma b_i$.

The standard error for $a + bx$ has been calculated according to:

$$SE(a + bx) = \left[\frac{1}{n}\left(SD^2(\bar{y}_i) + (x - \bar{x})^2 \cdot SD^2(b_i)\right)\right]^{1/2} \qquad \text{(Eq. 6)}$$

where $SD^2(\bar{y}_i)$ and $SD^2(b_i)$ is the variance of the mean value of y and the regression coefficients, respectively, calculated from the individual regression lines. $\bar{x}$ is the overall mean value of dissolution half-lives. A 95% confidence interval for the overall regression line has been calculated as follows:

$$(a + bx) \pm t^{0.95}_{n(k-2)} \cdot \mathrm{SE}(a + bx) \qquad \text{(Eq. 7)}$$

where $t^{0.95}_{n(k-2)}$ is the value of the Student's t distribution with $n(k - 2)$ degrees of freedom appropriate for a 95% interval.

The standard error of a new observation of y in a random subject with $x = x_0$ has been calculated as:

$$SE(a + bx_0) = \left[\left(1 + \frac{1}{n}\right)\left(SD^2(\bar{y}_i) + (x_0 - \bar{x})^2 \cdot SD^2(b_i)\right)\right]^{1/2} \qquad \text{(Eq. 8)}$$

The corresponding 95% prediction interval is:

$$(a + bx_0) \pm t^{0.95}_{n(k-2)} \cdot SE(a + bx_0) \qquad \text{(Eq. 9)}$$

This procedure is developed in analogy to that described in Brownlee (20).

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