(as in carboxylic acid anions) may be destabilizing, positive charge (as in protonated amines) will always be destabilizing, but delocalized negative charge (as in phenol anions) may be stabilizing.

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Improvement of the Oral Bioavailability of Digitalis Glycosides by Cyclodextrin Complexation

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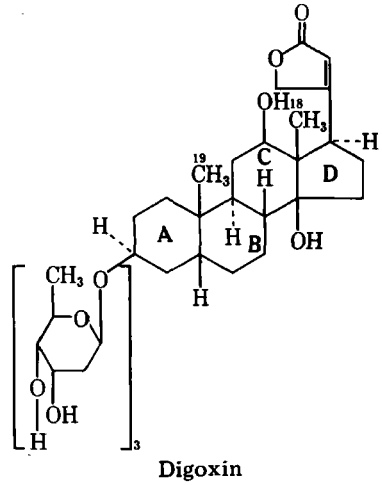
Abstract □ Inclusion complexes of the digitalis glycosides digitoxin, digoxin, and methyl digoxin with three cyclodextrins (α-, β-, γ-homologues) in water and in the solid state were studied by a solubility method, IR and ^{1}H-NMR spectroscopy, and X-ray diffractometry. Solid complexes (in a molar ratio of 1:4) of the digitalis glycosides with γ-cyclodextrin were prepared and their *in vivo* absorption examined. The rapidly dissolving form of the γ-cyclodextrin complex significantly increased plasma levels of digoxin (~5.4-fold) after oral administration to dogs.

Keyphrases □ Bioavailability—oral, digoxin, digitoxin, methyl digoxin, complexation with cyclodextrins □ Digoxin—complexation with cyclodextrins, oral bioavailability, digitoxin, methyl digoxin □ Cyclodextrins—complexation with digitalis glycosides, oral bioavailability, digoxin, digitoxin, methyl digoxin

The bioavailability of the digitalis glycosides from commercial tablets varies significantly (1–3). The main cause of this variability appears to be related to such factors as low water solubility (4–6) and chemical instability in acidic media (7–9). Cyclodextrins have been used extensively to improve various physicochemical properties of drug molecules (10–12) by forming inclusion complexes in which the drug molecules are included in the relatively hydrophobic cavity of the cyclodextrins (13).

The present study describes the inclusion complexes of the digitalis glycosides digitoxin, digoxin, and methyl digoxin with the three cyclodextrins (α-, β-, γ-homologues). Complex formation in water and in the solid state was studied by a solubility method, IR and ^{1}H-NMR spectroscopy, and X-ray diffractometry. Plasma levels of digoxin were determined after the oral administration of the digoxin–γ-cyclodextrin complex to dogs.



Digoxin

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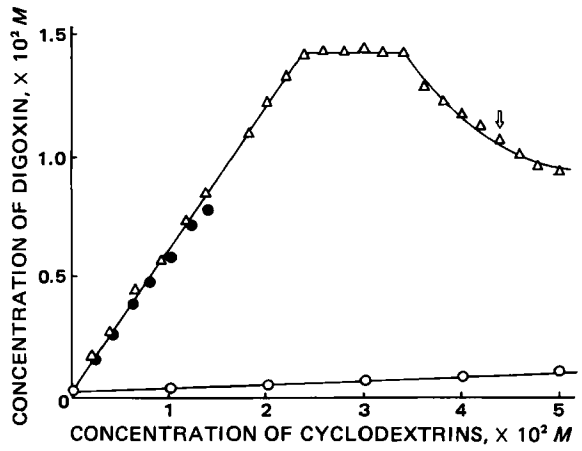

Figure 1—*Phase solubility diagrams of the digoxin–cyclodextrin systems in water at 25°. Key: (○) α-cyclodextrin; (●) β-cyclodextrin; and (△) γ-cyclodextrin. The arrow indicates the experimental conditions for the preparation of solid γ-cyclodextrin complexes (see* text*).*

EXPERIMENTAL

Materials—Digitoxin[1], digoxin[2], methyl digoxin[2], and the α-, β-, and γ-cyclodextrins[3] were used as supplied. All other materials and solvents were analytical reagent grade. Deionized double-distilled water was used.

Solubility Studies—Solubility measurements were carried out according to Higuchi and Lach (14). Excess amounts of digitalis glycosides were added to an aqueous solution containing various concentrations of cyclodextrins and were shaken at 25 ± 0.5°. After equilibrium was reached (~7 days), an aliquot was centrifuged and pipetted through a cotton filter. A 0.5-ml aliquot was diluted with ethanol–water (1:1, v/v) and analyzed spectrophotometrically[4]. An apparent stability constant, K', was calculated from the initial linear portion of the phase solubility diagram according to the following (15):

$$K' = \frac{\text{slope}}{\text{intercept} \cdot (1 - \text{slope})}$$

Preparation of Solid Complexes—The solid complexes were prepared by mixing appropriate amounts of the cyclodextrin and digitalis glycoside in water. Appropriate mixtures were chosen by examining the descending curvature of the B_s-type phase solubility diagram (see Fig. 1). For example, 3.25 g of digoxin and 14.3 g of γ-cyclodextrin were added to 250 ml of water, and the mixture was stirred at 25° for 7 days. The complex, which precipitated as a microcrystalline powder, was removed by filtration and dried under vacuum at 60° for 48 hr. This powder corresponded to a 1:4 digoxin–γ-cyclodextrin complex, which had a molecular weight of 5965.

Spectroscopic Studies—The IR spectra[5] were measured as potassium bromide pellets. ^{1}H-NMR spectra[6] were measured in D_2O using Fourier transform methodology (16). An average of 6000 accumulations with 8192 data points were made at a sweep width of 2000 Hz. The ^{1}H-chemical shifts were assigned values based on the external standard 3-(trimethylsilyl)propanesulfonic acid sodium salt with an accuracy of ±0.0012 ppm. The powder X-ray diffraction spectra[7] were obtained by scanning at 1°/min in terms of 2θ angle.

Dissolution Studies—The digoxin powder (150 mg, 100 mesh) was compressed into a cylindrical tablet (diameter 10 mm) at a pressure of ~200 kg/cm^2. The release of drug was measured using a rotating disk apparatus (17) in 0.05 M KCl–HCl solution (pH 1.52) at 37°. At appropriate intervals, 1-ml samples were removed, extracted with chloroform, and assayed by high-performance liquid chromatography (HPLC). The dissolution behavior of 1:4 digitalis glycoside–γ-cyclodextrin complexes (150 mg, 100 mesh) was examined in a similar manner.

HPLC Analysis—The chromatograph[8] was operated at a flow rate of 0.7 ml/min. The separation utilized a column[9] (40 mm × 25 cm) with methanol–water (58:42) as a mobile phase, and the eluant was monitored spectrophotometrically at 220 nm. Components were quantitated by measuring peak heights and comparing the height with an internal standard containing a known amount of prednisolone[2].

***In Vivo* Absorption Studies**—Six female beagle dogs, with an average weight of 11 kg, were used. A tablet was administered orally with 20 ml of water after an overnight fast at intervals of at least 1 week. The administration sequence was based on a crossover matrix designed to minimize any residual or cumulative effects of the preceding dose. The formulations of the tablets were as follows: 98–99% (w/w) lactose as a diluent, 1.0% (w/w) magnesium stearate as a lubricant, and 0.1% (w/w) digoxin or 0.76% (w/w) digoxin–γ-cyclodextrin complex. Plasma samples were obtained at timed intervals, and digoxin concentrations were determined by enzyme immunoassay[10] (18).

Table I—Apparent Stability Constants (M^{-1}) of Digitalis Glycoside–Cyclodextrin Complexes Determined by the Solubility Method in Water at 25°

Digitalis Glycoside	Cyclodextrin α	Cyclodextrin β	Cyclodextrin γ
Digoxin	180	11200	12200
Digitoxin	290	17000	63600
Methyl digoxin	400	11400	13600

RESULTS AND DISCUSSION

Solubility Study—The complexing behavior of digitalis glycosides with cyclodextrins in water was studied by a solubility method. The phase solubility diagrams obtained for digoxin with three cyclodextrins in water are shown in Fig. 1. In the case of α-cyclodextrin, the solubility of digoxin increased slightly in a linear fashion as a function of the α-cyclodextrin concentration, and the resulting solubility curve can be classified as type A_L (15). The solubility plot for the β-cyclodextrin system is qualitatively similar to that for α-cyclodextrin, where a large increase in digoxin solubility was obtained within the solubility limit of β-cyclodextrin (~1.6 × 10^{-2} M). On the other hand, γ-cyclodextrin showed a typical B_S-type solubility curve (15), where the initial ascending portion is followed by a plateau region and then a decrease in total digoxin solubility accompanied by precipitation of a microcrystalline complex. Although the shape of solubility curves in Fig. 1 cannot be completely explained in terms of a stoichiometric relationship (19), an apparent stability constant (K'), as a tentative measure of inclusion complexation, was estimated from the equation based on the assumption that a 1:1 complex is initially formed. Table I summarizes the K' values for digitalis glycoside–cyclodextrin systems calculated from the initial ascending portion of solubility diagrams. The cavity size dependencies of the present systems were clearly noted from the magnitude of K' values (γ- > β- > α-cyclodextrin).

Since the digitalis glycoside molecule is too large to be included within the cyclodextrin cavity, it is reasonable to assume that at least one complex with a stoichiometric host-to-guest molecular ratio greater than one may be formed, in particular for the higher concentrations of cyclodextrin. To gain insight into the stoichiometry of the γ-cyclodextrin system, the solid material that precipitated beyond the plateau region was analyzed (19). The analysis of the digoxin–γ-cyclodextrin system gave the following results for 10^2L_t (the total concentration of γ-cyclodextrin) and X_s (the mole fraction of digoxin in the solid): 3.6, 0.20; 4.0, 0.20; 4.2, 0.20; 4.4, 0.20; 4.8, 0.20; and 5.0, 0.20. These data indicate that 1:4 complex formation of digoxin with γ-cyclodextrin predominate beyond the plateau region, while the lower-order complexes (*i.e.*, 1:1, 1:2, and 1:3) may be formed around the initial increasing portion of the solubility diagram (Fig. 1). Similar results were obtained for γ-cyclodextrin with digitoxin and methyl digoxin. Since only the 1:4 solid complex was isolated from the descending curvature of the B_S-type solubility diagram (probably because of limited solubility), this form was used for further study.

NMR Study—^{1}H-NMR techniques (200 MHz) were employed to examine the inclusion mode in aqueous solution. Table II summarizes a typical example of the effects of cyclodextrins on some ^{1}H-chemical shifts (18-methyl and 19-methyl) of digoxin (20). Unfortunately, the other proton signals were too weak to be quantitatively analyzed under the experimental conditions used. In the presence of cyclodextrins, both

[1] Shionogi Pharmaceutical Co. Ltd., Osaka, Japan.
[2] Mitsubishi Yuka Pharmaceutical Co. Ltd., Ibaraki, Japan.
[3] Nippon Shokuhin Kako Ltd., Tokyo, Japan.
[4] Hitachi 556S; Hitachi Ltd., Tokyo, Japan.
[5] JEOL JIR-40; JEOL Ltd., Tokyo, Japan.
[6] JEOL JNM-FX 200; JEOL Ltd., Tokyo, Japan.
[7] Rigaku Denki Geiger Flex 2012; Rigaku Denki Co. Ltd., Tokyo, Japan.
[8] ATTO HSLC-013-4; ATTO Co., Tokyo, Japan.
[9] LiChrosorb RP-18 (5 μm); E. Merck, Darmstadt, West Germany.
[10] Digoxin batch assay Emit cad.; Syva Co., U.S.A.

Table II—Effects of Cyclodextrins on ^{1}H-Chemical Shifts of Some Digoxin Protons in Deuterium Oxide [a]

Protons[b]	Without Cyclodextrins	^{1}H Increment[c] with Cyclodextrins, ppm		
		α	β	γ
18-Methyl	0.772	0.038	0.055	0.040
19-Methyl	0.932	0.017	0.198	0.081

[a] Concentrations of digoxin and cyclodextrins were 1.0×10^{-4} *M* and 4.0×10^{-4} *M*, respectively. [b] Assigned according to Ref. 20. [c] The values are positive if any upfield shift of the resonance occurs.

signals moved downfield probably due to the steric perturbation through inclusion complexation (21). Interestingly, the downfield shift of the 19-methyl signal was apparently greater than that of the 18-methyl signal, particularly for β-cyclodextrin. This suggests that the A-ring moiety of the digoxin molecule may strongly interact with β-cyclodextrin. The magnitude of the downfield shifts of both methyl protons decreased in the order of β- > γ- > α-cyclodextrin. The effect of digoxin on ^{1}H-chemical shifts of cyclodextrins was also examined. All cyclodextrin protons located within or near the cavity (*e.g.*, H-3, H-5, or H-6) experienced a shielding effect, where the magnitude of the upfield shift decreased in the order of β- > γ- > α-cyclodextrin. These NMR data indicate that the digoxin molecule is located at the entrance of the α-cyclodextrin cavity, could penetrate further into the β-cyclodextrin cavity, and is loosely bound to γ-cyclodextrin.

Since β-cyclodextrin induced a chemical shift change that was the largest among the three cyclodextrins (Table II), ^{1}H-chemical shift displacements ($\Delta\delta$) of the 19-methyl signal of digoxin on addition of β-cyclodextrin were quantitatively examined. Figure 2 shows the plots of molar ratio of β-cyclodextrin–digoxin *versus* change in chemical shift (δ) of the 19-methyl signal. Above a molar ratio of 4 for β-cyclodextrin–digoxin, no further substantial changes of the chemical shifts occur. This suggests that the stoichiometric equivalence point is reached to form 1:4 complexes of digoxin with β-cyclodextrin. A similar stoichiometric relationship was expected for digoxin–γ-cyclodextrin, which is consistent with the result of the solubility study. Inspection of a space-filling molecular model[11] shows that four molecules of β- or γ-cyclodextrin are available for the complete inclusion of the digoxin; digoxin fits tightly into β-cyclodextrin channels and more loosely into the larger interior space of γ-cyclodextrin channels.

Evidences of Complex Formation in Solid State—To confirm the complexation of cyclodextrins with digitalis glycosides in the solid state, X-ray diffractometry and IR spectroscopy were employed and compared with the corresponding physical mixtures in the same molar ratio. Figure 3 shows the powder X-ray diffraction patterns of the digoxin–γ-cyclodextrin complex and their physical mixture. The diffraction pattern of the physical mixture was simply the superposition of each component, while that of γ-cyclodextrin complex was apparently different from each constituent and constituted a new solid phase. The γ-cyclodextrin complex gave a somewhat diffuse diffraction pattern, suggesting that it is less crystalline than the physical mixture. Figure 4 shows the IR spectra of the digoxin–γ-cyclodextrin complex in the carbonyl-stretching region of the C-23 carbonyl group in digoxin (22). In the case of the γ-cyclodextrin complex, the 1720 cm^{-1} band was found to shift to 1743 cm^{-1},

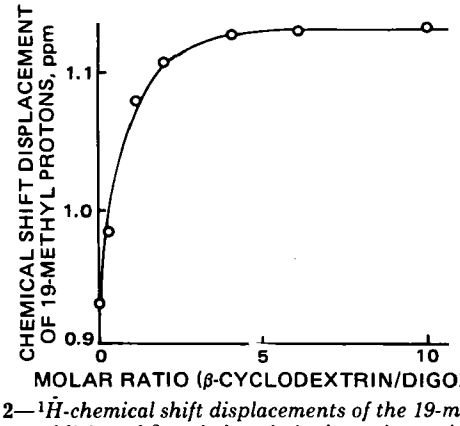

Figure 2—*^{1}H-chemical shift displacements of the 19-methyl signal of digoxin on addition of β-cyclodextrin in deuterium oxide.*

[11] Corey–Pauling–Koltun molecular model.

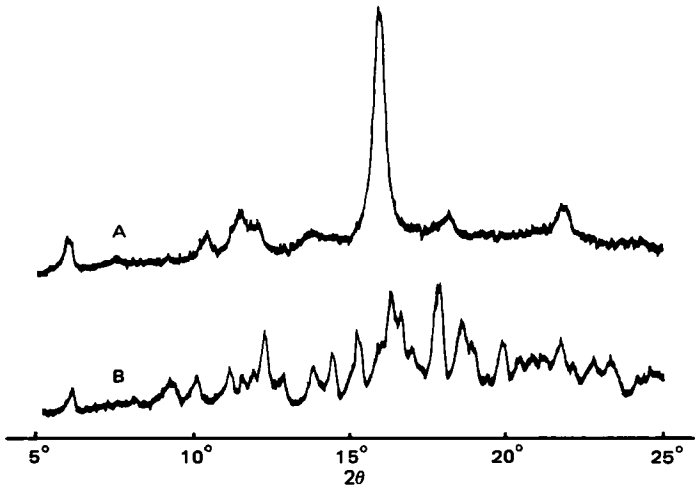

Figure 3—*Powder X-ray diffraction patterns of the digoxin–γ-cyclodextrin system. Key: (A) 1:4 complex of digoxin with γ-cyclodextrin; (B) physical mixture of digoxin and γ-cyclodextrin in 1:4 molar ratio.*

suggesting the dissociation of the intermolecular hydrogen bonds of digoxin through inclusion complexation. Similar results were obtained for other glycoside–γ-cyclodextrin systems. These data clearly indicate that the digitalis glycoside–γ-cyclodextrin complexes exist in the solid state.

Dissolution Behavior of Inclusion Complex—Figure 5 shows a typical example of the dissolution profiles of digoxin and 1:4 digoxin–γ-cyclodextrin complex from the rotating disk with constant surface area in acidic medium (pH 1.52) at 37°, where intact digoxin was quantitatively determined by HPLC. It is evident that the complexed form of digoxin dissolved much more rapidly (~100-fold) than digoxin itself. Similar results were obtained for other digitalis glycoside–γ-cyclodextrin systems. The observed increase in rate may be due to an increase in solubility and/or a decrease in crystallinity of the drug by inclusion complexation (23). It is interesting to note that the dissolution profile of the complex showed a negative curvature with time. This dissolution data may provide evidence for a complicated system, *i.e.*, decrease in concentration of intact digoxin due to acid hydrolysis or change in the tablet surface of the complexed digoxin during the dissolution process. This preliminary study revealed that γ-cyclodextrin significantly retarded the hydrolysis of digoxin in an acidic medium, which is described in detail in a separate paper (24). It seems most likely that the digoxin is precipitating on the surface of the tablet as it dissociates resulting in negative curvature of the dissolution curve, since the NMR data in Table II suggested that the inclusion of digoxin into the γ-cyclodextrin cavity is not tight. Although the complex appears to dissociate rather quickly after dissolution, the initial decrease in dissolution rate together with improved chemical stability in acidic medium suggests that the γ-cyclodextrin complexes of digitalis glycosides may have good oral bioavailability.

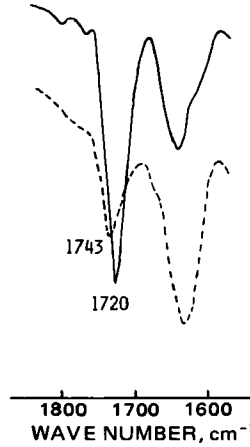

Figure 4—*IR spectra of the digoxin–γ-cyclodextrin system, measured by the potassium bromide disk method. Key: (- - - -) 1:4 complex of digoxin with γ-cyclodextrin; (—) physical mixture of digoxin and γ-cyclodextrin in 1:4 molar ratio.*

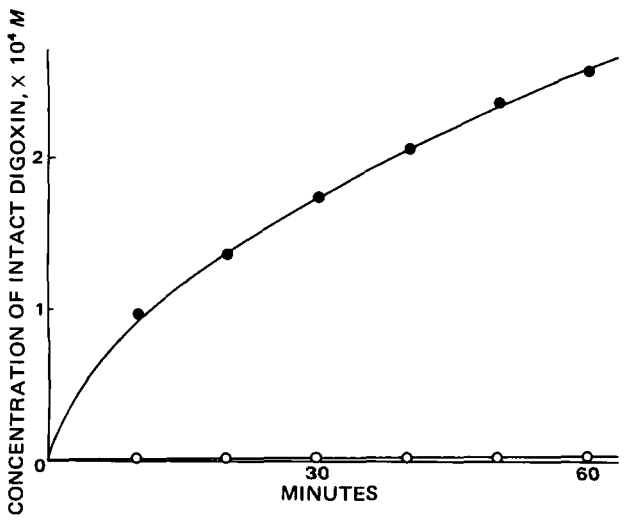

Figure 5—*Dissolution curves of digoxin and its γ-cyclodextrin complex in acidic medium (pH 1.52) at 37°, measured by the rotating disk method. Key: (○) digoxin; (●) 1:4 complex of digoxin with γ-cyclodextrin.*

Bioavailability of Digoxin–γ-Cyclodextrin Complex—The *in vivo* absorption study was undertaken to find out if the *in vitro* dissolution enhancement of digoxin from its γ-cyclodextrin complex increases the GI absorption of the drug. Finely powdered digoxin and its γ-cyclodextrin complex were compressed to tablets (average weight 50 mg) to give a final digoxin content of 0.1 or 0.05%. Figure 6 shows the mean plasma levels of digoxin following the oral administration of tablets to dogs, where the concentration of digoxin in the plasma sample was determined by enzyme immunoassay (18). When equivalent doses of digoxin (100 μg) were administered to dogs, the γ-cyclodextrin complex attained maximum plasma levels of 0.90 ± 0.14 mg/liter at 45 min, which was ~3 times higher than that of digoxin alone (25). The area under the plasma concentration–time curve (AUC) of the complex up to 24 hr was found to be 5.4 times as much as that of digoxin alone. Furthermore, the AUC of the γ-cyclodextrin complex containing 50 μg of digoxin was also found to be superior to that of 100 μg of digoxin alone. Therefore, the enhanced bioavailability of digoxin by γ-cyclodextrin complexation suggests that the complex offers a decrease in dose and fewer side effects in oral digitalis glycoside therapy. It should be noted also that the 1:4 complexation of digitalis glycosides with γ-cyclodextrin results in an ~8-fold increase in the molecular weight of the drug, which may facilitate the pharmaceutical preparation, particularly the content uniformity test.

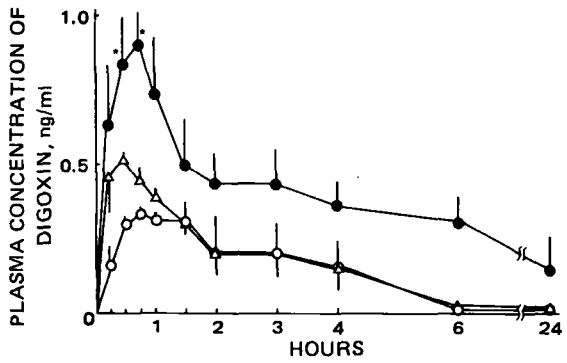

Figure 6—*Plasma levels of digoxin following the oral administrations of tablets containing digoxin or 1:4 digoxin–γ-cyclodextrin complex to dogs. Each point represents the mean ±SE of six dogs. Key: (○) 100-μg digoxin tablet; (●) γ-cyclodextrin complex tablet containing 100 μg of digoxin; (△) γ-cyclodextrin complex tablet containing 50 μg of digoxin; (*) $p < 0.01$, (●) versus (○).*

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