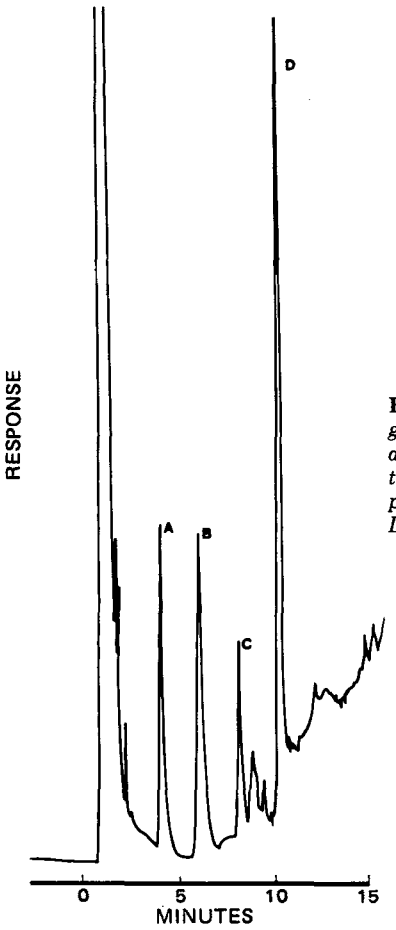

Figure 3—*Liquid chromatographic trace of the extract of a multivitamin–multimineral tablet. Key: A, niacinamide; B, pyridoxine; C, thiamine; and D, riboflavin.*

separation of all four water-soluble vitamins is possible (Figs. 1 and 2). There also is a baseline separation of the vitamins in the dimethyl sulfoxide extract (Fig. 3).

To validate the selectivity of the procedure, placebo formulations spiked with known amount of the vitamins were employed. The results are tabulated in Table III. All four vitamins were quantitatively recovered in both multivitamin and multivitamin–multimineral tablets.

To verify the applicability of this procedure further, various commercial multivitamin and multivitamin–multimineral tablets were assayed simultaneously by the HPLC and the USP methods (Tables IV and V). In each case, the precision of the HPLC procedure was greater than that of the current official procedure. This result may be attributed to the less complicated sample workup required by the former procedure. However, the results of the two methods compared favorably in terms of accuracy.

The described HPLC method appears to be attractive in terms of analysis time and should be adapted to various commercial products. The column lifetimes under these conditions of minimum sample preparation initially was a subject of concern. However, over 500 preparations were processed without change in the chromatographic characteristics of the system. This stability may be due in part to the small quantities of sample injected and in part to the proper care as recommended by the column supplier. The methodology for the HPLC determination of other vitamins is currently under investigation and will be published later.

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In Vivo–In Vitro Correlations with a Commercial Dissolution Simulator I: Methenamine, Nitrofurantoin, and Chlorothiazide

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Abstract □ Dissolution profiles were determined for nine methenamine, 14 nitrofurantoin, and six chlorothiazide dosage forms using a dissolution simulator. Various *in vivo–in vitro* correlations were examined. The best correlation for methenamine was between the maximum urinary excretion rate and the time for 15% dissolution. A good correlation for the 50-mg nitrofurantoin tablets was also found between cumulative percent of drug excreted in 12 hr and the percent dissolved in 1 hr. There were no significant correlations for the 100-mg nitrofurantoin dosage forms. Good correlations were also observed for the 250- and 500-mg chlorothiazide tablets between the percent of drug dissolved in 1 min or the time for 15% dissolution and the maximum excretion rate.

Keyphrases □ Dissolution—profiles for methenamine, nitrofurantoin, and chlorothiazide, dissolution simulator □ Dissolution simulator—profiles for methenamine, nitrofurantoin, and chlorothiazide □ Methenamine—dissolution profile using dissolution simulator □ Nitrofurantoin—dissolution profile using dissolution simulator □ Chlorothiazide—dissolution profile using dissolution simulator

Many methods developed to study the *in vitro* dissolution properties of dosage forms have been reviewed previously (1). The primary objective of most efforts to develop *in vitro* dissolution procedures is to provide data that can be related to the performance of oral dosage forms when administered to human subjects. One sophisticated

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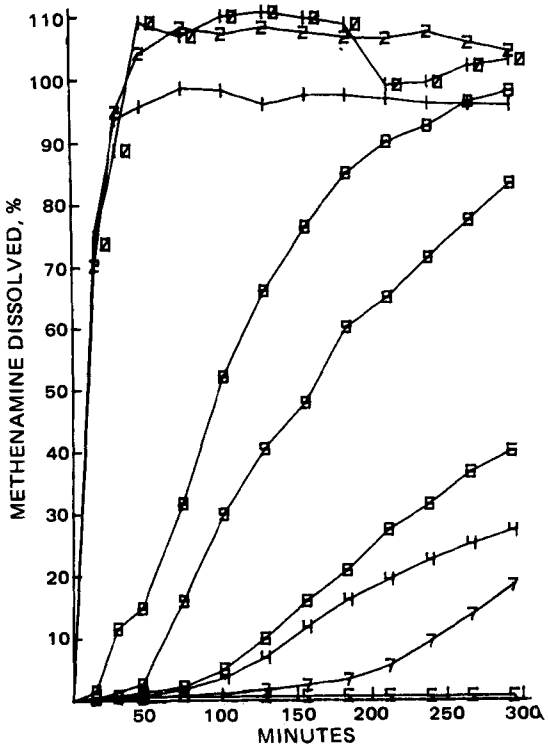

Figure 1—*Dissolution profiles for nine methenamine formulations. Each data point is the mean of two determinations. The simulated intestinal phase began after 32 min.*

instrument developed recently is a dissolution simulator[1], which is designed to be utilized in conjunction with an absorption simulator[2]. It is claimed (2) that these devices provide *in vitro* data that correlate well with *in vivo* bioavailability data. However, relatively little data demonstrating the applicability of these devices (3–5) have been published.

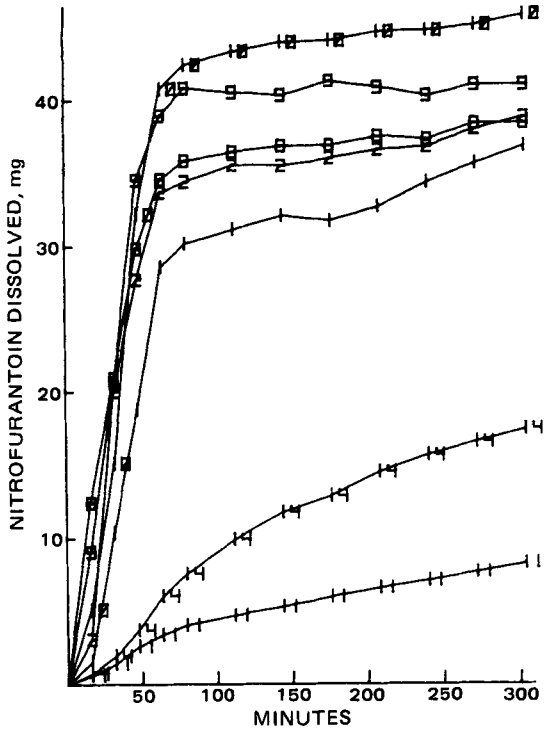

Figure 2—*Dissolution profiles for seven 50-mg nitrofurantoin formulations. Each data point represents the mean of two determinations. The intestinal phase began after 32 min.*

[1] SM 16752, Sartorius Filters, San Francisco, Calif.
[2] SM 16753, Sartorius Filters, San Francisco, Calif.

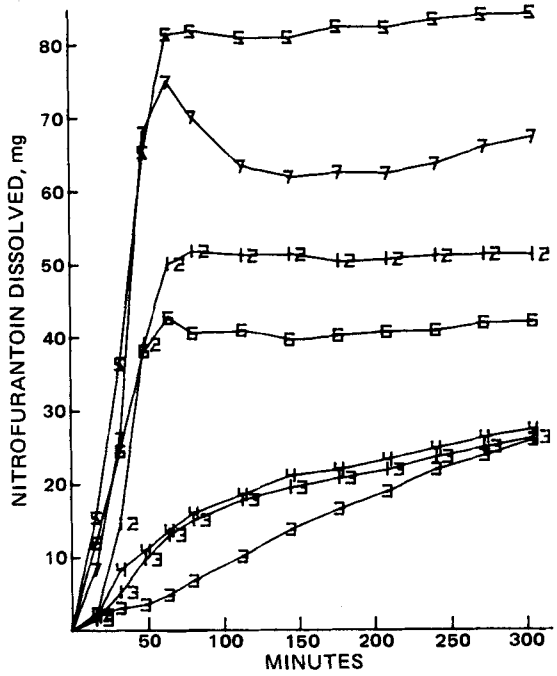

Figure 3—*Dissolution profiles for seven 100-mg nitrofurantoin formulations. Each data point represents the mean of two determinations. The intestinal phase began after 32 min.*

BACKGROUND

The dissolution simulator is composed of two identical dissolution systems that permit the simultaneous evaluation of two dosage forms.

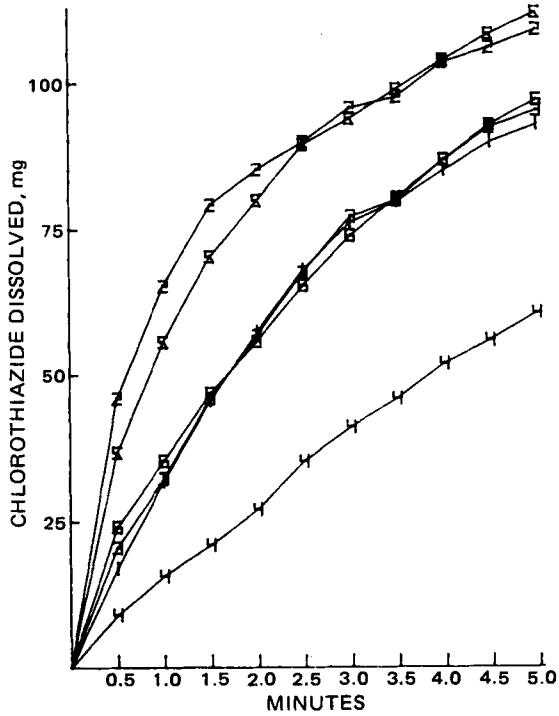

Figure 4—*Dissolution profiles for three 250-mg chlorothiazide tablet formulations (Products 1, 3, and 4) and three 500-mg chlorothiazide tablet formulations (Products 2, 5, and 6) in simulated gastric fluid. Each data point represents the mean of two determinations.*

The dissolution process takes place in two plastic cylinders. Dissolution fluid (100 ml) is placed in each cylinder, along with the dosage form and 70 g of glass beads. The cylinders rotate around a horizontal axis at 1.2 cpm to provide gentle agitation. The cylinder contents are maintained at 37°, and the pH of the dissolution fluid may be changed during the dissolution process by the addition of buffer salts. Samples ranging from 2.5 to 7.5 ml are automatically withdrawn and collected for analysis at sampling intervals of 0.5–60 min. The volume of dissolution fluid removed by sampling is replaced by fresh solution contained in a reservoir. The manufacturer recommends that the rate of sample withdrawal should be determined from data obtained in preliminary studies with the absorption simulator. The sampling rate for the dissolution simulator is directly related to the rate of drug diffusion in the absorption simulator.

The absorption simulator is essentially a dialysis cell designed to determine the diffusion rate of a drug, dissolved in either simulated gastric or intestinal fluid, across a membrane coated with a lipid substance into a pH 7.5 buffer. The diffusion rate is reported to be related to the rate of drug diffusion across the membranes of the GI tract.

Since little data were available to permit an evaluation of the correlation between *in vitro* dissolution data obtained with the dissolution simulator and the bioavailability of oral dosage forms in humans, a series of studies was initiated. The present study reports dissolution data for nine methenamine, 14 nitrofurantoin, and six chlorothiazide dosage forms. Each formulation was the subject of previous *in vivo* urinary excretion studies with healthy human subjects (6–8).

EXPERIMENTAL

Absorption Simulator Studies—Each drug was dissolved in 100 ml of simulated gastric fluid (pH 1.1) containing hydrochloric acid, sodium chloride, and aminoacetic acid or in simulated intestinal fluid (pH 6.5) containing phosphate buffer. The initial drug concentrations were 200 μg/ml for the methenamine and chlorothiazide solutions and 100 μg/ml for the nitrofurantoin. A membrane with an effective surface area of 80 cm^2 was used for both the simulated gastric and intestinal phase diffusion studies. The composition of the lipid coating of the membrane was different for the two studies, but the nature of the coating was not available from the supplier.

The gastric and intestinal phase studies used artificial gastric and intestinal membranes[3]. The drug diffused from the gastric or intestinal phase, across the membrane, into the simulated plasma phase, which was a pH 7.5 phosphate buffer. At least three samples were collected from each phase at equal time intervals, and each study was done in duplicate. The resulting data were employed to calculate a diffusion rate constant, which was subsequently used to estimate an appropriate sampling rate for the dissolution simulator, according to a previous method (9).

Dissolution Simulator Studies—Each dosage form of the three drugs was initially placed into the simulated gastric fluid. The sampling rates for each drug were as follows: methenamine, 2.5 ml every 15 min for 30 min; nitrofurantoin, 2.5 ml every 16 min for 32 min; and chlorothiazide, 7.5 ml every 0.5 min for 5 min. At the end of the gastric phase, phosphate buffer was added to the dissolution fluid to increase the pH to 6.5 for methenamine and nitrofurantoin. Dissolution studies of the chlorothiazide formulations were only studied using the gastric phase. The sampling rates during this intestinal phase were as follows: methenamine, 2.5 ml every 5.5 min for 278 min; and nitrofurantoin, 2.5 ml every 16 min for 240 min. Two units of each drug formulation were studied. In addition, six tablets of one lot of nitrofurantoin were used to measure the reproducibility of the dissolution process.

The methenamine dosage forms, identified previously in a 10-subject study (6), consisted of a compressed tablet with 500 mg of methenamine base (Product 1), a suspension containing 50 mg of methenamine mandelate/ml (Product 2), a 500-mg methenamine hippurate tablet (Product 10), and six formulations of enteric-coated methenamine mandelate (Products 4–9). A 10th dosage form (Product 3), given as a solution in a previous study (6), was not included.

The nitrofurantoin dosage forms, included in earlier *in vivo* studies using 14 subjects (7), were six 50-mg tablets of microcrystalline drug (Products 1, 2, 8, 9, 11, and 14) and six 100-mg tablets of microcrystalline drug (Products 3–6, 12, and 13). A 50-mg macrocrystalline capsule (Product 10) and a 100-mg macrocrystalline capsule (Product 7) also were studied.

The chlorothiazide tablets contained either 250 (Products 1, 3, and 4) or 500 (Products 2, 5, and 6) mg of chlorothiazide and also were identified in an earlier study in which each dosage form was administered to a group of 12 subjects (8).

[3] Artificial gastric lipid barrier, SM 15701, and artificial intestinal lipid barrier, SM 15702, Sartorius Filters, San Francisco, Calif.

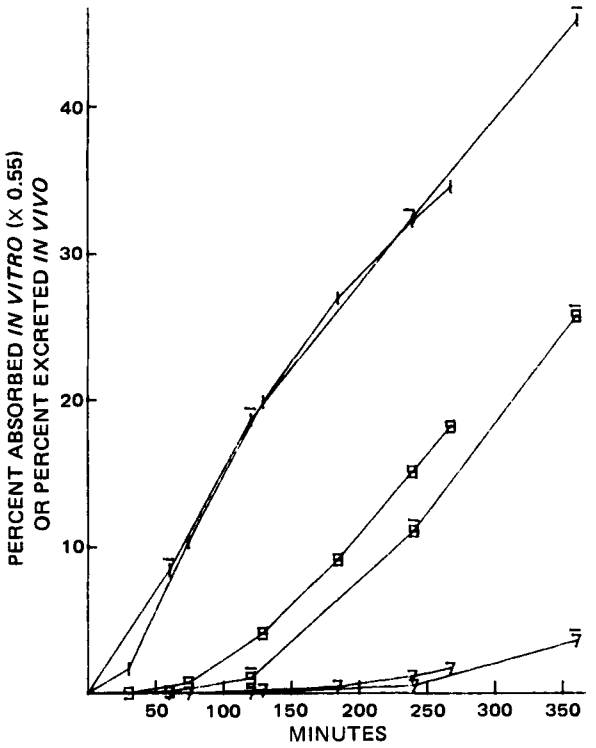

Figure 5—*Comparison of the total amount of methenamine withdrawn from the* in vitro *dissolution system (× 0.55) (Products 1, 7, and 9) and the cumulative amount of methenamine excreted in the* in vivo *studies (Products 1, 7, and 9) for three methenamine formulations. Each* in vitro *data point is the mean of two determinations. Each* in vivo *data point is the mean of 10 subjects.*

USP Dissolution Tests—Dissolution testing, using the USP rotating basket, was described previously for *in vivo* studies of the nitrofurantoin (7) and chlorothiazide (8) dosage forms. Studies using this dissolution apparatus were not conducted for the methenamine tablets.

Analytical Methods—Samples of methenamine solutions obtained with both the dissolution and absorption simulators were assayed by a colorimetric procedure based on a method developed to determine urine concentrations (10). Samples were diluted, and 0.5-ml aliquots were acidified with 0.5 ml of 6 *N* HCl and heated in a 70° water bath in a closed tube for 2 hr to hydrolyze the methenamine to formaldehyde. The solution was then cooled to 5°, and 1 ml of 0.1% tryptophan in 50% ethanol, 0.2 ml of 1% ferric chloride, and 1 ml of 90% sulfuric acid were added. The solution was heated for an additional 70 min at 70°.

After cooling to room temperature, the absorbance was determined at 575 nm and the methenamine concentration was calculated from a standard curve obtained by carrying aqueous solutions of known methenamine composition through the assay procedure. Preliminary studies indicated that the materials contained in the enteric coating of some of the products did not interfere in the assay at the dilution levels employed for the *in vitro* samples.

Nitrofurantoin samples obtained with either the absorption or dissolution simulator were diluted as necessary with pH 6.5 or 7.5 phosphate buffer, and the absorbance was determined at 278 nm. Standard curves also were prepared with nitrofurantoin solutions of known composition at pH 6.5 and 7.5.

Chlorothiazide samples were assayed at 278 nm after dilution with pH 6.5 or 7.5 phosphate buffer. Solutions of known chlorothiazide concentration also were assayed.

Treatment of Dissolution Data—Three types of data treatment were employed to evaluate the relationship between *in vitro* dissolution and *in vivo* urinary excretion data.

Simulated Absorption Data—The cumulative amount of drug excreted in the urine and the cumulative amount of dissolved drug withdrawn from the dissolution chamber were plotted *versus* time and were compared visually.

General Correlations—*In vivo* measurements, such as the cumulative

Table I—Diffusion Characteristics and Sampling Rates Determined with the Absorption Simulator

Drug	$K_d \times 10^3$ cm/min^{-1} (±SD)		Calculated Time, between Samples, min[b]		Actual Time between Samples, min	
	Gastric Phase[a]	Intestinal Phase[a]	Gastric Phase	Intestinal Phase	Gastric Phase	Intestinal Phase
Methenamine	0.28 (0.12)	0.64 (0.13)	29.5	5.5	15.0	5.5
Nitrofurantoin	0.16 (0.01)	0.34 (0.00)	67.9	16.0	16.0	16.0
Chlorothiazide	0.12 (0.00)	0.44 (0.04)	110.0	9.8	0.5	—

[a] Diffusion rate constant, calculated according to Stricker (9). [b] Theoretical sampling rates for the dissolution simulator, calculated from the absorption simulator diffusion rate according to Stricker (9).

percent of drug excreted at specific times and maximum urinary excretion rates, were correlated with *in vitro* data such as the cumulative percent of drug dissolved and the time for a specific percent of drug to dissolve *in vitro*. The latter values for $T_{15\%}$, $T_{50\%}$, and $T_{70\%}$ were determined using the logarithmic-probability method of Sullivan *et al.* (11).

Quadrant Analysis—This relatively new approach (12, 13) utilizes *in vivo* data from individual subjects rather than just mean data from a group of subjects. The performance of each dosage form in each subject is evaluated relative to a reference dosage form. An arbitrary guideline, *e.g.*, 75%, is selected, and the percent of subjects exhibiting a relative bioavailability of at least 75% is determined and plotted *versus* the *in vitro* dissolution measurement of interest for each test dosage form. For example, if five out of 10 subjects exhibit a maximum urinary excretion rate for a test product that is at least 75% that of a reference product, 50% would be used for correlation with the *in vitro* measurement for that specific dosage form. The other formulations included in the *in vivo* and *in vitro* evaluations would be treated similarly to develop a possible correlation.

RESULTS AND DISCUSSION

Absorption Simulator—The apparent first-order diffusion rate constants determined in simulated gastric and intestinal fluids using the absorption simulator are given in Table I. A direct comparison of the diffusion rates for a given drug in both gastric and intestinal fluid is not possible because the composition of the membranes was different for the two fluids.

The diffusion rate constants, which according to Stricker (9) are related to the *in vivo* drug absorption rate, were used to compute the optimum sampling intervals for the *in vitro* dissolution studies. The resulting sampling times are given in Table I. Since the dissolution simulator is supposed to involve dissolution studies using gastric fluid for 30 min to simulate gastric residence time, the calculated sampling intervals for methenamine, nitrofurantoin, and chlorothiazide were not appropriate. Thus, arbitrary sampling times were chosen for the gastric fluid dissolution studies of these drugs. In particular, the 0.5-min time used for chlorothiazide was based on a preliminary study that indicated that the gastric dissolution fluid was rapidly saturated with this drug within 5 min. Furthermore, because of the limited solubility of chlorothiazide, the dissolution study was terminated after 5 min.

The results obtained with the absorption simulator indicated that it was of little value for these three drugs in the determination of appropriate sampling intervals for the dissolution studies. While the absorption simulator may be useful in the study of diffusion characteristics of drugs through lipid membranes, such studies were beyond the scope of the present investigation.

Dissolution Profiles—Figures 1–4 illustrate the dissolution rate profiles for the various drug products as determined in the dissolution simulator. The enteric-coated methenamine formulations (Products 4–9) shown in Fig. 1 exhibited a delay in the onset of dissolution until the dissolution fluid pH was increased from 1.1 to 6.5. These dissolution profiles were utilized to develop the correlations described later with the previously determined *in vivo* data.

While the dissolution of only two units of each formulation was routinely studied for each drug, six tablets of one 50-mg nitrofurantoin product were used in one instance to evaluate the reproducibility of the dissolution process. The mean amount of nitrofurantoin dissolved after 16 min was 9.2 mg (range 8–11 mg); after 32 min, the mean was 21.3 mg (range 20–22 mg); and after 48 min, the mean was 34.7 mg (range 34–36 mg). Generally, this good agreement among the six replicates also was seen between the duplicate determinations for each formulation of the three drugs.

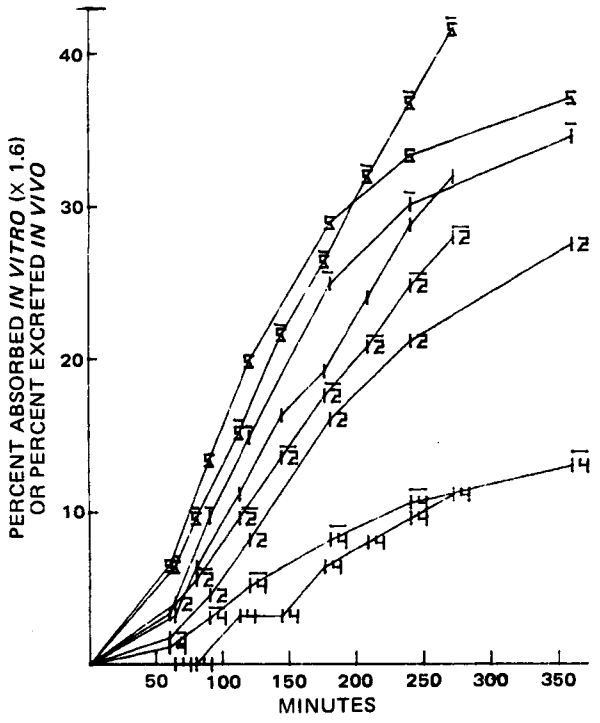

Figure 6—*Comparison of the total amount of nitrofurantoin withdrawn from the* in vitro *dissolution system (× 1.6) and the cumulative amount of nitrofurantoin excreted in the* in vivo *studies for four nitrofurantoin tablet formulations. Each* in vitro *data point (Products 1, 5, 12, and 14) is the mean of two determinations. Each* in vivo *data point (Products 1, 5, 12, and 14) is the mean of 14 subjects.*

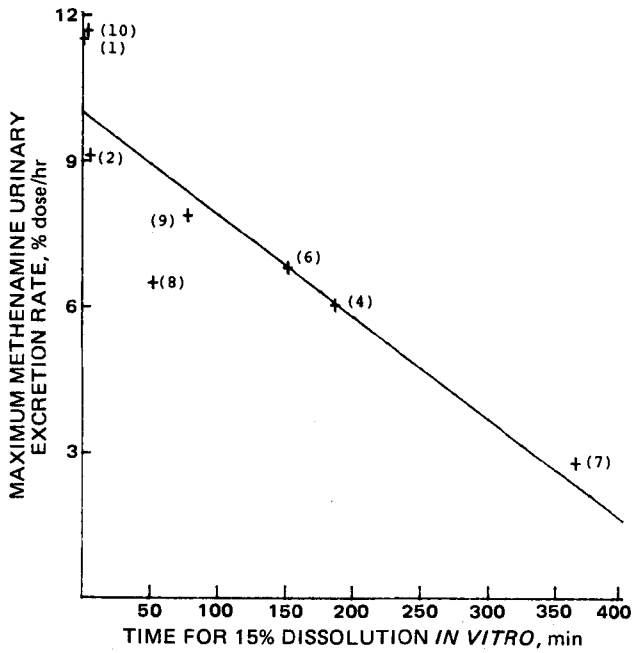

Figure 7—In vitro–in vivo *correlation for eight methenamine formulations,* $r = -0.894$ ($p < 0.01$). *Each data point represents the mean of two* in vitro *and 10* in vivo *values.*

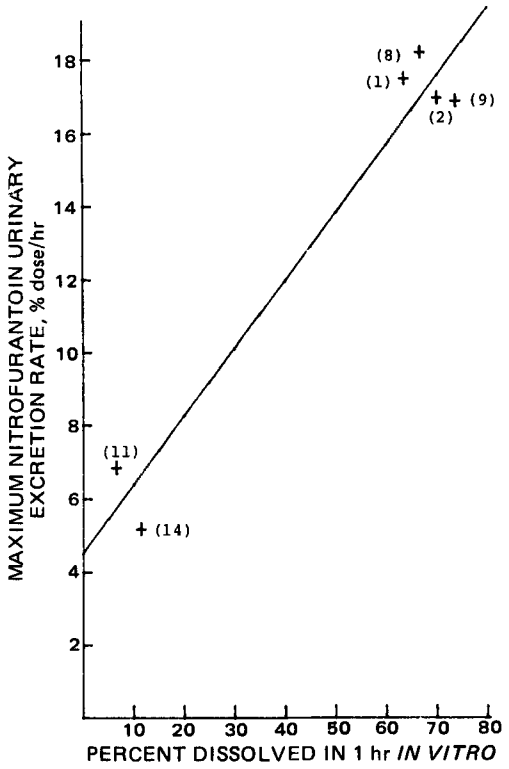

Figure 8—In vitro–in vivo *correlation for five 50-mg nitrofurantoin tablet formulations,* r = *0.976* (p < *0.01*). *Each data point represents the mean of two* in vitro *and 14* in vivo *values.*

Simulated Absorption Profiles—The dissolution simulator is reported by the manufacturer to be capable of providing data that can simulate *in vivo* absorption profiles (2). To evaluate this claim, plots were constructed of cumulative urinary excretion of each drug and the cumulative amount of dissolved drug withdrawn from the dissolution chamber at the corresponding times. Since the dissolution studies for the chlorothiazide tablets were carried out only for 5 min, simulation of *in vivo* data was not possible for these dosage forms.

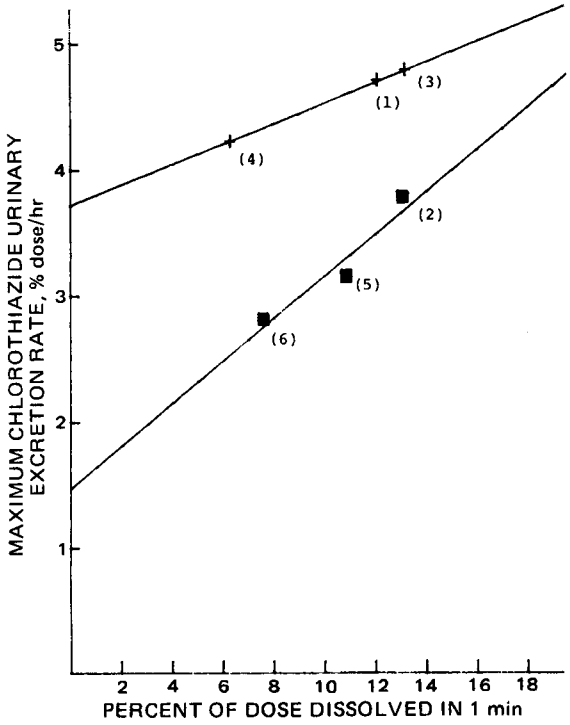

Figure 9—In vitro–in vivo *correlations for three 250- (+) and three 500- (■) mg chlorothiazide tablet formulations,* r = *0.999* (p < *0.05*) *and* r = *0.962* (p > *0.10*), *respectively. Each data point represents the mean of two* in vitro *and 12* in vivo *values.*

Methenamine Tablets—All data were expressed as the percent of dose to normalize for differences in methenamine content among products. Product 1, a compressed tablet of methenamine, was selected as a reference, and plots were made of the cumulative percent of dissolved drug withdrawn from the dissolution chamber and the mean cumulative percent of drug excreted in the urine of the 10 subjects receiving the drug in an earlier study (6). The *in vitro* curves paralleled the *in vivo* data but were somewhat higher. An empirical correction factor of 0.55, applied to the *in vitro* data, resulted in good correspondence between the two data sets.

The data shown in Fig. 5 illustrate the comparison between the *in vitro* data, corrected by a factor of 0.55, and the corresponding *in vivo* urinary excretion data for Product 9, with an intermediate dissolution rate, and Product 7, with a relatively slow dissolution rate. A similar good correspondence also was obtained for Products 4–6 and 10. The relationship between *in vitro* and *in vivo* data was not nearly as good for Products 2 and 8, with the *in vitro* data predicting greater urinary excretion than was actually seen.

Nitrofurantoin Dosage Forms—Figure 6 illustrates good correspondence between the *in vivo* and *in vitro* data for Products 1, 5, 12, and 14. Each *in vitro* data point was adjusted by a factor of 1.6, based on the observed parallelism for the *in vivo* and *in vitro* data for Product 9, which was taken as the reference product. Similar good correspondence was observed for Products 2 and 8, but there was a significant difference between the *in vivo* and *in vitro* data sets for Products 3, 4, 6, 11, and 13. As will be discussed, Products 7 and 10, the macrocrystalline capsules, exhibited dissolution characteristics that were quite different from the microcrystalline tablets. Therefore, these two dosage forms were not included with the microcrystalline formulations in this data treatment.

General Correlations—These correlations attempted to relate various *in vitro* and *in vivo* measurements. Only the best correlations will be discussed, although other correlations were examined.

Methenamine Tablets—The best correlation, $r = 0.928$ ($p < 0.01$), was seen between the area under the dissolution–time curve at 4.5 hr *versus* the cumulative percent excreted at 6 hr. When using $T_{15\%}$ as the *in vitro* measurement, good correlations were observed with the cumulative

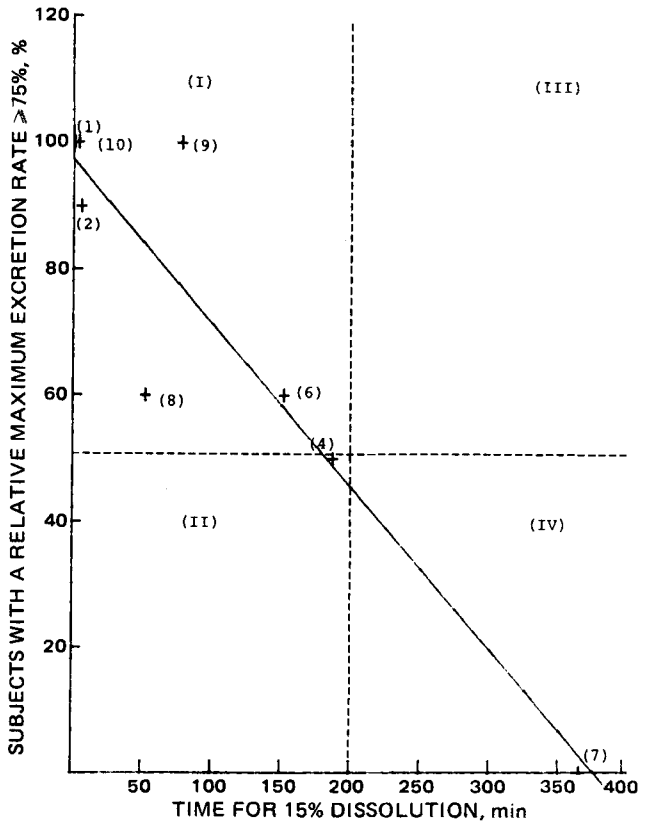

Figure 10—*Quadrant analysis for eight methenamine formulations,* r = *−0.93* (p < *0.01*). *Each data point represents the mean of two* in vitro *and 10* in vivo *values. Product 9 was the reference product.*

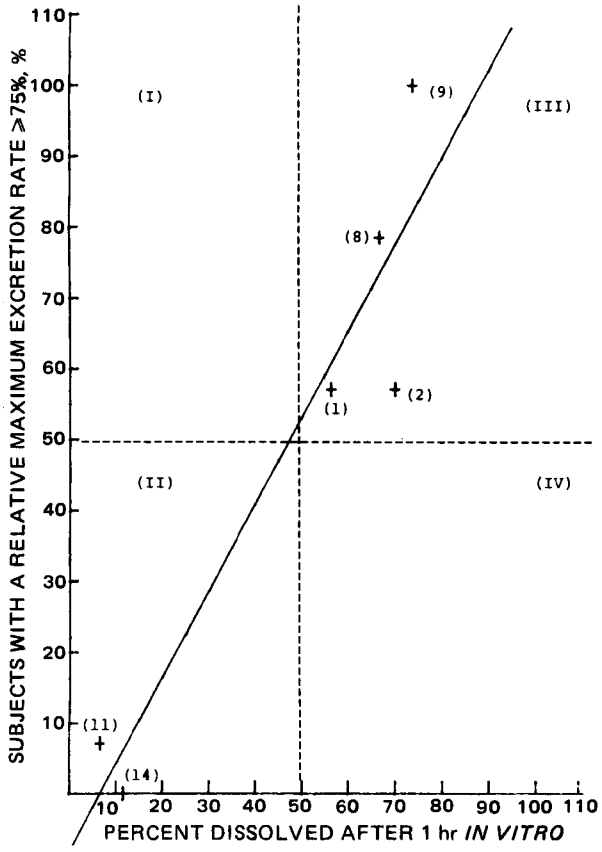

Figure 11—*Quadrant analysis for six 50-mg nitrofurantoin tablet formulations,* r = *0.943 (*p < *0.01). Each data point represents the mean of two* in vitro *and 14* in vivo *values. Product 9 was the reference product.*

percent excreted at 48 hr, $r = -0.875$ ($p < 0.01$), and the maximum excretion rate, $r = -0.894$ ($p < 0.01$) (Fig. 7). Since Products 4, 5, and 7 were <30% dissolved after 5 hr, correlations with $T_{\%}$ values beyond 15% could not be used. Product 5 is not illustrated because <1% of the drug had dissolved after 5 hr, and the tablet retained its original shape during the dissolution period.

Nitrofurantoin—Attempts to obtain *in vivo–in vitro* correlations that included Products 7 and 10 were not successful. Both dosage forms exhibited relatively good dissolution properties (Figs. 3 and 4). However, these two products ranked below eight of the other products in earlier bioavailability studies involving determination of cumulative urinary excretion. Thus, these two products, which represented capsule formulations of the macrocrystalline drug, were omitted from the correlations.

The percent of drug excreted in 12 hr and the maximum urinary excretion rate were correlated with the percent of drug dissolved at various times. Generally, the correlations with the 100-mg tablets were less satisfactory than with the 50-mg tablets. The correlation coefficient for the 100-mg tablets, using a variety of measurements, was <0.8 ($p < 0.10$), while correlation coefficients exceeded 0.93 ($p < 0.01$) for the 50-mg tablets. The best correlation for the 50-mg tablets is shown in Fig. 8, $r = 0.976$ ($p < 0.01$), using the maximum excretion rate and the percent of drug dissolved in 1 hr. Cumulative percent excreted in 12 hr also correlated well with the 1-hr dissolution value, $r = 0.960$ ($p < 0.01$). The poorer correlation with the 100-mg tablets may be due to the fact that the maximum excretion rates for the six tablets were much closer than for the 50-mg tablets. Previous investigators (14) noted a similar poorer correlation for 100-mg nitrofurantoin tablets, using the same lots as employed in the present study, but obtained *in vitro* data with a dissolution–dialysis system.

As part of the earlier *in vivo* studies (7), the individual tablets also were subjected to dissolution testing as described in USP XVIII. The present dissolution simulator data correlated much better than the earlier USP data for the 50-mg tablets, $r = 0.673$ ($p > 0.10$), although the degree of correlation was approximately the same for the 100-mg tablets using either the dissolution simulator, $r = 0.704$ ($p < 0.10$) or the USP rotating basket, $r = 0.793$ ($p < 0.10$).

Chlorothiazide Tablets—The data obtained for these dosage forms demonstrated one major limitation in the design of the dissolution simulator, namely that the total volume of dissolution fluid was limited to 100 ml. As shown in Fig. 4, this fluid was rapidly saturated with dissolving drug; as a result, dissolution was not continued beyond 5 min. The best correlations for both the 250- and 500-mg tablets were seen between the cumulative percent of drug dissolved in 1 min and the maximum excretion rate (Fig. 9). The data for the 250- and 500-mg tablets were not combined into a single correlation because the *in vivo* data did not exhibit a dose proportionality in urinary excretion, with a lower percent recovery for the 500-mg tablets (8).

The correlation was significant for the 250-mg tablets, $r = 0.999$ ($p < 0.05$), but not for the 500-mg tablets, $r = 0.962$ ($p < 0.10$). Good correlations, $r > 0.9$, existed between the maximum excretion rate or the cumulative percent excreted at 24 hr and $T_{15\%}$ or the percent dissolved at 2.5 min for the 250-mg tablets. However, these correlations were based on only three data points; the general applicability of the relationships need to be established for a larger group of dosage forms.

A good correlation, $r = 0.999$ ($p < 0.05$), also was obtained for the 250-mg tablets between the maximum excretion rate and the time for 30% dissolution using the USP rotating basket. However, for the 500-mg tablets, the correlation was much poorer for these same parameters, $r = 0.773$ ($p > 0.10$). Thus, on the basis of cost and convenience, the USP method is preferred over the dissolution simulator for the 250-mg tablets since both procedures result in comparable *in vitro–in vivo* correlations. However, the correlations for the 500-mg tablets were somewhat better for the dissolution simulator compared to the USP method.

Quadrant Analysis—This approach evaluates *in vivo* data using each subject as his or her own control, relating the bioavailability of a dosage form in a given subject to the performance of a reference dosage form in the same subject. For illustration, an arbitrary specification was selected such that 50% of the subjects participating in an *in vivo* study should exhibit a relative bioavailability of at least 75% for a test dosage form to be considered acceptable. The *in vivo* data were plotted *versus* an *in vitro* measurement for the individual dosage forms, and an *in vitro* dissolution specification was selected. While the *in vitro* specifications described for the three drugs under consideration are admittedly somewhat arbitrary,

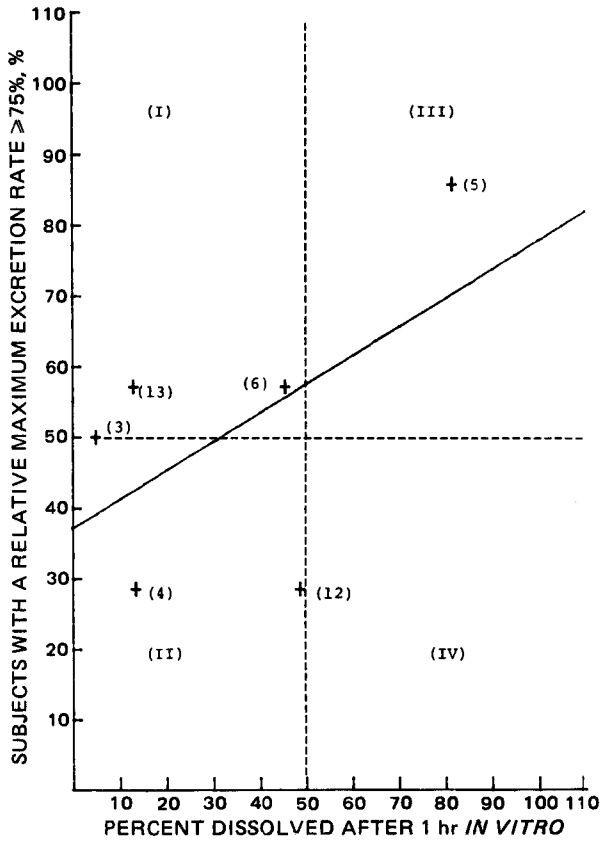

Figure 12—*Quadrant analysis for six 100-mg nitrofurantoin tablet formulations,* r = *0.557 (*p > *0.1). Each data point represents the mean of two* in vitro *and 14* in vivo *values. Product 9 was the reference product.*

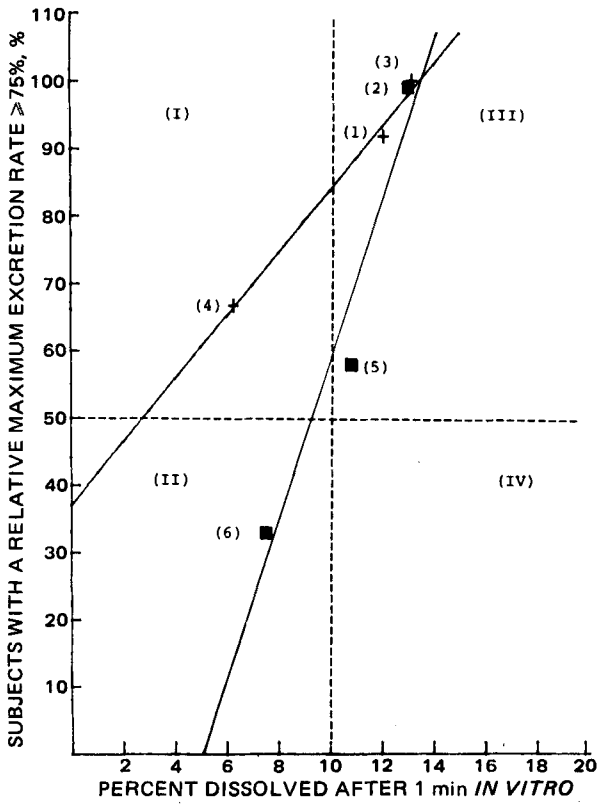

Figure 13—*Quadrant analysis for three 250- (+) and three 500- (■) mg chlorothiazide tablet formulations, r = 0.995 (p < 0.1) and r = 0.967 (p > 0.1), respectively. Each data point represents the mean of two* in vitro *and 12* in vivo *values.*

they serve to illustrate an alternative approach to setting such specifications.

Methenamine—Figure 10 illustrates the results of the quadrant analysis approach for methenamine. The selection of 200 min as the maximum permissible time to achieve 15% dissolution was based on the *in vivo* data, which indicated that Products 5 and 7 were unacceptable. Note that Product 5 does not appear in this figure since it did not achieve 15% dissolution within 6 hr. With the exception of Products 5 and 7, all other dosage forms were confined to Quadrant I and thus exhibited acceptable bioavailability and dissolution properties.

Nitrofurantoin—Figures 11 and 12 show the quadrant analysis for the 50- and 100-mg dosage forms, respectively. With the exception of Products 11 and 14, all other dosage forms represented in Fig. 11 were in Quadrant III and thus met both the *in vivo* and *in vitro* specifications. Using the same specifications for the 100-mg tablets illustrated in Fig. 12 did not yield a satisfactory result. Only Product 5 was in Quadrant III; Products 3, 6, and 13 failed the *in vitro* specification, although each exhibited adequate bioavailability. Furthermore, Product 12 nearly passed the dissolution requirement, although only 30% of the subjects exhibited a maximum urinary excretion rate that was at least 75% that of the reference product.

Chlorothiazide—A dissolution specification of 10% dissolution within 1 min was selected for the 250- and 500-mg tablets shown in Fig. 13. With this criterion, Product 4 exhibited an adequate excretion rate for the 250-mg tablets but failed the dissolution specification. For the 500-mg tablets, Product 6 failed both the *in vitro* and *in vivo* specifications. Because of the small number of dosage forms and the brief duration of the dissolution test, these data may be of limited value in establishing the utility of this approach as a predictor of the bioavailability of chlorothiazide dosage forms.

Study Limitations—All dissolution studies were carried out on production lots used in previous *in vivo* bioavailability studies. Thus, some formulations had been stored at room temperature for up to 4 years, and in some instances the expiration date indicated on the package had been exceeded. Furthermore, only a few tablets or capsules remained for many of the products, and it was not possible to reassay them for content and content uniformity. As a result, drug content and/or dissolution characteristics of these dosage forms may have changed since the earlier studies.

Evidence suggesting that significant changes in the characteristics of the dosage forms had not occurred may be obtained by comparing the extent of dissolution observed in the present studies with that obtained previously. The maximum amount of methenamine recovered during the dissolution studies with the dissolution simulator corresponded with the labeled quantity of drug in the product for those dosage forms that totally dissolved. With nitrofurantoin, a range of 7–80% of the labeled amount of drug was dissolved after 80 min in the dissolution simulator. In the earlier study (7), between 10 and 70% of the drug was dissolved after 90 min using the USP procedure. Furthermore, the two products with the slowest dissolution rate in the dissolution simulator were the same two products that dissolved the slowest in the previous study (7). Similarly, the extent of dissolution of the chlorothiazide tablets in the dissolution simulator was quite similar to the results obtained earlier (8) using the USP apparatus.

CONCLUSIONS

The absorption simulator was evaluated as a tool to determine optimum sampling times for dissolution studies using the dissolution simulator. It was determined that the sampling times computed from the absorption simulator were not essential for the subsequent dissolution studies.

Reasonably good correlations generally were observed between the *in vitro* data obtained with the dissolution simulator and the *in vivo* data obtained in earlier human studies. However, the general applicability of the device to a wide variety of drugs is limited because only two dosage units can be tested simultaneously. Moreover, the 100-ml capacity of the dissolution system is insufficient for drugs with very low water solubility. The dissolution properties of the methenamine, chlorothiazide, and 50-mg nitrofurantoin tablets could all be satisfactorily correlated with one or more *in vivo* parameters. The correlations for the 100-mg nitrofurantoin tablets and the macrocrystalline nitrofurantoin capsule dosage forms were less satisfactory.

Attempts also were made to predict urinary excretion profiles using the *in vitro* data. After application of an adjustment factor, good predictions were seen for the majority of the methenamine dosage forms and seven of the 14 nitrofurantoin formulations. The method was not applicable for the chlorothiazide tablets.

Finally, *in vivo–in vitro* correlations were examined using a quadrant analysis approach. The method was shown to be of value in setting *in vitro* specifications that have at least some relevance to the *in vivo* performance of the dosage form.

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High-Performance Liquid Chromatographic Determination of Free Myxin and Its Reduction Product as Impurities in Cuprimyxin-Containing Creams

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Received November 28, 1980, from the *Quality Control Department, Hoffmann–La Roche Inc., Nutley, NJ 07110.* Accepted for publication February 20, 1981.

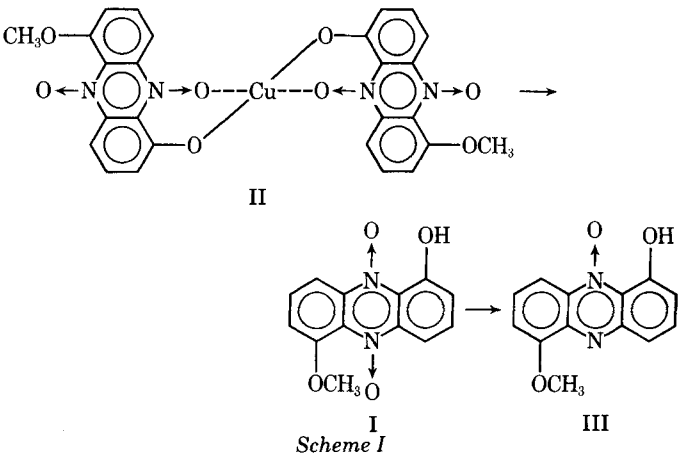
Abstract □ A stability-indicating high-performance liquid chromatographic method was developed that can detect and quantitate low levels of the two most likely breakdown products of cuprimyxin. These degradation compounds, free myxin and its reduction product, can be determined in topical cream preparations in which cuprimyxin is formulated at the 0.5% (w/w) level. The method requires a simple two-step extraction, the addition of an internal standard, and chromatography on an amine-bonded column.

Keyphrases □ Myxin—high-performance liquid chromatographic assay in cuprimyxin-containing creams □ Cuprimyxin—high-performance liquid chromatographic determination of free myxin and its reduction product □ High-performance liquid chromatography—determination of free myxin and its reduction product in cuprimyxin-containing creams □ Antibacterial agents—high-performance liquid chromatographic determination of myxin and its reduction product in cuprimyxin-containing creams

Myxin (6-methoxy-1-phenazinol 5,10-dioxide) (I) was first isolated from the soil (1) and identified as a phenazine-type product from the broth of a myxobacter (2). Its correct structure was established by Weigele and Leimgruber (3), and it was realized in its most effective antimicrobial form as the cupric complex (II) (4, 5). When applied topically, II was found to have considerable antibacterial, antiyeast, and antifungal activity in veterinary applications without the irritation side effects experienced with I (6). It was formulated in a cream and tested *in vitro* against Gram-positive and Gram-negative bacterial and fungal pathogens (7) and yeast infections (8), *in vivo* as a cream for otic and ophthalmic infections (9), and as a suspension with hydrocortisone acetate for the treatment of otitis (10). The apparent biological mechanism of action involves alteration of the invading bacterial DNA template (11).

BACKGROUND

A polarographic investigation found the electrochemical behavior of I to be a function of pH, reducing to 6-methoxy-1-phenazinol at pH < 3, to the anionic species 6-methoxyphenazinol at pH > 9, and to the intermediate 6-methoxy-1-phenazinol 10-oxide (III) at pH 3–9 (12). Compound III can be further reduced by intramolecular hydrogen bonding to 6-methoxy-1-phenazinol. The major degradation product of I in acid media and in pH 3–9 buffer solutions was found to be III[1]. Although the copper complex of II is readily dissociated in acid media to form I (Scheme I), pharmaceutical creams containing excess copper ions minimize dissociation when formulated at pH 5.7–6.2 (13).



Scheme I

The rapid conversion of II to I was used to assay for II by measurement of the amount of I found spectrophotometrically[2] and by TLC (14). Microbiological assay methods for determining II directly from seeded agar plates also were reported[3].

The determination of I and III as probable impurities in formulations involving II is difficult for two reasons: (*a*) the relative ease of conversion of II to its free form (I), and (*b*) the inability to differentiate I and III spectrophotometrically. A separation technique that minimizes dissociation of the copper complex is essential. This paper describes the development of a high-performance liquid chromatographic (HPLC) method that meets these requirements.

EXPERIMENTAL

Apparatus—A constant-flow solvent delivery system[4] was connected to a loop injector[5]. An amine-bonded silica column[6] was coupled to a spectrophotometric detector[7] set at 280 nm. A 10-mv recorder[8] was set at a chart speed of 50 cm/hr.

Reagents and Chemicals—Distilled-in-glass grade ethyl acetate[9] and heptane[9] and reagent grade acetic acid[10] were used. Samples and

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[6] Chromegabond NH_2 (10 μm), 30 cm × 4.6 mm i.d., ES Industries, Marlton, N.J.

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