$$S_T = 52.048 - 0.324s - 0.955P - 0.232s^2 + 0.016sP - 0.053s^2 - 0.003s^2P \quad \text{(Eq. 6)}$$

By computer simulation, the three-dimensional contour systems were produced from these equations. The mathematical models are shown in Figs. 7 and 8, respectively. As was found from disintegration time measurements, decreasing the compaction pressure increased the generated surface area of the tablet fragment with all formulations. However, with this test, the highest values of surface area occurred with formulations containing 2.5% intra–12.5% extragranular starch and 15% intragranular starch. On the basis of this test, these formulations should be selected, not the one containing 15% extragranular starch, as found from BP disintegration test determinations. This test also shows that there are large differences in surface area values for starch combinations less than 10% intra–5% extragranular, a fact not shown by simple disintegration time measurements. In addition, the common practice of incorporating 5% extragranular starch into a formulation actually produced the lowest values of surface area, again a fact not shown by simple disintegration time measurements.

This new type of disaggregation test appears to be particularly sensitive. It is capable of monitoring small formulation differences with a high degree of precision and of producing an index that is more directly relatable to tablet dissolution. The official disintegration test, however, may produce misleading results, because the insensitivity of the test can mask real and significant differences between tablets. The new disaggregation test reported here thus appears to provide a more sensitive tool for evaluating the disintegrating properties of tablets.

CONCLUSIONS

The authors consider that the official disintegration test is only sufficiently sensitive to detect gross differences between tablets. An alternative and more sensitive technique is proposed; it utilizes an automated counter and measures the surface area generated per tablet during disintegration. This new disaggregation method was used to show that the optimum starch combination in a tablet formulation is either 2.5% intra–12.5% extragranular or 15% intragranular starch alone. Furthermore, the distribution of starch will not affect the resultant hardness of the tablets and, to achieve maximum generation of surface area, tablets should be compacted at as low a pressure as possible.

REFERENCES

(1) K. L. Kelly and M. W. Green, *Bull. Nat. Formul. Comm.*, **13**, 48(1945).

(2) A. A. Noyes and W. R. Whitney, *J. Am. Chem. Soc.*, **19**, 930(1897).

(3) H. Nogami, J. Hasegawa, and Y. Nakai, *Chem. Pharm. Bull.*, **7**, 331(1959).

(4) E. Suito and N. Hirai, *J. Chem. Soc. Jpn.*, **72**, 713(1951).

(5) Y. Nakai and Y. Kubo, *Chem. Pharm. Bull.*, **8**, 634(1960).

(6) Y. Nakai, *ibid.*, **8**, 641(1960).

(7) H. Nogami, J. Hasegawa, and M. Mujamoto, *ibid.*, **15**, 297(1967).

(8) J. C. Sanders, *Pharm. Weekbl.*, **104**, 485(1969).

(9) E. Sandell, *Acta Pharm. Suec.*, **7**, 55(1970).

(10) E. Shotton and G. S. Leonard, *J. Pharm. Pharmacol.*, **24**, 798(1972).

(11) T. Allen, "A Critical Evaluation of the Coulter Counter," Particle-Size Analysis Conference, Bradford, United Kingdom, 1966.

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[x] To whom inquiries should be directed.

Dissolution Characteristics and Oral Absorption of Digitoxin and Digoxin Coprecipitates

RANJIT K. REDDY, SAID A. KHALIL [*], and M. WAFIK GOUDA [x]

Abstract □ A marked increase in the dissolution rates of digitoxin and digoxin was attained by dispersing the drugs in two inert solid carriers, poloxamer 188 and deoxycholic acid. The 1 and 10% (w/w) drug–carrier solid dispersions were prepared by the solvent method. The former dissolved significantly faster than the latter. The oral administration of 10% (w/w) digitoxin–carrier coprecipitates to mice significantly increased toxicity. This observed increase is attributed to an increase in the rate and, possibly, the extent of oral absorption of the drug. Although a 10% coprecipitate of digoxin in both carriers showed an increase in the dissolution rate, no increase in oral toxicity was observed. X-ray diffraction patterns indicated that both digitoxin and deoxycholic acid undergo crystalline modifications due to treatment by the solvent, but the exact nature of the drug–carrier solid dispersions was not revealed.

Keyphrases □ Digitoxin—coprecipitates with inert solid carriers, dissolution and oral absorption, mice □ Digoxin—coprecipitates with inert solid carriers, dissolution and oral absorption, mice □ Dissolution—digitoxin and digoxin, coprecipitates with inert solid carriers, mice □ Absorption, oral—digitoxin and digoxin, coprecipitates with inert solid carriers, mice □ Coprecipitates—digitoxin and digoxin with inert solid carriers, dissolution and oral absorption, mice □ Dosage forms—digitoxin and digoxin coprecipitates with inert solid carriers, dissolution and oral absorption, mice

It is well documented that the bioavailability of digoxin from commercial tablet dosage forms is not uniform (1–7). Furthermore, the absorption efficiency from tablets is considerably less than from an oral solution (6, 8). Solid dosage forms of digitoxin are also suspected of exhibiting bioavailability differences (9).

Mortar grinding increased the dissolution rate of three digoxin samples studied (10). Tablets and capsules of digoxin made after crushing material that passed the BP requirements gave higher area under the concentration–time curves than did formulations made of the same material before crushing (11). It was concluded

that particle size may be an important determinant of digoxin bioavailability. A comparison of human plasma digoxin levels after administration of tablets made from particles of 3.7-, 12-, and 22-μm diameter showed that the smaller the particle size, the higher was the plasma level (12). A number of reports (6, 7, 13) correlated digoxin tablet dissolution rate *in vitro* to biological availability, showing that the absorption of this drug is dissolution rate limited.

As noted previously (14), if a drug is more rapidly and/or more completely absorbed from solution than from a solid form, its absorption is quite likely to be dissolution rate limited. Slow dissolution results in incomplete, erratic, and unpredictable absorption, and the potential for variability in absorption increases greatly with incompletely absorbed drugs. The purpose of this study was to improve the dissolution of digitoxin and digoxin to reduce the variability in their absorption. The usefulness of solid dispersions of poorly soluble drugs in inert carriers for increasing their dissolution rate has been widely reported (15–28). Soluble carriers such as povidone, polyethylene glycol, urea, citric acid, and succinic acid and poorly soluble ones such as deoxycholic acid, lithocholic acid, and cholic acid have been used.

In this report, the effects of coprecipitating digitoxin and digoxin with two carriers, poloxamer 188 and deoxycholic acid, on the *in vitro* dissolution rate and oral absorption in mice are presented. Deoxycholic acid increased the dissolution and absorption of reserpine (23, 26) and nitrofurantoin (27). To the authors' knowledge, poloxamer 188 has not been used previously to prepare solid dispersions of drugs. It is a block polymer of ethylene oxide and propylene oxide, with an average molecular weight of 8350. It is highly water soluble (>10 g/100 ml at 25°) and is currently used medicinally as a fecal softener. It is a nonionic surfactant which was shown to have no membrane effects (29). High molecular weight polymers are expected to form interstitial solid solutions with many drugs (22). Because it possesses these properties, poloxamer 188 was investigated for its possible use as a matrix for solid-dispersing insoluble drugs.

EXPERIMENTAL

Materials—Digoxin[1], digitoxin[2], poloxamer 188[3], and deoxycholic acid[4] were used as received. All other chemicals were either USP or analytical grade.

Preparation of Coprecipitates—The 1 and 10% (w/w) drug–carrier coprecipitates were prepared by dissolving the two components in 95% alcohol. The solvent was removed under vacuum in a rotary evaporator[5] at room temperature; the vacuum was maintained overnight. The material was then scraped and screened, and the fraction that passed through an 80-mesh sieve and was retained on a 100-mesh sieve was used. Precipitated digitoxin and digoxin were prepared by treating them in a similar manner in the absence of any carrier. Physical mixtures composed of 1 and 10% (w/w) of the drugs and carriers were prepared by mixing the components on paper with a spatula.

Analysis of Drug in Coprecipitates—Two to four accurately weighed fractions of the coprecipitate were dissolved in alcohol and, after suitable dilution with water, were subjected to the described assay procedure. The drug content was between 98.8 and 102.2%.

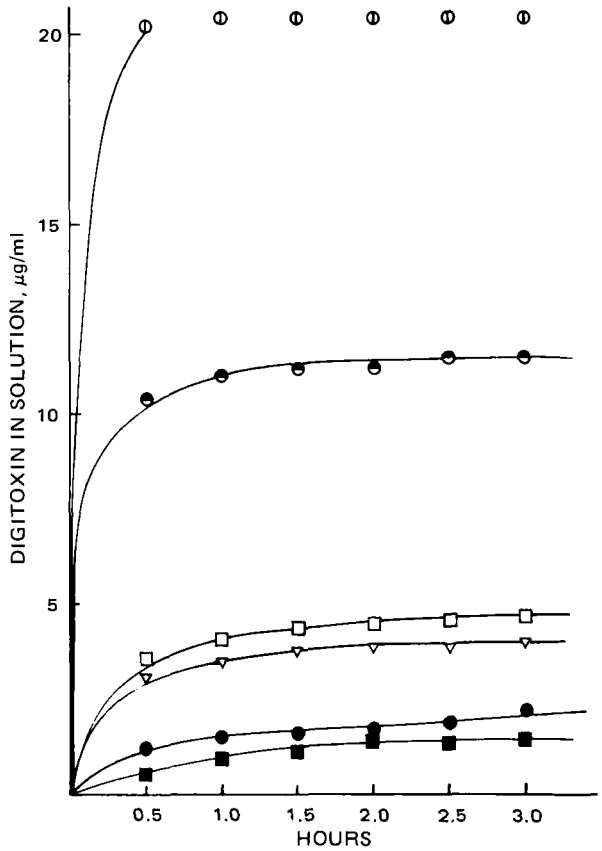

Figure 1—*Dissolution of digitoxin from poloxamer 188 test preparations. Key:* ■, *untreated drug;* ●, *treated drug;* ▽, *10% physical mixture;* □, *1% physical mixture;* ⊖, *10% coprecipitate; and* ⊙, *1% coprecipitate.*

Dissolution Rates—The dissolution characteristics (under nonsink conditions) of (*a*) pure digitoxin and digoxin, (*b*) precipitated digitoxin and digoxin, (*c*) digitoxin and digoxin in 1 and 10% (w/w) coprecipitates, and (*d*) physical mixtures of the drugs with the carriers were determined. In all cases, 80–100-mesh powders (particle size 149–177 μm) containing 15 mg of drug were used. All dissolution experiments were carried out with samples not more than 2 days old.

The beaker method of Levy and Hayes (30), with slight modification, was employed. The dissolution medium consisted of 500 ml of water maintained at 37° in a 1-liter beaker immersed in a constant-temperature water bath. Stirring was provided by a three-blade, 4.45-cm diameter polyethylene stirrer[6] rotating at 60 rpm and dipped in the water to 3.5 cm. The test system was added to the dissolution medium, and 5-ml samples were removed as a function of time and analyzed for drug content. Five milliliters of the dissolution medium was added back to the beaker after each sampling. Each experiment was done in duplicate. A cumulative correction was made for the previously removed samples (31).

Solubility Determinations—The equilibrium solubility of digitoxin and digoxin in water at 37° was determined, and the effect of the carriers on the solubility of the drugs was studied. Excess drug was placed in erlenmeyer flasks along with 20 ml of water or 20 ml of water and carriers. The flasks were tightly stoppered and equilibrated in a water bath shaker[7]. Equilibrium solubility was determined by repetitive sampling.

Assay Procedure—The colorimetric method of Mesnard and Devaux (32) was employed, with slight modification, to assay for the amount of digitoxin and digoxin in solution. The presence of the carriers did not interfere with the assay. To 3 ml of drug solution was

[1] Lanoxin, lot 57177, Burroughs Wellcome Co.
[2] Lot W11097, supplied by Eli Lilly and Co.
[3] Pluronic F-68, supplied by Wyandotte Chemical Co.
[4] Nutritional Biochemicals Corp.
[5] Rotavapor-R, Brinkmann Instruments.
[6] Nalge, No. 6160.
[7] Metabolyte, New Brunswick Scientific.

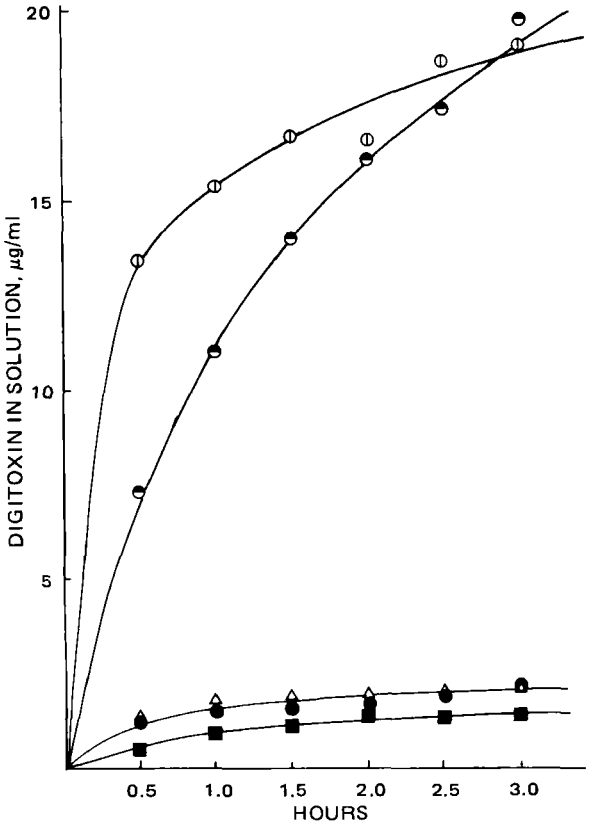

Figure 2—*Dissolution of digitoxin from deoxycholic acid test preparations. Key: ■, untreated drug; ●, treated drug; △, 10 and 1% physical mixtures; ⊖, 10% coprecipitate; and ⊙, 1% coprecipitate.*

added 0.5 ml of 0.01 *N* periodic acid in 0.1 *N* H_2SO_4. After allowing a 10-min oxidation period, 1 ml of 2% sodium arsenite solution in 0.5 *N* HCl was added to stop the oxidation. Four milliliters of the mixture was added to 4 ml of a 0.6% aqueous solution of thiobarbituric acid, adjusted to pH 2, in a 10-ml volumetric flask. The flask was heated in a boiling water bath for 20 min, after which it was cooled to room temperature and the volume was made up with distilled water. The absorbance was determined at 530 nm[8] against a blank treated similarly. The slopes obtained from Beer's law plots were used to determine drug concentrations in the dissolution and solubility studies and in analyzing the coprecipitate systems for drug content.

The direct spectrophotometric assay for digitoxin was tried first. Filtration of the sample through the membrane filters[9] used drastically increased the absorbance of the solution at 220 nm.

Protocol for *In Vivo* Studies—The GI absorption characteristics of digitoxin and digoxin were assessed by comparing the relative oral toxicity of pure drugs, 10% (w/w) physical mixtures with the carriers, and 10% (w/w) coprecipitates. Male albino mice[10], 16–30 g, were deprived of feed 16–18 hr prior to drug administration. Water was freely allowed. The drugs were administered by gastric intubation as a suspension in 0.5% (w/v) methylcellulose (100 cps) aqueous vehicle.

The concentration of the suspension was adjusted such that the dose to be administered would be contained in a volume of 0.2 ml (digitoxin) and 0.4 ml (digoxin)/10 g of body weight. The animals were returned to cages after drug administration, and food and water were allowed *ad libitum*. The number of deaths in the next 168 hr was noted. The 1% coprecipitates were not investigated because the dose to be administered would have been prohibitive.

X-Ray Diffraction Studies—Powdered samples were packed firmly on an aluminum slide having a cavity with a glass window. All diffraction spectra[11] were obtained by scanning at 2°/min in terms of a 2θ angle.

RESULTS AND DISCUSSION

Digitoxin Coprecipitates—The dissolution characteristics of digitoxin–poloxamer 188 test preparations are illustrated in Fig. 1. Digitoxin in both 1 and 10% coprecipitates dissolved at a significantly faster rate than the pure drug. The 1% coprecipitate released the drug much faster than the 10% coprecipitate. The former contained 11 times as much poloxamer 188 as the latter. Complete dissolution of the carrier in the 1% coprecipitates yielded a 0.297% solution, about three times the critical micelle concentration (CMC) value of this surfactant (33).

Surfactants increase the dissolution rate of drugs because of lowering of interfacial tension, which results in an increase in the effective surface area of the drug, or due to micellar solubilization. Therefore, the effect of two poloxamer 188 concentrations, 0.027 and 0.297% (corresponding to the 10 and 1% coprecipitates, respectively), on the dissolution of digitoxin was studied. Digitoxin dissolved at a faster rate in the presence of both concentrations of the surfactant. Equilibrium solubility in these surfactant solutions at 37° was determined. No difference between the solubility of digitoxin in water (0.62 mg/100 ml) and in the surfactant solutions was observed. This result indicates the lack of drug–carrier interaction in the concentrations employed. The slightly faster dissolution of the drug in the more concentrated poloxamer 188 solution (Fig. 1) might be due to better wetting of the drug.

Figure 2 shows the dissolution of digitoxin from the deoxycholic acid test systems. Both coprecipitates dissolved much faster than the pure drug, and the 1% coprecipitate dissolved faster than the 10% coprecipitate, at least in the initial stages. The dissolution of digitoxin from both the 1 and 10% physical mixtures proceeded at equal rates. The studies with physical mixtures indicated that the mere presence of the carrier in an amount equivalent to that present in the coprecipitates was not responsible for the enhanced dissolution of digitoxin from the coprecipitates. Equilibrium solubility studies indicated that there was no interaction between digitoxin and deoxycholic acid.

Digitoxin is known to form solvates with alcohol which are stable *in vacuo* (34). Since drug solvates may have markedly different rates

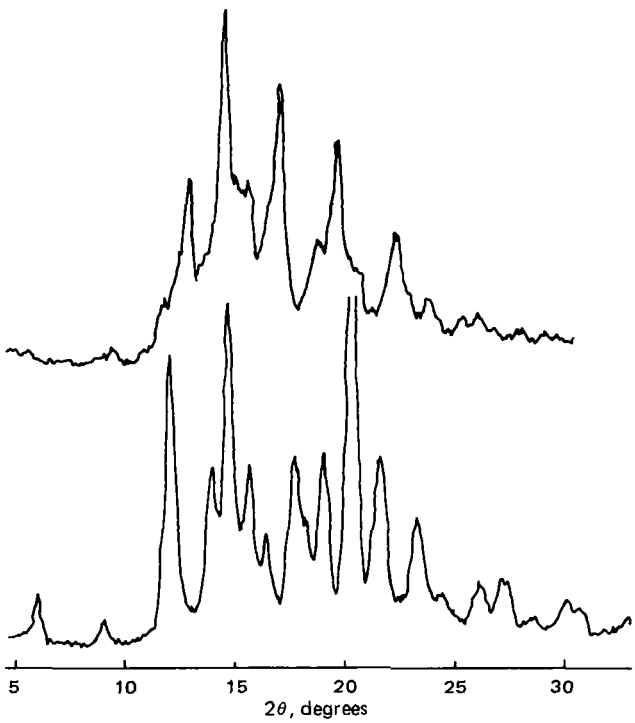

Figure 3—*X-ray diffraction spectra of treated (top) and untreated (bottom) digitoxin.*

[8] Beckman ACTA CIII.
[9] Millipore, 0.45-µm filters.
[10] Horton Laboratories, Oakland, Calif.
[11] Norelco, Philips Electronic.

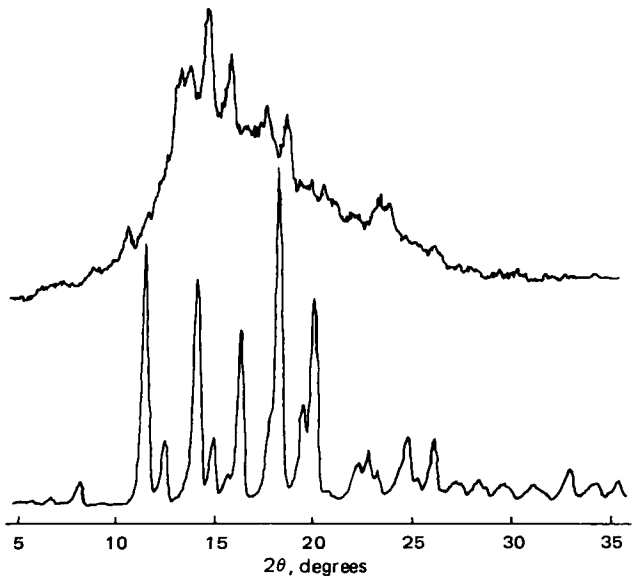

Figure 4—*X-ray diffraction spectra of untreated (top) and treated (bottom) deoxycholic acid.*

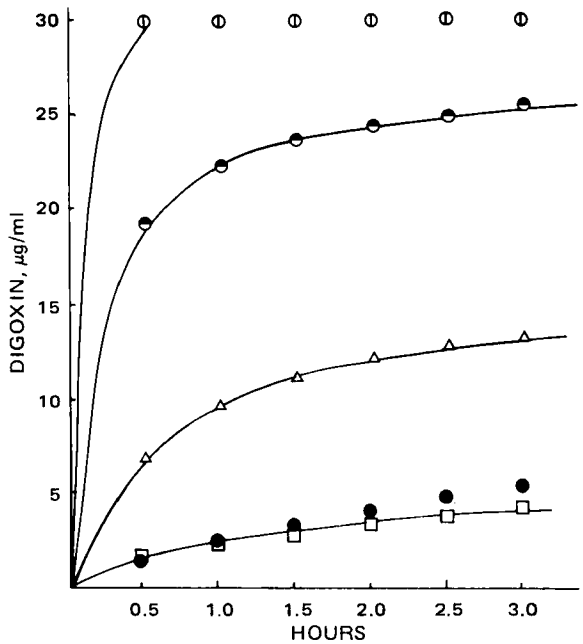

Figure 5—*Dissolution of digoxin from poloxamer 188 test preparations. Key: ●, treated drug; □, untreated drug; △, 10 and 1% physical mixtures; ⊖, 10% coprecipitate; and ⊙, 1% coprecipitate.*

of dissolution compared to the pure drugs (35), the effect of treating (dissolving in alcohol and removing the solvent *in vacuo*) digitoxin on its dissolution was studied. As shown in Fig. 1, treated digitoxin dissolved at a slightly faster rate than did the untreated sample. To determine if digitoxin had undergone any crystalline modifications due to solvent treatment, X-ray diffraction spectra were obtained. Treated digitoxin (Fig. 3) had a diffraction spectrum different from the untreated digitoxin, indicating the possibility of polymorphic modification or solvate formation. The X-ray diffraction pattern ruled out the possibility that solvent treatment changes digitoxin to an amorphous form. The small difference in the dissolution rates of the pure and precipitated digitoxin does not account for the considerably faster dissolution of the drug from the coprecipitates.

Since these facts indicate that the enhanced dissolution of digitoxin from the coprecipitates cannot be fully accounted for as due to surface tension lowering by the carriers, drug–carrier complexation, or solvate formation, other factors must be considered. The enhanced dissolution of the coprecipitated digitoxin could be due to its presence in an amorphous state, as a high energy polymorph, in a microcrystalline state, or as a solid solution. The X-ray diffraction technique has been used to study solid dispersions (36–38). In the present study, the digitoxin coprecipitates as well as the physical mixtures showed the diffraction patterns of the carriers only. Simple dilution with the carriers masked the diffraction peaks of digitoxin. Hence, the X-ray studies neither confirmed nor ruled out any of the mentioned possibilities as the specific cause of enhanced drug dissolution from the coprecipitates. The X-ray diffraction pattern of deoxycholic acid after solvent treatment (Fig. 4) indicated a change in its crystalline nature. There was no change in the X-ray spectrum of poloxamer 188 due to solvent treatment.

Both the poloxamer 188 and deoxycholic acid coprecipitates achieved supersaturation, but there was no indication of drug precipitation and decline toward the equilibrium value, as was the case with a digitoxin–povidone coprecipitate (28). A distinct difference existed in the pattern of dissolution of the poloxamer 188 (Fig. 1) and deoxycholic acid (Fig. 2) coprecipitates. The drug from the former went into solution rapidly during the first 30 min, but there was almost no change in the amount of the drug in solution after this period. In contrast, the deoxycholic acid coprecipitates released digitoxin over a greater time. This difference in the release pattern of the drug could be due to the differences in the solubilities of the two carriers in water. Since poloxamer 188 is highly soluble, it dissolves rapidly and all drug dispersed in it comes in contact with the dissolution medium. Deoxycholic acid, because of its low solubility, does not let all of the dispersed drug come in contact with the dissolution medium immediately. The shapes of the dissolution curves of the deoxycholic acid coprecipitates (Fig. 2) suggest that the initial steeply rising portion may represent the dissolution of the drug present on the surface of the carrier or the dissolution of a high energy or amorphous form of the drug. Once this surface-dispersed or high energy form dissolves, the dissolution is rate limited by the leaching out of the drug in the interior of the carrier or by the erosion of the drug crystals.

Preliminary *in vivo* absorption studies were undertaken to find if the *in vitro* dissolution enhancement of digitoxin from its coprecipitates increases in the GI absorption of the drug. Drug absorption from the test preparations was assessed indirectly by determining their oral toxicity. Oral drug absorption was assessed by determining the number of deaths in mice given the same dose of digitoxin as pure drug, as a 10% drug–carrier physical mixture, and as a 10% coprecipitate (Table I).

The administration of digitoxin as the poloxamer 188 coprecipitate caused a significant ($p < 0.001$) increase in its oral toxicity, but the administration of the physical mixture did not result in any significant change. Administration of up to 2.7 g of poloxamer 188 alone/kg did not result in any toxicity to the mice, in agreement with the reported toxicity of poloxamer 188 (39). The mice that received the poloxamer 188 coprecipitate died at a much earlier time than those receiving the other two preparations. While 62% of the deaths resulting from the coprecipitate occurred between 12 and 36 hr after administration, only 33% of the deaths due to the pure drug occurred in 72 hr.

Table I also summarizes the results of the toxicity studies with

Table I—Oral Toxicity of Various Digitoxin Preparations in Mice[a]

Test System	Number of Animals Dead[b]	Mortality, %
Digitoxin	6	20
Digitoxin–poloxamer 188[c] coprecipitate	29	97
Digitoxin–deoxycholic acid[c] coprecipitate	30	100
Digitoxin–poloxamer 188[c] physical mixture	11	37
Digitoxin–deoxycholic acid[c] physical mixture	9	30
Poloxamer 188[d]	0	0
Deoxycholic acid[e]	0	0

[a] A dose of 70 mg of digitoxin/kg was administered as a suspension in 0.5% methylcellulose. Thirty animals were used for each test system. [b] Animals were observed for 7 days postadministration. [c] A 700-mg/kg dose was administered containing 10% (w/w) digitoxin. [d] A 2.7-g/kg dose was used. [e] A 630-mg/kg dose was used.

Table II—Effect of Poloxamer 188 and Deoxycholic Acid on the Solubility of Digoxin in Water at 37°

Test System	Solubility, mg/100 ml
Water	3.47
Poloxamer 188 in concentration equivalent to 10% coprecipitate	4.77
Poloxamer 188 in concentration equivalent to 1% coprecipitate	5.38
Deoxycholic acid in concentration equivalent to 10% coprecipitate	4.62
Deoxycholic acid in concentration equivalent to 1% coprecipitate	4.25

deoxycholic acid test preparations. Like the poloxamer 188 coprecipitate, the deoxycholic acid coprecipitate caused a significant ($p < 0.001$) increase in oral toxicity. Once again, the mice receiving the coprecipitate died much earlier than those receiving the other two test forms. Of 30 mice that received only deoxycholic acid in amounts present in the coprecipitate, none died. Therefore, the inherent toxicity of deoxycholic acid is not a contributing factor to the enhanced toxicity of the coprecipitated drug.

Although these toxicity studies suffer from the limitation of being a single-dose method, they do indicate a highly significant increase in the rate and, possibly, the extent of oral absorption of digitoxin from the coprecipitates.

Digoxin Coprecipitates—The dissolution characteristics of digoxin from poloxamer 188 test systems are illustrated in Fig. 5. The coprecipitates dissolved significantly faster than the pure drug, and the 1% coprecipitate dissolved faster than the 10% coprecipitate. Precipitating digoxin from ethanol did not significantly alter the dissolution of the drug. The X-ray diffraction patterns of the treated and untreated digoxin were similar. Although the physical mixtures showed a significant enhancement of drug dissolution, there was no difference between the 1 and 10% mixtures.

Equilibrium solubility studies (Table II) showed that poloxamer 188 increased drug solubility, indicating the possibility of micellar complexation. It is evident, however, that although the surface tension-lowering and solubilizing effects of poloxamer 188 do contribute to the enhanced dissolution of the coprecipitated drug, these effects do not fully account for the differences in dissolution of the pure and coprecipitated drug.

The dissolution of digoxin from deoxycholic acid coprecipitates (Fig. 6) proceeded at a faster rate compared to the dissolution of the drug alone. The 1:9 and 1:99 physical mixtures also dissolved faster than the drug. The dissolution of the drug from both physical mixtures proceeded at a rate that was higher than its dissolution from the 10% coprecipitate. Equilibrium solubility studies (Table II) indicated that there was an interaction between digoxin and deoxycholic acid. Deoxycholic acid is known to form inclusion compounds with many drugs (40), including those with a steroidal nucleus (41). It seems possible that the presence of an extra hydroxyl group in the digoxin molecule (compared to digitoxin) is favoring an interaction between the drug and the carrier in the coprecipitate. In the 10% coprecipitate,

Table III—Oral Toxicity of Various Digoxin Preparations in Mice[a]

Test System	Number of Animals Dead[b]	Mortality, %
Digoxin	1	3
Digoxin–poloxamer 188[c] coprecipitate	0	0
Digoxin–deoxycholic acid[c] coprecipitate	30	100
Digoxin–poloxamer 188[c] physical mixture	0	0
Digoxin–deoxycholic acid[c] physical mixture	26	87
Deoxycholic acid[d]	27	90

[a] A dose of 300 mg of digoxin/kg was administered as a suspension in 0.5% methylcellulose. Thirty animals were used for each test system. [b] Animals were observed for 7 days postadministration. [c] A 3-g/kg dose was administered containing 10% (w/w) digoxin. [d] A 2.7-g/kg dose was used.

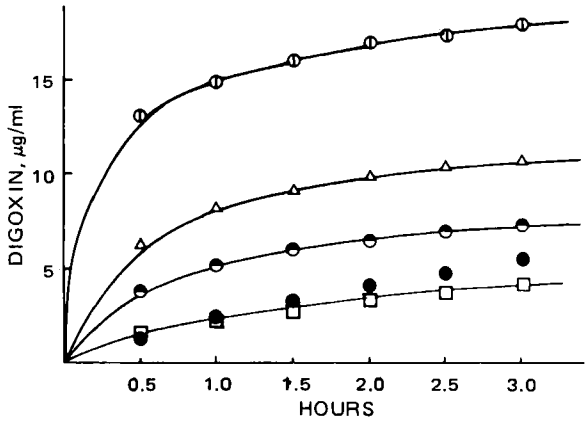

Figure 6—*Dissolution of digoxin from deoxycholic acid test preparations. Key: ●, treated drug; □, untreated drug; △, 10 and 1% physical mixtures; ◓, 10% coprecipitate; and ◔, 1% coprecipitate.*

such an interaction seems to have a negative effect on the increase in the dissolution rate obtained through coprecipitation.

As discussed under digitoxin coprecipitates, dilution of digoxin with the carriers caused loss of diffraction peaks of the drug. Therefore, the X-rays did not aid in elucidating the exact nature of the coprecipitated drug. It is reasonable to assume, however, that coprecipitation changes the nature of digoxin in the same way as mentioned under digitoxin coprecipitates.

The data in Table III indicate that for this particular dose of digoxin (300 mg/kg), the digoxin–poloxamer 188 coprecipitate was no more toxic to mice than was the drug alone. The drug suspension administered to mice contained 6.75% poloxamer 188, which is above its CMC. Surfactants hinder drug absorption due to micellar complexation (42), and poloxamer 188 retarded the absorption of phenolsulfonphthalein from the rat intestine (29). It is, therefore, possible that entrapment of the digoxin in the surfactant micelles was the reason for the lack of enhancement of toxicity of the digoxin–poloxamer 188 coprecipitate. The oral toxicity of the administered dose of digoxin as a 10% digoxin–deoxycholic acid coprecipitate (Table III) did not increase significantly beyond that of deoxycholic acid alone (in the amount present in the coprecipitate). It is probable that the toxicity of the carrier is masking any increase in toxicity of the coprecipitate. More sensitive tests of bioavailability, such as plasma level curves, might differentiate the potencies of the various test systems.

The 1% coprecipitates of both digitoxin and digoxin dissolved faster than the 10% coprecipitates. Because the ratio of drug to carrier was smaller in the 1% systems, precipitation of the drug could have been hindered more than in the 10% systems, resulting in a finer particle size of the drugs. The difference in the dissolution rates also could be due to the presence of the drugs as solid solutions in the 1% coprecipitates.

In summary, coprecipitation of digitoxin and digoxin with two inert carriers, poloxamer 188 and deoxycholic acid, resulted in a dramatic increase in the dissolution rate of the two cardiac glycosides. The increased dissolution of the coprecipitated digitoxin caused an increase in its oral toxicity in mice, but the increased dissolution of the coprecipitated digoxin did not cause such an increase.

REFERENCES

(1) J. Lindenbaum, M. D. Mellow, M. O. Blackstone, and V. P. Butler, Jr., *N. Engl. J. Med.*, **285**, 1344(1971).

(2) T. G. Vitti, D. Banes, and T. E. Byers, *ibid.*, **285**, 1433(1971).

(3) V. Manninen, J. Melin, and G. Hartel, *Lancet*, **2**, 434 (1971).

(4) T. R. D. Shaw, M. R. Howard, and J. Hammer, *ibid.*, **2**, 303(1972).

(5) P. F. Binnion and M. McDermott, *ibid.*, **2**, 592(1972).

(6) J. G. Wagner, M. Christensen, E. Sakmar, D. Blair, J. D. Yates, P. K. Willis, III, A. J. Sedman, and R. G. Stoll, *J. Am. Med. Assoc.*, **224**, 199(1973).

(7) J. Lindenbaum, M. P. Butler, Jr., J. E. Murphy, and R. M.

Creswell, *Lancet*, **1**, 1215(1973).
(8) D. H. Huffmann and D. L. Azarnoff, *J. Am. Med. Assoc.*, **222**, 957(1972).
(9) F.D.C. Reports, "The Pink Sheet," **36** (28)(1974).
(10) A. T. Florence, E. G. Salole, and J. B. Stenlake, *J. Pharm. Pharmacol.*, **26**, 480(1974).
(11) A. J. Jounela and A. Sothman, *Lancet*, **1**, 202(1973).
(12) T. D. R. Shaw, J. E. Carless, M. R. Howard, and K. Raymond, *ibid.*, **2**, 209(1973).
(13) B. F. Johnson, H. Greer, T. McCrerie, C. Bye, and A. Fowle, *ibid.*, **1**, 1473(1973).
(14) M. Gibaldi, "Introduction to Biopharmaceutics," Lea & Febiger, Philadelphia, Pa., 1971, p. 21.
(15) K. Sekiguchi and N. Obi, *Chem. Pharm. Bull.*, **9**, 866 (1961).
(16) T. Tachibana and A. Nakamura, *Kolloid-Z. Polym.*, **203**, 130(1965).
(17) A. H. Goldberg, M. Gibaldi, and J. L. Kanig, *J. Pharm. Sci.*, **54**, 1145(1965).
(18) *Ibid.*, **55**, 482(1966).
(19) *Ibid.*, **55**, 487(1966).
(20) A. H. Goldberg, M. Gibaldi, J. L. Kanig, and M. Mayersohn, *J. Pharm. Sci.*, **55**, 581(1966).
(21) M. Mayersohn and M. Gibaldi, *ibid.*, **55**, 1323(1966).
(22) W. L. Chiou and S. Riegelman, *ibid.*, **58**, 1505(1969).
(23) M. Gibaldi, S. Feldman, and T. R. Bates, *ibid.*, **57**, 708(1968).
(24) *Ibid.*, **60**, 1569(1971).
(25) E. I. Stupak and T. R. Bates, *J. Pharm. Sci.*, **61**, 400(1972).
(26) M. H. Malone, H. I. Hochman, and K. A. Nieforth, *ibid.*, **55**, 972(1966).
(27) R. B. Stoll, T. R. Bates, and J. Swarbrick, *ibid.*, **62**, 65(1973).
(28) E. I. Stupak and T. R. Bates, *ibid.*, **62**, 1806(1973).
(29) S. N. Malik, D. H. Canham, and M. W. Gouda, *ibid.*, **64**, 987(1975).
(30) G. Levy and B. A. Hayes, *N. Engl. J. Med.*, **262**, 1053 (1960).
(31) D. E. Wurster and P. W. Taylor, *J. Pharm. Sci.*, **54**, 670(1965).
(32) P. Mesnard and G. Devaux, *C. R.*, **253**, 497(1961).
(33) W. Saski and S. G. Shah, *J. Pharm. Sci.*, **54**, 71(1965).
(34) "The Merck Index," 8th ed., Merck and Co., Rahway, N.J., 1968, p. 364.
(35) E. Shefter and W. I. Higuchi, *J. Pharm. Sci.*, **52**, 781(1963).
(36) W. L. Chiou and S. Niazi, *ibid.*, **60**, 1333(1971).
(37) *Ibid.*, **62**, 498(1973).
(38) W. L. Chiou, *J. Pharm. Sci.*, **60**, 1406(1971).
(39) "Pluronic® Polyols Toxicity and Irritation Data," 3rd ed., BASF Wyandotte Corp., Wyandotte, Mich.
(40) S. G. Frank, *J. Pharm. Sci.*, **64**, 1585(1975).
(41) J. L. Lach and W. A. Pauli, *ibid.*, **55**, 32(1966).
(42) A. T. Florence, *J. Pharm. Pharmacol.*, **22**, 265(1970).

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* Present address: Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

[x] To whom inquiries should be directed.

Electrochemical Evidence for Interaction between Chlorpromazine Hydrochloride and Trifluoperazine Hydrochloride and the Flavin Coenzymes

LENA A. DeLISSER-MATTHEWS [x] and ALI KHALAJ

Abstract □ Polarographic and chronopotentiometric methods were applied to study the effects of the phenothiazine tranquilizers chlorpromazine hydrochloride and trifluoperazine hydrochloride on the electrochemical behavior of the flavin coenzymes flavin mononucleotide and flavin adenine dinucleotide. The effects of the drugs were measured mainly by decreases in the diffusion currents, i_d, developed in the polarographic experiments and by a similar decrease in the chronopotentiometric constant, $i_0\tau^{1/2}$, in the chronopotentiometric experiments when the coenzymes were reduced in the presence of the added drugs. The observed interference with the redox properties of the coenzymes could conceivably be related to the reported ability of the drugs to inhibit respiration and produce their tranquilizing effect.

Keyphrases □ Chlorpromazine hydrochloride—effect on electrochemical behavior of flavin coenzymes, polarographic and chronopotentiometric investigation □ Trifluoperazine hydrochloride—effect on electrochemical behavior of flavin coenzymes, polarographic and chronopotentiometric investigation □ Flavin coenzymes—electrochemical behavior, effect of chlorpromazine and trifluoperazine hydrochlorides □ Electrochemical behavior—flavin coenzymes, effect of chlorpromazine and trifluoperazine hydrochlorides □ Polarography—investigation of effect of chlorpromazine and trifluoperazine hydrochlorides on electrochemical behavior of flavin coenzymes □ Chronopotentiometry—investigation of effect of chlorpromazine and trifluoperazine hydrochlorides on electrochemical behavior of flavin coenzymes □ Tranquilizers—chlorpromazine and trifluoperazine hydrochlorides, effect on electrochemical behavior of flavin coenzymes □ Coenzymes, flavin—electrochemical behavior, effect of chlorpromazine and trifluoperazine hydrochlorides

One mechanism proposed to account for the tranquilizing action of the phenothiazine drugs involves the possible interaction between the drugs and certain electron-transferring coenzymes of the respiratory chain. The inhibitory effects of chlorpromazine on respiration were suggested to result from complex formation between chlorpromazine and flavin adenine dinucleotide (1). The studies involved observation of changes in the fluorescent and spectrophotometric properties of the coenzymes upon the addition of the drug.

Similar inhibitory effects were reported in studies of the effects of chlorpromazine upon the mitochondrial systems of both the brain and heart of rats (2, 3). It was