Enhanced Absorption and Dissolution of Reserpine from Reserpine–Polyvinylpyrrolidone Coprecipitates

ELLIOT I. STUPAK* and THEODORE R. BATES▲

Abstract □ The *in vivo* absorption patterns of pure reserpine, a 1:5 w/w reserpine–polyvinylpyrrolidone physical mixture, and a 1:5 w/w reserpine–polyvinylpyrrolidone coprecipitate were compared by following the cumulative amounts of reserpine equivalents excreted in the urine as a function of time. The results of these urinary excretion studies demonstrated that both the rate and extent of absorption of reserpine in the rat were significantly enhanced following the oral administration of the coprecipitate; the fraction of the oral dose absorbed was three times greater with the 1:5 reserpine–polyvinylpyrrolidone coprecipitate than with either the pure drug or the 1:5 reserpine–polyvinylpyrrolidone physical mixture. The *in vitro* dissolution characteristics of the three test preparations at 37° correlated very well with the *in vivo* absorption data.

Keyphrases □ Reserpine, absorption—from reserpine–polyvinylpyrrolidone coprecipitates, correlation with *in vitro* dissolution rates, rats □ Polyvinylpyrrolidone–reserpine coprecipitates—enhanced reserpine absorption, correlation with *in vitro* dissolution rates, rats □ Coprecipitates, reserpine–polyvinylpyrrolidone—effect on *in vivo* absorption, correlation with *in vitro* dissolution, rats □ Bioavailability—reserpine polyvinylpyrrolidone coprecipitates, *in vivo* absorption correlated with *in vitro* dissolution, rats

Several investigations (1–15) demonstrated that the formation of solid dispersions or coprecipitates of relatively water-insoluble drugs with various pharmacologically inert carriers can increase significantly their *in vitro* dissolution rates. However, the use of polymeric materials, especially polyvinylpyrrolidone, as carriers in coprecipitate systems has received limited attention in the literature (7, 8, 13–15).

In theory, an enhancement in the dissolution rate of a drug should facilitate its GI absorption rate if the absorption process is dissolution rate limited. Although the *in vitro* dissolution properties of some coprecipitate systems have been characterized in some detail, the literature is almost devoid of investigations designed to assess the *in vivo* absorption characteristics of these systems. The need for such *in vivo* studies is well recognized, since an enhancement of the *in vitro* dissolution rate of a hydrophobic drug from a solid dispersion may not necessarily lead to similar increases in the *in vivo* rate and/or extent of drug absorption.

In a recently published report from this laboratory (14), polyvinylpyrrolidone, a water-soluble polymer, in the form of a coprecipitate with reserpine was shown to enhance markedly the rate of solution of this water-insoluble drug. On the basis of these *in vitro* findings, it was decided to characterize and compare the *in vivo* absorption patterns of reserpine, a 1:5 w/w reserpine–polyvinylpyrrolidone physical mixture, and a 1:5 w/w reserpine–polyvinylpyrrolidone coprecipitate system in the rat. It was anticipated that the results from such a study would provide the type of information necessary to establish the usefulness of polyvinylpyrrolidone solid dispersions in promoting the rate and/or extent of absorption of reserpine and, possibly, other hydrophobic drugs.

EXPERIMENTAL

Materials—The reserpine[1] and polyvinylpyrrolidone[2] employed in this study were pharmaceutical grade. The polyvinylpyrrolidone used had an average molecular weight of 40,000. Reagent grade *p*-toluene sulfonic acid[3], sodium bicarbonate, sodium carbonate, citric acid, trisodium citrate, acetone, chloroform, and glacial acetic acid were used as received.

Test Preparations—The 1:5 w/w reserpine–polyvinylpyrrolidone coprecipitate system was prepared by dissolving both components in chloroform and subsequently evaporating off the organic solvent *in vacuo*. The residue was then dried to constant weight *in vacuo* and screened, and the reserpine–polyvinylpyrrolidone weight ratio was analytically confirmed (14). The 1:5 w/w physical mixture was prepared by gently triturating appropriate quantities of reserpine (6–30-μ crystals) and polyvinylpyrrolidone in a glass mortar. Pure reserpine, possessing a particle-size range of 6–30 μ, served as the third test preparation.

***In Vivo* Absorption Rate Studies**—In all *in vivo* experiments, adult, male, Sprague-Dawley[4] rats, weighing 300–400 g., were used as the test animals. The rats were fasted (with water allowed *ad libitum*) for 20–24 hr. prior to initiation of the absorption experiments. At the time of dosing, the fasted rats were lightly anesthetized with ether; a 0.25% methylcellulose[5] suspension of the test preparation, at a reserpine dosage level of 25 mg./kg. of body weight, was administered directly into the stomach by oral intubation. In a limited number of experiments involving only pure reserpine, the dosage level was varied from 25 to 100 mg./kg. The dosing volume employed was 3.0 ml./kg. The animals were then placed into restraining cages and urine specimens were collected at 2, 4, 8, 12, 24, and 48 hr. The volume and pH of each specimen were recorded and an aliquot was frozen until assayed in duplicate (see *Assay Procedure*). The total cumulative amount of reserpine equivalents excreted in the urine at each collection period was calculated and expressed on the basis of percent of dose administered in order to correct the data for animal weight variations. Each animal received all three test preparations in a random crossover fashion, allowing at least a 1-week interval between preparations.

To obtain an appreciation for the magnitude of blank urine values, seven animals were dosed with either the methylcellulose suspension vehicle alone or in combination with an appropriate quantity of polyvinylpyrrolidone. Urine specimens were collected at 24 and 48 hr. and treated in the same manner as drug-containing urine specimens. The mean blank values ($\pm SE$) thus obtained were quite small (*i.e.*, 12.9 ± 0.7 mcg./24 hr. and 29.7 ± 2.9 mcg./48 hr.). However, the blank value for each animal was used in correcting all of its urinary excretion data.

Assay Procedure for Reserpine and Its Metabolites in Urine—The fluorometric procedure of Jakovljevic *et al.* (16) for the determination of reserpine in pure aqueous media and pharmaceutical

[1] Supplied by Ciba Pharmaceutical Co., Summit, NJ 07901
[2] Plasdone-C (K-30); supplied by the General Aniline and Film Corp., New York, NY 10020
[3] Eastman Organic Chemicals, Rochester, N. Y.
[4] Blue Spruce Farms, Altamont, NY 12009
[5] Methocel 60-HG (4000 cps.); Dow Chemical Co., Midland, Mich.

dosage forms was appropriately modified to make it suitable for biological fluids (*e.g.*, urine and plasma). The modified method involves the separation of reserpine and its metabolites (reserpic acid and methyl reserpate) from the urine specimens *via* a two-step solvent extraction procedure and subsequent quantitative determination.

One milliliter of urine was shaken with 4.0 ml. of a protein precipitant solution composed of a 1:1 v/v ratio of acetone and 1.2% acetic acid, and the mixture was centrifuged. A 2.0-ml. aliquot of the clear supernate was withdrawn and mixed with 0.5 ml. of a 1 *M* citrate buffer (pH 4.0), and the mixture was extracted with 15.0 ml. of chloroform. The phases were separated *via* centrifugation, a 1.0-ml. aliquot of the aqueous phase was withdrawn for use in the second extraction step, and the chloroform phase was retained for the fluorometric determination of reserpine and reserpic acid. For the second extraction step, which removes methyl reserpate, the 1.0-ml. aqueous aliquot from the first extraction was mixed with 0.6 ml. of a pH 9.0 carbonate buffer (equal volumes of saturated solutions of sodium carbonate and sodium bicarbonate) and 1.5 ml. of water and subsequently extracted with 15.0 ml. of chloroform. The mixture was centrifuged to separate the phases, the aqueous phase was aspirated off, and the chloroform phase was retained for fluorescence development.

The fluorescence of the chloroform phase obtained from each of the two extraction steps was developed by heating an appropriately sealed test tube containing 2.0 ml. of the chloroform phase, 3.0 ml. of glacial acetic acid, and 5.0 ml. of a 1% w/v solution of *p*-toluenesulfonic acid in glacial acetic acid in a boiling water bath for exactly 10 min. Subsequently, each tube was cooled rapidly in an ice bath to room temperature and the fluorescence of the sample was measured on a fluorometer[6], using a number 7-60 primary filter (360-400 nm.) and a combination of a number 8 secondary filter (480 nm. and above) and a number 2 density filter (1%).

The fluorometer was set at a sensitivity of 10× for all fluorometric determinations. Reserpine and its two major metabolites, methyl reserpate and reserpic acid, strongly fluoresce when treated in this manner. However, trimethoxybenzoic acid, which results from the biotransformation of reserpine to methyl reserpate, does not fluoresce under these assay conditions.

On each assay day, standard solutions of reserpine over the concentration range of 0.02-0.06 mcg./ml. of final reading solution were simultaneously assayed with unknown urine samples, and a linear calibration curve was constructed. Recoveries of 95-100% were obtained upon adding reserpine to blank urine.

When necessary, the sensitivity of the fluorescence readings were increased by withdrawing a larger aliquot of the chloroform phase from either extraction step, evaporating the solution to dryness at room temperature under reduced pressure, and redissolving the residue in 2.0 ml. of chloroform.

***In Vitro* Dissolution Rate Studies**—The dissolution apparatus consisted of a 500-ml. three-necked round-bottom flask, containing 350 ml. of 0.005 *M* acetic acid (pH 3.65) and 0.005% polysorbate 80. The solution was maintained at 37 ± 0.1° and agitated at 60 r.p.m. by means of a Teflon stir blade (70 mm. diameter) connected to a constant-speed stirring mechanism[7]. Due to the extremely low aqueous solubility of reserpine, it was necessary to employ the dilute acetic acid solution to maintain sink conditions. Polysorbate 80 was added to reduce the interfacial tension between reserpine particles and the dissolution medium. The concentration of surfactant employed did not affect the solubility of reserpine in the acidic medium.

At frequent time intervals subsequent to the introduction of a quantity of test preparation equivalent to 10 mg. of reserpine into the dissolution medium, a 5.0-ml. sample was removed with the aid of a filter pipet and replaced with 5.0 ml. of fresh dissolution medium. The amount of reserpine in solution at each time interval, appropriately corrected for this dilution effect (17), was determined spectrophotometrically at 268 nm. using a recording spectrophotometer[8]. Polyvinylpyrrolidone, in the concentrations present in the assay samples, was found not to interfere with the determination of reserpine.

[6] Turner fluorometer, model 111 (G. K. Turner Associates, Palo Alto, CA 94303).

[7] Servodyne Laboratory Stirrer, Cole-Parmer Instrument Co., Chicago, IL 60648

[8] Beckman DB-G, Beckman Instruments Inc., Fullerton, CA 92634

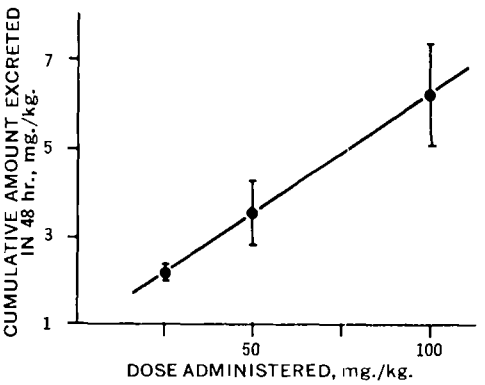

Figure 1—*Relationship between the initial oral dose of reserpine and the cumulative amount of reserpine equivalents excreted in the urine after 48 hr. Each point represents the mean of seven animals. Bars denote standard errors of means.*

All dissolution rate experiments were performed in triplicate.

RESULTS AND DISCUSSION

***In Vivo* Absorption Studies**—A preliminary study was conducted to ascertain whether the absorption or metabolism of reserpine was saturable in the dose range employed. For this purpose, suspensions containing 25, 50, and 100 mg./kg. of reserpine were orally administered to the seven test animals, and the cumulative amount (expressed as milligrams per kilogram of body weight) of reserpine equivalents excreted in the urine in 48 hr. was determined. The mean data, plotted on rectilinear coordinates, are shown in Fig. 1. The excellent linearity of the resultant least-squares plot suggests that, over the dose range examined, the GI absorption and elimination of reserpine are not dose dependent. The fraction of the total amount of reserpine equivalents excreted as methyl reserpate in 48 hr. was calculated, and the mean values (±*SE*) were found to be 0.867 (±0.010), 0.882 (±0.014), and 0.881 (±0.005) for the 25-, 50-, and 100-mg./kg. doses, respectively. The constancy of these values is a good indication that the metabolism of reserpine is not capable of being saturated over this dose range.

The cumulative amounts (expressed as percent of dose) of reserpine equivalents excreted in the urine as a function of time following the oral administration of reserpine alone and as a 1:5 w/w physical mixture and coprecipitate with polyvinylpyrrolidone are

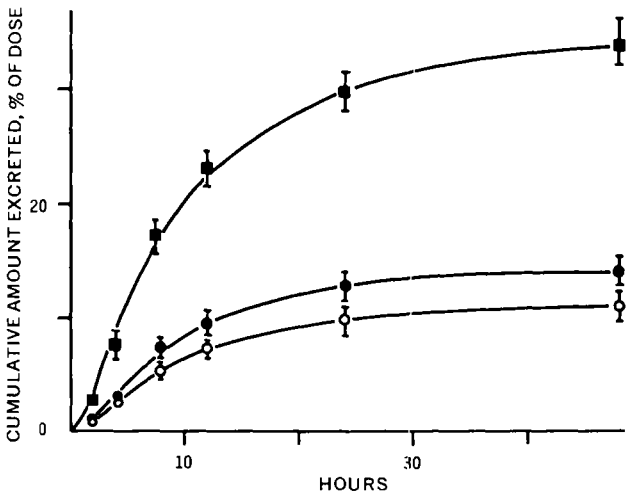

Figure 2—*Cumulative amount of reserpine equivalents excreted following oral administration of reserpine (○), a 1:5 w/w reserpine-polyvinylpyrrolidone physical mixture (●), and a 1:5 w/w reserpine-polyvinylpyrrolidone coprecipitate (■). Each point represents the mean of seven animals. Bars denote standard errors of means.*

Table I—Effect of Coprecipitation with Polyvinylpyrrolidone on Cumulative Amount of Reserpine Equivalents Excreted in 4 hr. (Percent of Dose Administered)

Rat Number	Reserpine	1:5 w/w Reserpine–Polyvinylpyrrolidone Physical Mixture	1:5 w/w Reserpine–Polyvinylpyrrolidone Coprecipitate
1	4.1	2.7	8.1
2	2.0	3.5	10.7
3	3.8	4.0	12.1
4	1.6	3.4	7.4
5	3.3	3.5	3.8
6	1.5	1.6	8.5
7	3.8	4.2	4.6
Mean	2.9 (0.4)[a]	3.3 (0.3)	7.9 (1.1)
	N.S.	$p < 0.01$	
	$p < 0.01$		

[a] Standard error of the mean in parentheses.

shown in Fig. 2. Each point represents the mean value for seven animals. It is apparent from these curves that, at every time period, a significantly greater amount of reserpine was absorbed into the body from the coprecipitate than from either of the other test preparations.

Two time periods were selected to illustrate the effect of coprecipitating reserpine with polyvinylpyrrolidone on the rate and the extent of drug absorption. The cumulative 4-hr. urinary excretion data were used to reflect the effect on the initial absorption rate, while the 48-hr. time period was used as a measure of the extent of reserpine absorption. Tables I and II list the percent of dose excreted by each animal for each test preparation at 4 and 48 hr., respectively. Also recorded in these tables are the mean and standard error of the mean (*SE*) for each test preparation and the results of the *t* tests for differences between paired samples. The mean values at the 4-hr. time period (Table I) indicate that the cumulative amount excreted and, hence, the initial absorption rate of reserpine are enhanced two- to threefold when administered in the form of a coprecipitate with polyvinylpyrrolidone as compared to either of the other test systems. The difference between the coprecipitate and either of the other two test preparations is significant at the 1% level, while the difference between the means of the pure drug and physical mixture is not statistically significant.

Similarly, inspection of the 48-hr. urinary excretion data (Table II) reveals that two to three times as much drug is excreted from the coprecipitate than from either the physical mixture or the pure drug. Thus, following the oral administration of the coprecipitate, the apparent bioavailability of reserpine is increased almost threefold as compared to either of the other two test preparations, the difference being highly significant ($p < 0.001$). The difference between the physical mixture and the pure drug is, once again, not

Table II—Effect of Coprecipitation with Polyvinylpyrrolidone on the Cumulative Amount of Reserpine Equivalents Excreted in 48 hr. (Percent of Dose Administered)

Rat Number	Reserpine	1:5 w/w Reserpine–Polyvinylpyrrolidone Physical Mixture	1:5 w/w Reserpine–Polyvinylpyrrolidone Coprecipitate
1	12.7	10.9	36.6
2	13.6	15.4	39.1
3	11.4	13.4	36.3
4	5.9	17.0	33.8
5	12.8	10.1	37.2
6	6.1	17.8	26.7
7	12.2	13.7	26.4
Mean	10.7 (1.2)[a]	14.0 (1.1)	33.7 (1.9)
	N.S.	$p < 0.001$	
	$p < 0.001$		

[a] Standard error of the mean in parentheses.

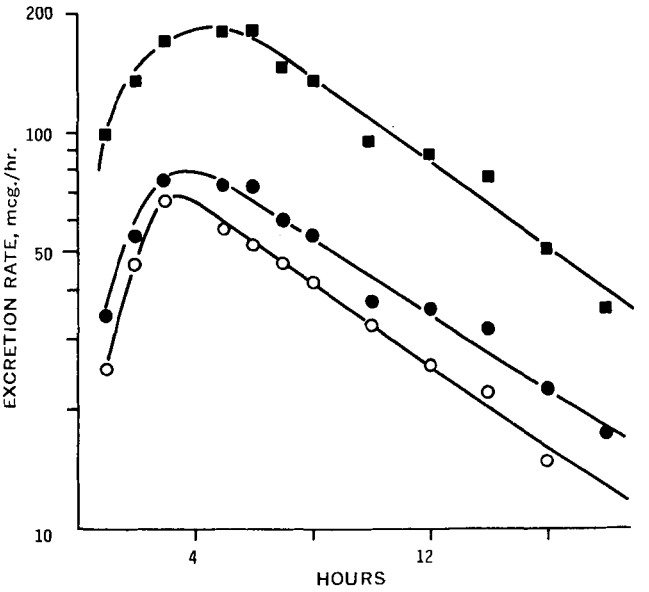

Figure 3—*Urinary excretion rate of methyl reserpate after oral administration of reserpine* (○), *a 1:5 w/w reserpine–polyvinylpyrrolidone physical mixture* (●), *and a 1:5 w/w reserpine–polyvinylpyrrolidone coprecipitate* (■). *Each point represents the mean of seven animals. Data are corrected for animal weight differences.*

significant. The data suggest that it would take only one-third of the dose of reserpine in the form of a 1:5 w/w coprecipitate with polyvinylpyrrolidone to obtain the same extent of absorption as that observed with the pure drug. This can be further exemplified by the curves shown in Fig. 3, which represent plots of the logarithm of the mean urinary excretion rate (expressed as micrograms per hour) of methyl reserpate *versus* time for the three test preparations. The resulting curves are analogous to those which one would expect from increasing doses of the same drug. That is, there are significant peak height differences, but the peak excretion rate occurs at approximately the same time and the mean half-lives (±*SE*) obtained from the terminal portion of the three curves are identical statistically, being 6.14 (±0.33), 5.90 (±0.38), and 6.03 (±0.47) hr. for the pure drug, physical mixture, and coprecipitate,

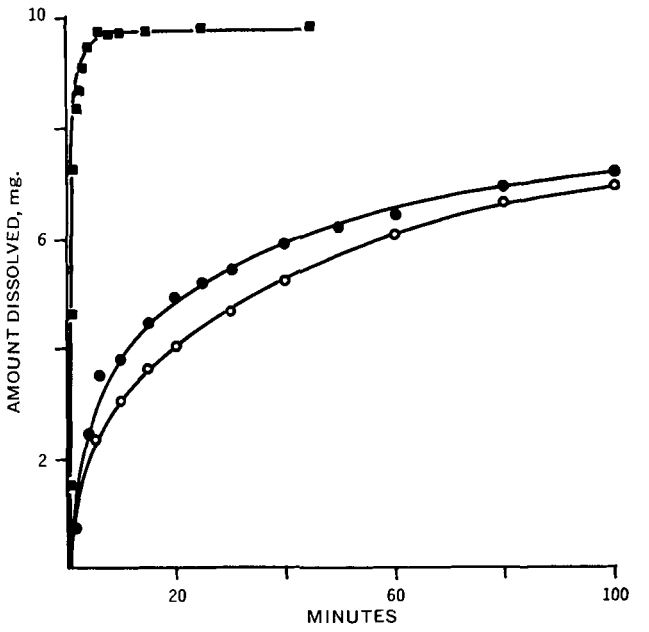

Figure 4—*Dissolution rates of reserpine from test preparations at 37°. Key:* ○, *pure reserpine;* ●, *1:5 w/w physical mixture; and* ■, *1:5 w/w coprecipitate.*

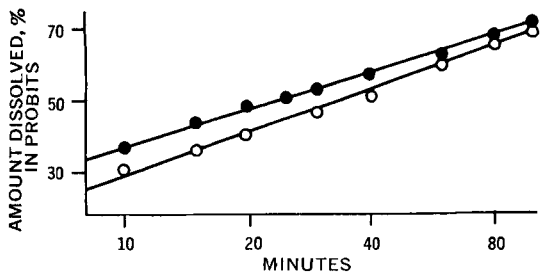

Figure 5—*Log-normal probability plots of dissolution data from reserpine test preparations at 37°. Key: ○, pure reserpine; and ●, 1:5 w/w physical mixture.*

respectively. The times of occurrence of peak urinary excretion rates and the half-life values are quite similar to those obtained by Glazko *et al.* (18) from blood level studies with orally dosed rats. It is apparent that the urinary excretion rate constant of the major metabolite of reserpine in the rat is much larger than the elimination rate constant of the parent drug and, therefore, can be used to reflect the blood level of its precursor (reserpine).

The fractions of the total amount of reserpine equivalents excreted as methyl reserpate in 48 hr. ($\pm SE$) were determined to be 0.836 (±0.011) for pure reserpine, 0.864 (±0.007) for the physical mixture, and 0.870 (±0.004) for the coprecipitate system. The near equality of these mean values indicates that the metabolism of reserpine is not affected by the presence of polyvinylpyrrolidone in either the coprecipitate or the physical mixture.

***In Vitro* Dissolution Rate Studies**—Based on the *in vivo* absorption data, the potentiation noted in both the rate and the extent of absorption of reserpine from the coprecipitate system cannot be attributed to any biological or physiological changes induced by the presence of polyvinylpyrrolidone but rather to some physicochemical process occurring within the GI fluids. Therefore, particulate dissolution rate experiments were undertaken to establish whether a correlation existed between the *in vitro* dissolution rates and *in vivo* absorption characteristics of reserpine from the test preparations. The dissolution rate profiles for the three test samples, under sink conditions, are shown in Fig. 4. Each point represents the average of three determinations, with each of the three runs falling within a 5% range of the mean. It is evident from the plot that, at each time interval, marked differences exist between the amount of reserpine in solution from the coprecipitate and the other two test preparations. To demonstrate quantitatively these differences, the dissolution half-live for each test preparation was determined by the log-probit method suggested by Wagner (19). Log-probit plots of the dissolution data for the three test preparations are shown in Figs. 5 and 6. The half-lives as determined from these plots are 34 min. for the pure drug, 24 min. for the physical mixture, and *only* 1 min. for the coprecipitate system.

In Vitro–In Vivo Correlation—A linear correlation was found to exist between the amount of reserpine dissolved in 10 min. and the

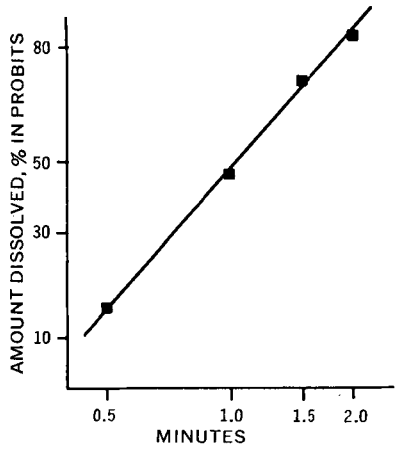

Figure 6—*Log-normal probability plot of dissolution data from 1:5 w/w reserpine–polyvinylpyrrolidone coprecipitate at 37°.*

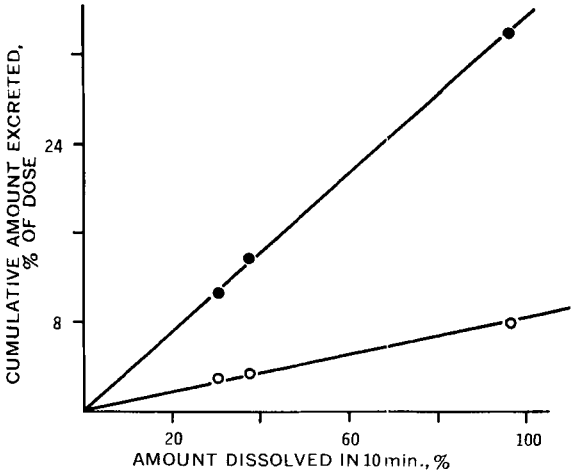

Figure 7—*Correlation between percent reserpine in solution after 10 min. and the mean cumulative percent of reserpine equivalents excreted in the urine 4 hr. (○) and 48 hr. (●) following oral administration of the three test preparations.*

cumulative amount of reserpine equivalents excreted at all experimental time periods. Representative correlation plots using the 4- and 48-hr. urinary excretion data are shown in Fig. 7. Both lines were drawn by the method of least squares, and the corresponding equations for each line are given by Eqs. 1 and 2:

$$\text{4-hr. } \textit{in vivo} \text{ data:} \quad y = 0.080x + 0.205 \quad \text{(Eq. 1)}$$

$$\text{48-hr. } \textit{in vivo} \text{ data:} \quad y = 0.347x + 0.327 \quad \text{(Eq. 2)}$$

In both cases, the correlation coefficients are greater than 0.99. The excellent *in vitro–in vivo* correlation strongly suggests that the dissolution rate is the controlling factor in the amount of reserpine absorbed from the GI tract and subsequently excreted.

The present investigation clearly established that coprecipitation of reserpine with polyvinylpyrrolidone can markedly enhance the absorption characteristics of this hydrophobic drug and that these *in vivo* increases can be quantitatively correlated with *in vitro* dissolution rates.

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Absorption and Excretion of Riboflavin in the Rat: An Unusual Example of Nonlinear Pharmacokinetics

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Abstract □ An estimation of the availability of riboflavin after oral administration of riboflavin-5′-phosphate is greatly complicated in the rat because of the occurrence of a complex and marked nonlinear, dose-dependent excretion of the vitamin. The total urinary recovery of riboflavin after intraperitoneal administration of riboflavin-5′-phosphate was found to be highly dose dependent in both normal and vitamin-deficient rats as well as in deficient rats with ligated bile ducts. The elimination of the vitamin involves at least two nonlinear processes occurring simultaneously and having opposite effects on the dose–total urinary recovery relationship. One process involves biliary excretion which increases disproportionately with increasing body levels of riboflavin. The other process appears to be a binding of the vitamin to tissues which function kinetically as deep compartments. Apparently, the higher the body level of the vitamin, the smaller is the fraction that can be "immobilized" in the compartments and the larger is the fraction that can be detected in the urine. Over the dose range studied, the nonlinear tissue binding phenomenon is predominant over the nonlinear biliary excretion in the riboflavin-deficient rat. The converse is true in the normal, nondeficient animal.

Keyphrases □ Riboflavin—absorption and excretion after oral administration of riboflavin-5′-phosphate, nonlinear pharmacokinetics, rats □ Pharmacokinetics, nonlinear—absorption, excretion of riboflavin, rats □ Absorption, riboflavin—nonlinear pharmacokinetics, rats □ Excretion, riboflavin—nonlinear pharmacokinetics, rats

The extensive studies of Levy and Jusko (1, 2) with riboflavin revealed that the GI absorption of this vitamin in man deviates markedly from the classical principles of passive transport. For example, the urinary recovery of riboflavin as a function of dose after oral administration of riboflavin or riboflavin-5′-phosphate to fasted healthy humans shows that the absorption process is capacity limited and easily saturable. Urinary excretion data further suggest that the absorption of the vitamin is limited to the proximal small intestine. When the vitamin is administered with food, absorption of riboflavin is enhanced and saturation of intestinal absorption is evident only with doses exceeding 30 mg. The site specificity and capacity-limited characteristics of riboflavin absorption suggest that the vitamin is absorbed by a specialized transport process rather than by passive diffusion. More recently, Jusko *et al.* (3) suggested that bile may play a role in the absorption of riboflavin. After oral administration of a saturation dose of the vitamin (150 mg./m.^{2}) to children with biliary obstruction, the absorption of riboflavin was significantly impaired as compared to that observed in normal children of similar ages. Surgical correction of biliary atresia in two patients led to increased absorption of the vitamin.

The characteristics of riboflavin absorption in the rat appear to be quite different than those observed in man. For example, although riboflavin seems to be absorbed by a specialized transport process in man, Spencer and Zamcheck (4) observed that the isolated rat intestine fails to transport riboflavin against a concentration gradient. Studies in this laboratory indicate that transfer of riboflavin across the rat everted intestine is unaffected by classic inhibitors such as cyanide and dinitrophenol. More importantly, the marked capacity-limited absorption of riboflavin observed in man has not been seen in intact rats even at doses (on a weight basis) considerably larger than those employed in man. After oral administration of up to 8-

Table I—Blank Values Expressed in Terms of Apparent Riboflavin in Rat Urine Collected under Different Experimental Conditions

Experimental Conditions				
Number of Animals	Nutritional Status	Dietary State	Bile Duct	Amount Excreted, mcg.[a]
5	Riboflavin deficient	Fasted	Intact	11 ± 2
8	Riboflavin deficient	Fasted	Ligated	10 ± 2
5	Riboflavin deficient	Fed	Intact	15 ± 2
5	Normal	Fasted	Intact	69 ± 12
5	Normal	Fed	Intact	151 ± 36

[a] Mean amount of apparent riboflavin ± *SD* of the mean, in rat urine collected over a 72-hr. period.