alanine (XIII, 5%). A chemical method was developed for the separation of the amino acid VIII from the phenolic products present in the hydrolysis mixture.

2. Under basic conditions, hydrolysis of the acetamidomalonate ester IX gave low yields (5–7%) of the required amino acid VIII. However, no phenolic side-products were formed under this condition.

3. Synthesis of the formamidomalonate ester XVI and its hydrolysis under basic conditions were carried out in an effort to realize higher yields of trimethoxyphenylalanine (VIII). The hydrolysis of the ester XVI also gave unsatisfactory yields of the target amino acid VIII.

4. An efficient alternative synthesis of trimethoxyphenylalanine (VIII) was achieved which involves the formation of the hydantoin derivative XXIII and its hydrolysis under basic conditions (66% yield).

5. GC–mass spectrometric examination of the peyote amino acid fraction gave no evidence for the presence of trioxygenated phenylalanine analogs, VIII and XI–XIII. This observation suggests that the carboxyl group may not survive beyond the stage of dopa during the biosynthesis of the trioxygenated peyote alkaloids.

REFERENCES

(1) G. J. Kapadia, G. S. Rao, M. H. Hussain, and B. K. Chowdhury, *J. Heterocycl. Chem.*, **10**, 135(1973).

(2) G. J. Kapadia, M. H. Hussain, and G. S. Rao, *J. Pharm. Sci.*, **61**, 1172(1972).

(3) G. J. Kapadia, G. S. Rao, E. Leete, M. B. E. Fayez, Y. N. Vaishnav, and H. M. Fales, *J. Amer. Chem. Soc.*, **92**, 6943(1970).

(4) G. J. Kapadia and R. J. Highet, *J. Pharm. Sci.*, **57**, 191 (1968).

(5) G. J. Kapadia and N. J. Shah, *Lloydia*, **30**, 287(1967).

(6) R. M. Acheson, D. P. Dearnaley, A. O. Plunkett, and V. C. Porter, *J. Chem. Soc.*, **1963**, 2085.

(7) R. J. Highet and P. F. Highet, *J. Org. Chem.*, **30**, 902(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 23, 1973, from the *Department of Biomedicinal Chemistry, College of Pharmacy and Pharmacal Sciences, Howard University, Washington, DC 20001*

Accepted for publication July 18, 1973.

Presented in part to the International Congress of Pharmaceutical Sciences, Washington, D. C., September 1971.

Supported by the National Institute of Mental Health, Grant MH 15573.

The authors are grateful to Dr. H. M. Fales for helpful discussions.

* Present address: Laboratory of Chemical Pharmacology, National Heart and Lung Institute, National Institutes of Health, Bethesda, MD 20014

▲ To whom inquiries should be directed.

Enhanced Absorption of Digitoxin from Orally Administered Digitoxin–Polyvinylpyrrolidone Coprecipitates

ELLIOT I. STUPAK* and THEODORE R. BATES▲

Abstract □ The general applicability of the polyvinylpyrrolidone coprecipitation technique as a method for enhancing the GI absorption of orally administered hydrophobic drugs was explored with the cardiac glycoside digitoxin. The relative absorption characteristics of digitoxin alone and as a 1:9 (w/w) physical mixture and coprecipitate with polyvinylpyrrolidone were determined indirectly by measuring their oral LD_{50} values in rats. The *in vivo* data obtained provided evidence that digitoxin was absorbed from the coprecipitate at a significantly faster rate and was present in the body at a much higher level than when equivalent doses of either the drug alone or as a physical mixture with polyvinylpyrrolidone were orally administered. For example, one must orally administer approximately 11 times as much pure drug to reach the same amount of drug in the body as that attained following the administration of the drug as a coprecipitate. A correlation was found between the *in vitro* dissolution rates of these test systems at 37° and their *in vivo* toxicities.

Keyphrases □ Digitoxin absorption—enhanced using orally administered coprecipitate with polyvinylpyrrolidone, compared to separate drug and physical mixture □ Polyvinylpyrrolidone coprecipitate with digitoxin—enhanced *in vivo* absorption, compared to separate drug and physical mixture □ Coprecipitates, digitoxin–polyvinylpyrrolidone—enhanced *in vivo* drug absorption □ Drug absorption, digitoxin—enhanced using orally administered polyvinylpyrrolidone coprecipitate

Among the techniques that can potentially enhance the dissolution rate and, hence, the rate and/or extent of absorption of hydrophobic drugs is the formation of coprecipitates with pharmacologically inert, polymeric materials such as polyvinylpyrrolidone. This physicochemical drug modification offers the advantage of possibly enabling one to administer the drug orally in a form from which it is most available for GI absorption. Although several investigations (1–6) have demonstrated, *in vitro*, that the solubility and/or dissolution rates of drugs can be increased in this manner, little information is available in the literature related to the *in vivo* absorption pattern of drugs orally administered as polyvinylpyrrolidone coprecipitates. Recently, however, it was demonstrated (6) that both the rate and extent of absorption of the water-insoluble drug reserpine could be markedly enhanced when orally administered to rats in the form of a 1:5 (w/w) coprecipitate with polyvinylpyrrolidone.

The purpose of the present investigation was to ascertain, *in vivo*, the general applicability of the polyvinylpyrrolidone coprecipitation technique. To accomplish this aim, the absorption characteristics of digitoxin, a 1:9 (w/w) digitoxin–polyvinylpyrrolidone physical mix-

Table I—Relationship between the Oral Dose and Lethality of Digitoxin Alone and as a 1:9 (w/w) Physical Mixture with Polyvinylpyrrolidone

Test System	Dose of Drug[a], mg./kg.	Number of Animals Dosed	Number of Animals Dead[b]	Mortality, %
Digitoxin	60	15	5	33.3
	85	15	7	46.7
	110	15	10	66.7
	185	15	12	80.0
Digitoxin–polyvinylpyrrolidone physical mixture, 1:9 (w/w)	50	15	2	13.3
	75	15	4	26.7

[a] Dose administered as a suspension in 0.50% (w/v) methylcellulose at a constant dosing volume of 5.0 ml./kg. [b] Animals observed for 72 hr. postadministration.

ture, and a 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate were quantitatively studied by comparing their relative toxicities in the rat. The solubility and dissolution behavior of these systems were also examined.

EXPERIMENTAL

Materials—The digitoxin[1] and polyvinylpyrrolidone[2] employed in this study were pharmaceutical grade. The polyvinylpyrrolidone had an average molecular weight of 40,000. All other chemicals used were reagent grade and were used as received.

Preparation of Test Systems—The 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate and physical mixture were prepared in the manner previously described (6).

Unless otherwise specified, the gross particle size of the test systems was 297–420 μ for the 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate and 10–40 μ for the pure digitoxin and the drug contained in the 1:9 (w/w) physical mixture.

Protocol for *In Vivo* Absorption Studies—The GI absorption characteristics of digitoxin were assessed by comparing the relative oral toxicity of the drug from suspensions containing the pure drug and the 1:9 (w/w) drug–polyvinylpyrrolidone physical mixture and coprecipitate. Adult, male, Sprague–Dawley rats[3], weighing 150–250 g., were used as the test animals. The animals were fasted for 20–24 hr. prior to the oral administration of each test system as a suspension in a 0.50% (w/v) methylcellulose[4] aqueous vehicle. The dosing volume was held constant at 5.0 ml./kg. Fifteen rats were dosed with 60, 85, 110, or 185 mg./kg. of pure drug or an amount of the 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate containing 6, 7.5, 9, or 10 mg./kg. of digitoxin. The 1:9 drug–polyvinylpyrrolidone physical mixture was orally administered at drug dosage levels of 50 and 75 mg./kg.

The animals were housed in cages with wide, wire-mesh floors to prevent coprophagy and were observed for 72 hr. postdrug administration. At the end of this period, the total number of deaths was recorded. Throughout the entire experimental period, the animals were allowed free access to water; 8 hr. postdrug administration, the animals were placed back on food. The toxicity data were used to calculate the oral LD_{50} value for the test systems according to the method of Litchfield and Wilcoxon (7).

Protocol for *In Vitro* Dissolution Rate Studies—The stirrer-flask method (6) was used to assess the dissolution characteristics of the test preparations at 37°. The dissolution medium consisted of 350 ml. of a Clark–Lubs pH 7.4 phosphate buffer containing 0.005% (w/v) polysorbate 80 USP, and it was agitated at 150 r.p.m. At frequent intervals, subsequent to the introduction of a quantity of test preparation equivalent to 20 mg. of digitoxin, 3-ml. samples were removed from the flask with a filter pipet and immediately replaced with 3 ml. of fresh dissolution medium. The samples were assayed colorimetrically at 532 nm. for digitoxin content (8). The presence of polymer or polysorbate 80 in the samples did not interfere with the quantitative determination of digitoxin. All particulate dissolution rate experiments were performed in duplicate.

Table II—Relationship between the Oral Dose and Lethality of Digitoxin from a 1:9 (w/w) Coprecipitate with Polyvinylpyrrolidone

Dose of Drug[a], mg./kg.	Number of Animals Dosed	Number of Animals Dead[b]	Mortality, %
6.0	15	2	13.3
7.5	15	7	46.7
9.0	15	11	73.3
10.0	15	13	86.7

[a] Dose administered as a suspension in 0.50% (w/v) methylcellulose at a constant dosing volume of 5 ml./kg. [b] Animals observed for 72 hr. postadministration.

Equilibrium Solubility Determinations—The equilibrium solubility of digitoxin was determined at 37° in water, pH 7.4 phosphate buffer, and pH 7.4 phosphate buffer containing 1.0% (w/v) polyvinylpyrrolidone. Each system also contained 0.005% (w/v) polysorbate 80. Excess quantities of digitoxin were placed into 50-ml. glass-stoppered flasks together with 25.0-ml. portions of the solvent systems listed. All flasks were closed securely and mechanically shaken at 37° until equilibrium was attained. Equilibrium was established by repetitive sampling and was found to occur within 12–24 hr. The equilibrated samples were subjected to filtration[5] (0.45-μ pore size) at 37°, the filtrates were suitably diluted when necessary, and the concentration of drug in solution was determined colorimetrically by the procedure of Mesnard and Devaux (8).

RESULTS

Oral Toxicity Studies—The results of the oral toxicity experiments (Tables I and II) were used to construct the log–probability plots shown in Figs. 1 and 2. Based on these linear plots, the oral LD_{50} values for the drug alone and the 1:9 drug–polyvinylpyrrolidone coprecipitate and their corresponding 95% confidence intervals were calculated and found to be 88 (67.7–114) and 7.80 (7.03–8.66) mg./kg., respectively. An examination of these oral LD_{50} values indicates that one must orally administer approximately 11 times as much pure drug to reach the same amount of drug in the body as that attained following the administration of the drug as a coprecipitate. In addition, most of the rats given toxic doses of the digitoxin–polyvinylpyrrolidone coprecipitate usually developed characteristic neuromuscular signs and died at a much earlier time

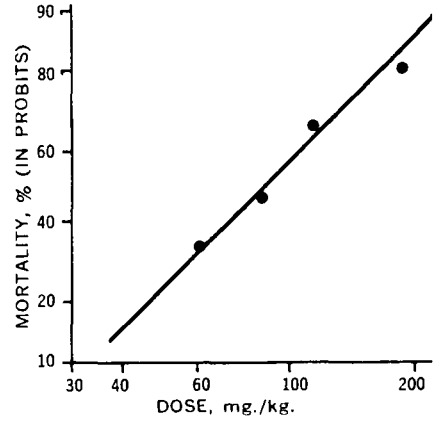

Figure 1—*Relationship between the oral dose and lethality of digitoxin.*

[1] Supplied by Wyeth Laboratories, Philadelphia, Pa.
[2] Plasdone-C (K30), supplied by General Aniline and Film Corp., New York, NY 10020
[3] Obtained from Blue Spruce Farms, Altamont, NY 12009
[4] Methocel 60-HG, Dow Chemical Co., Midland, Mich.
[5] Millipore.

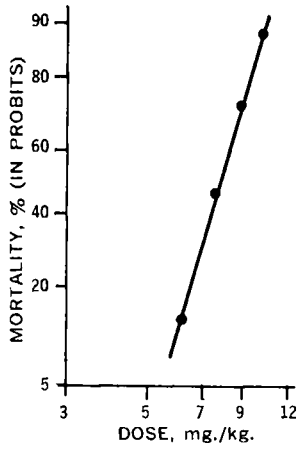

Figure 2—*Relationship between the oral dose and lethality of digitoxin from a 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate.*

than those animals dosed with the pure drug. For example, 75% of the deaths that resulted from the oral administration of a 10-mg./kg. dose of the drug as a polyvinylpyrrolidone coprecipitate occurred within 5–10 hr., with the remaining animals dying between 24 and 48 hr. On the other hand, 50% of the rats dosed with 185 mg./kg. of the pure drug died between 12 and 24 hr. and 50% between 24 and 48 hr.

Although visual inspection of the slopes of the linear plots shown in Figs. 1 and 2 suggests a lack of parallelism, the toxicity data, when statistically tested for parallelism according to the method of Litchfield and Wilcoxon (7), revealed that the two lines do not show any significant departure from parallelism ($p < 0.05$).

The 1:9 (w/w) digitoxin–polyvinylpyrrolidone physical mixture was orally administered at two drug dosage levels to two groups of rats to establish whether a simple, mechanical mixture of the two components would affect the oral toxicity of digitoxin. A comparison of the results of these toxicity tests with those obtained with the drug alone (Table I) reveals that the toxicity of digitoxin is essentially unaltered when administered as a suspension with polyvinylpyrrolidone.

Dissolution Rate and Equilibrium Solubility Studies—*In vitro* dissolution rate studies were conducted at 37° on particulate samples of the three digitoxin test preparations to establish whether this physicochemical parameter could be used to reflect the differences observed previously among their *in vivo* absorption characteristics. The rate of solution of a sample of pure *precipitated* digitoxin (particle-size range of 1–50 μ), prepared in the same manner as the coprecipitate system, was also evaluated. The results of these *in vitro* dissolution rate experiments, performed at pH 7.4 and under nonsink conditions, are shown in Figs. 3 and 4.

An examination of the dissolution rate profiles for the pure drug and the 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate

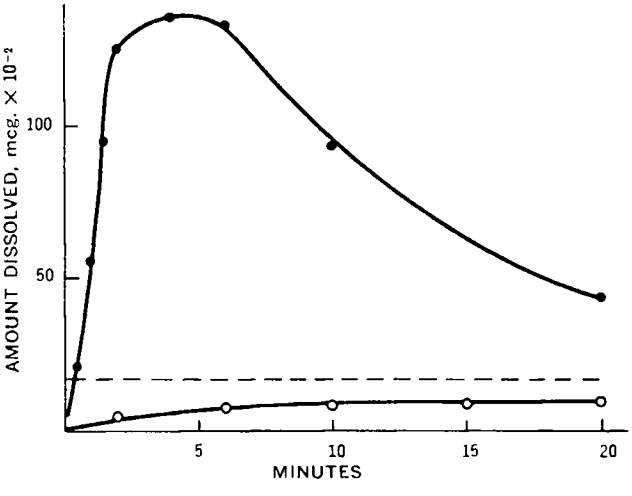

Figure 3—*Dissolution rates of digitoxin test preparation at 37°. Key: ○, pure digitoxin; ●, 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate; and - - -, equilibrium solubility of digitoxin in dissolution medium.*

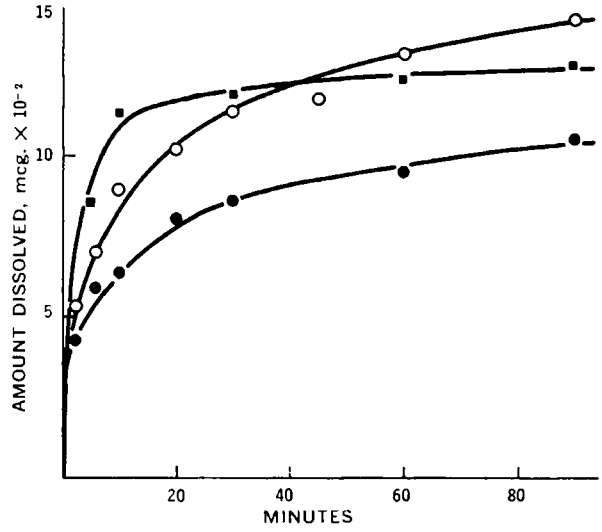

Figure 4—*Dissolution rates of digitoxin test preparations at 37°. Key: ○, Pure digitoxin; ●, digitoxin precipitated from chloroform; and ■, 1:9 (w/w) digitoxin polyvinylpyrrolidone physical mixture.*

(Fig. 3) reveals that the amount of drug in solution from the coprecipitate system rapidly and markedly exceeds the equilibrium solubility of the drug. After reaching a peak value in a relatively short period (*i.e.*, less than 5 min.), the amount of drug in solution slowly declines toward the equilibrium solubility value. However, even after 2 hr., the amount of digitoxin in solution from the coprecipitate system is nearly twice the equilibrium solubility value. On the other hand, the pure drug dissolves at a significantly slower rate and gradually approaches the equilibrium solubility value. The equilibrium solubility of digitoxin was unaffected by the amount of polyvinylpyrrolidone in solution from the coprecipitate system.

The peak, nonequilibrium (dynamic), solubility value for digitoxin from the 1:9 (w/w) coprecipitate, as determined from the dissolution rate experiments (Fig. 3), was 38.7 mcg./ml. or 13.5 mg./350 ml. of dissolution medium. This value exceeds the equilibrium solubility of the drug by a factor of 7–8 times. The magnitude of this factor compares favorably with the ratio of 11.3 obtained by comparing the oral LD_{50} value calculated from the pure drug to that for the 1:9 (w/w) digitoxin–polyvinylpyrrolidone coprecipitate.

The dissolution profiles for precipitated digitoxin (1–50 μ), pure digitoxin (10–40 μ), and the 1:9 (w/w) digitoxin–polyvinylpyrrolidone physical mixture are depicted in Fig. 4. The rates of solution of digitoxin from these three systems are quite similar in magnitude and markedly less than that previously noted for digitoxin from the 1:9 (w/w) coprecipitate (Fig. 3).

The equilibrium solubility of digitoxin at 37°, determined in various aqueous systems, was 4.8 mcg./ml. in water and pH 7.4 buffer and 4.9 mcg./ml. in pH 7.4 buffer containing 1% (w/v) polyvinylpyrrolidone. The concentration of polyvinylpyrrolidone used in this study was considerably higher than that which could be realized from the complete dissolution of the amount of polymer present in the samples of either the physical mixture or coprecipitate subjected to dissolution rate experimentation (1 *versus* 0.0514%).

DISCUSSION

Drug absorption may be assessed indirectly by quantitatively determining its oral toxicity (9, 10). This method is based on the fact that the toxicity of an orally administered drug is directly proportional to the logarithm of the amount of drug in the body or its blood level (11) which, in turn, is directly proportional to its rate of absorption from the GI tract. The two parameters most commonly employed to reflect the toxicity of a drug are its oral LD_{50} and LT_{50} (median lethal time of death) values.

In the present investigation, digitoxin alone or as a 1:9 (w/w) physical mixture or coprecipitate with polyvinylpyrrolidone was orally administered at various drug dosage levels to fasted rats and the percent mortality was determined as a means of comparing the absorption characteristics of the drug from the various test systems. The markedly lower oral LD_{50} value observed for the coprecipitated

drug as compared to the pure drug does not necessarily imply that digitoxin, when administered alone, is less available to the body than when administered in the form of polyvinylpyrrolidone coprecipitate. However, it strongly suggests that the peak level of digitoxin in the body after oral administration of the coprecipitate is far higher than that achieved following an equal dose of pure drug. This conclusion can be readily appreciated if one considers the case of a hydrophobic drug whose passive absorption is rate limited by the rate at which solution is effected within the GI fluids. In this type of absorption pattern, any change in the rate of solution of the drug in the GI fluids would produce a corresponding change in its absorption rate. Since dissolution must precede absorption, a faster dissolving form of the drug should be absorbed at a more rapid rate and possibly accumulate in the body to a greater extent than an *equal* dose of a slowly dissolving form of the same pharmacological agent (12).

The inherent toxicity of polyvinylpyrrolidone can be readily eliminated as a factor contributing to the marked decrease in the oral LD_{50} value for digitoxin from the coprecipitate. In this connection, it has been demonstrated that pure polyvinylpyrrolidone elicits no untoward effects when orally administered to rats in doses of up to 100 g./kg. The intravenous LD_{50} value for polyvinylpyrrolidone is also very high, being 12–15 g./kg. (13). This information, coupled with the results obtained following the oral administration of the physical mixture, indicates that the increased toxicity of digitoxin from the 1:9 (w/w) coprecipitate is not due to the presence of polyvinylpyrrolidone *per se* but to an increase in the amount of drug in the body produced by a marked enhancement in the rate at which drug absorption occurs from this test system.

Several *in vitro* dissolution rate and solubility experiments were performed to establish which physicochemical mechanism could best account for the marked enhancement observed in the dissolution rate of digitoxin from the 1:9 (w/w) drug–polyvinylpyrrolidone coprecipitate as compared to the pure drug. The mechanisms considered were: (*a*) the formation of a digitoxin–chloroform solvate during the preparation of the coprecipitate system, (*b*) an increase in the solubility of the drug due to the formation of a digitoxin–polyvinylpyrrolidone complex, and (*c*) the formation of a polymorphic or amorphous form of the drug as a result of coprecipitation with polyvinylpyrrolidone.

The formation of drug solvates has been shown to affect the dissolution rate of hydrophobic drugs significantly (14). In this connection, digitoxin has been shown to form solvates with ethanol and water, which are stable *in vacuo* at temperatures up to 118° (15). Since chloroform was used to prepare the digitoxin–polyvinylpyrrolidone coprecipitate system, the possibility existed that the enhanced absorption and dissolution characteristics of digitoxin from this system resulted from the formation of a 1:1 chloroform solvate. To explore this possibility, the dissolution rate of digitoxin precipitated from chloroform without polyvinylpyrrolidone was investigated.

The differences observed in the rates of solution of pure digitoxin and precipitated digitoxin are small (Fig. 4) and can most probably be attributed to differences in the particle-size distribution of the two test systems and/or some inhibition in the rate of solution brought about by the presence of a minute amount of chloroform in the precipitated drug crystals. The results of this experiment suggest that the increases noted in the dissolution of digitoxin from the 1:9 (w/w) polyvinylpyrrolidone coprecipitate cannot be due to the formation of a drug–chloroform solvate during the preparation of the coprecipitate system.

The dissolution characteristics of the 1:9 (w/w) digitoxin–polyvinylpyrrolidone physical mixture almost coincide with those obtained with the pure drug (Fig. 4). The slight increase noted in the initial rate of solution of digitoxin from the physical mixture, as compared to the pure drug, is most likely due to the ability of the hydrophilic polymer to enhance the wettability of the hydrophobic digitoxin particles. These results indicate that the mere presence of polyvinylpyrrolidone, at a concentration equal to that present in the 1:9 (w/w) coprecipitate, is not responsible for the enhanced dissolution rate of digitoxin from the coprecipitate. Also, the equilibrium solubility studies indicate that the increased solubility and dissolution rate of digitoxin from the 1:9 (w/w) drug–polyvinylpyrrolidone coprecipitate system are most probably not the result of the formation of a water-soluble complex between the drug and polyvinylpyrrolidone.

The similarity between the dissolution and solubility behavior of digitoxin from the 1:9 (w/w) drug polyvinylpyrrolidone coprecipitate and that previously observed with reserpine–polyvinylpyrrolidone coprecipitate systems (16), coupled with the fact that both drugs form *glass solutions* with the polymer, strongly suggests that a similar mechanism is operable. Accordingly, it may be proposed that a high energy form of digitoxin, most probably amorphous in nature, is formed as a result of coprecipitating the drug with polyvinylpyrrolidone and that its increased solubility characteristics are responsible for the marked enhancement in drug dissolution and absorption experienced with the coprecipitate system.

CONCLUSIONS

The data obtained in the present study demonstrate that coprecipitation with polyvinylpyrrolidone increases the solubility and dissolution rate of digitoxin and thereby significantly enhances the *in vivo* absorption of this hydrophobic drug. These observations, as well as those noted previously with reserpine (6, 16), indicate that it is quite possible that this technique might have general applicability to a wide variety of relatively water-insoluble drug entities whose absorption from the GI tract can be characterized as slow, erratic, and/or incomplete. The advantages of utilizing this technique in the pharmacological screening of new drugs for oral activity are numerous and self-evident. Its clinical applications are currently being explored in these laboratories.

REFERENCES

(1) I. M. Stone, U.S. pat. 3,089,818 (1963).

(2) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **55**, 1323 (1966).

(3) T. R. Bates, *J. Pharm. Pharmacol.*, **21**, 710(1969).

(4) A. P. Simonelli, S. C. Mehta, and W. I. Higuchi, *J. Pharm. Sci.*, **58**, 538(1969).

(5) G. V. Svoboda, M. J. Sweeney, and W. D. Walkling, *ibid.*, **60**, 333(1971).

(6) E. I. Stupak and T. R. Bates, *ibid.*, **61**, 400(1972).

(7) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99(1949).

(8) P. Mesnard and G. Devaux, *Compt. Rend.*, **253**, 497(1961).

(9) T. R. Bates and M. Gibaldi in "Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, p. 95.

(10) W. F. White and O. Gisvold, *J. Amer. Pharm. Ass., Sci. Ed.*, **41**, 42(1952).

(11) L. W. Dittert, H. J. Adams, F. Alexander, C. W. Chong, T. Ellison, and J. V. Swintosky, *J. Pharm. Sci.*, **57**, 1146(1968).

(12) M. Gibaldi, "Introduction to Biopharmaceutics," Lea & Febiger, Philadelphia, Pa., 1971, pp. 6–8.

(13) "PVP–Polyvinylpyrrolidone," General Aniline and Film Corp., New York, N. Y., 1964, p. 15.

(14) E. Shefter and W. I. Higuchi, *J. Pharm. Sci.*, **52**, 781(1963).

(15) "The Merck Index," 8th ed., Merck and Co., Rahway, N. J., 1968, p. 364.

(16) E. I. Stupak, Ph.D. thesis, University of Connecticut, Storrs, Conn., 1972.

ACKNOWLEDGMENTS AND ADDRESSES

Received April 13, 1973, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication July 6, 1973.

* Present address: Schering Corp., Bloomfield, NJ 07003

▲ To whom inquiries should be directed.