Ionic strength and ionic specificity were found to have a significant influence on the rectal absorption of gentamicin. Sodium was more effective than potassium in promoting rectal absorption, but the enhancing effect of sodium salicylate could not be totally explained on the basis of ionic strength. These data, while helping to elucidate some parameters that affect rectal drug absorption, may offer potential insights into new formulation designs for systemic delivery of water-soluble drugs from the rectal compartment.

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Effect of Quinidine on Digoxin Distribution and Elimination in Guinea Pigs

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Abstract ☐ The effect of quinidine on the distribution and elimination of digoxin was examined by comparing the change in the steady-state volume of distribution (Vd_{ss}), determined both from *in vivo* plasma elimination and tissue distribution and *in vitro* serum binding studies, with that in the total body clearance (CL_{tot}) determined from biliary, renal, and metabolic clearances in guinea pigs. The plasma disappearance of digoxin after a 250-μg/kg iv dose followed a triexponential decline in both the control and quinidine-treated guinea pigs. In the quinidine-treated guinea pigs, the pharmacokinetic parameters Vd_{ss} and CL_{tot} significantly decreased to approximately half of that for the control guinea pigs. The tissue-to-plasma partition coefficients (K_p) of all tissues studied, *i.e.* liver, heart, muscle, and brain, at 6 hr after bolus injection of digoxin decreased in the presence of quinidine. The serum free fraction and the plasma-to-blood concentration ratio of digoxin in the therapeutic range did not show a significant alteration in the presence of quinidine. This suggested that the decrease of K_p is due mainly to the inhibition of tissue distribution of digoxin by quinidine. The biliary clearance (CL_B) and renal clearance (CL_R) also significantly decreased in the presence of quinidine. It was concluded that quinidine caused a inhibition of digoxin in the tissue binding or uptake, which significantly decreased the K_p values of digoxin; this result may explain the significant decrease of Vd_{ss}. Moreover quinidine may be the cause of a reduction of biliary, renal, and metabolic clearances, which significantly decrease the CL_{tot} of digoxin.

Keyphrases ☐ Quinidine—effect on the distribution and elimination of digoxin, guinea pigs ☐ Digoxin—pharmacokinetics, effect of quinidine coadministration, guinea pigs ☐ Pharmacokinetics—digoxin in the guinea pig, effect of quinidine coadministration

When quinidine is given to patients (1–5), dogs (6), or guinea pigs (7) receiving digoxin, the serum digoxin concentration increases. Reduction in the total body clearance (CL_{tot}) (5, 6) and the volume of distribution (Vd) (5, 6, 8) of digoxin has been observed and accounts for the elevated digoxin concentration. Quinidine has been reported to diminish the renal clearance (CL_R) of digoxin in humans (2, 4, 5, 8, 9) and dogs (10, 11) without significantly altering the glomerular filtration rate as measured by the creatinine clearance. Doherty *et al.* (12) reported that quinidine reduced the canine skeletal and heart muscle concentrations of digoxin, while increasing concentrations in the plasma and brain. Straub *et al.* (13) showed that quinidine reduced the number of digitalis-binding sites, as determined by *in vitro* binding studies with Na^+,K^+-ATPase from bovine heart membrane. Evidence has been reported that quinidine was capable of decreasing the affinity for digoxin of cardiac glycoside receptor sites on purified Na^+,K^+-ATPase in guinea pigs and on intact human erythrocyte membranes (14).

The present study examined the effect of quinidine on the distribution and elimination of digoxin by comparing the changes in Vd and CL_{tot} *in vivo*, which were determined from the tissue distribution, metabolism, excretion, serum protein binding, and plasma-to-blood distribution ratio. As a model animal for digoxin–quinidine interaction in the human, the guinea pig, a species in which digoxin distribution appears similar to that observed in the human, was selected.

EXPERIMENTAL

Digoxin[1] and quinidine sulfate[2] were used. Tritiated digoxin, labeled at the 12α-position (14.0 Ci/mmole)[3], which was found to be at least 99% pure by TLC, was used as the radioactive compound. All other reagents were commercially available and analytical grade.

Animal Experiments—Adult male Hartley guinea pigs[4], weighing 280–300 g, were used. Under light ether anesthesia, the jugular vein and carotid artery were cannulated with polyethylene tubing[5]. For the biliary and urinary excretion studies, bile fistula and urinary bladder cannulation were used to collect samples of bile and urine, respectively. Cannulated animals were kept in restraining cages with access to water under normal housing conditions prior to the experiments.

The guinea pigs were simultaneously given 250 μg/kg of digoxin (containing 100 μCi/kg of 12α-[^{3}H]digoxin) in 40% ethanol solution and 25 mg/kg of quinidine sulfate in physiological saline through the jugular vein cannula. The digoxin solution containing 40% ethanol was administered alone to the control guinea pigs. Blood samples (0.25 ml) were obtained for the determination of digoxin at 1, 5, 30, 60, 120, 180, 240, 300, and 360 min, and for the determination of quinidine (in different animals) at 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min in heparinized poly-

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Tokyo Kasei Co., Tokyo, Japan.
[3] New England Nuclear Co., Boston, Mass.
[4] Nihon Seibutsu Zairyo, Tokyo, Japan.
[5] PE-10 for jugular vein and PE-50 for carotid artery: Clay Adams, Becton, Dickinson & Co., Parsippany, N.J.

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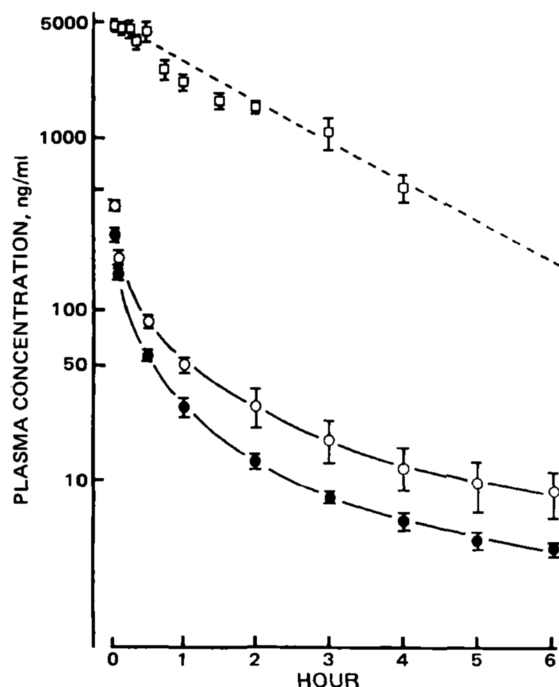

Figure 1—*Plasma disappearance curves of digoxin after a 250-μg/kg iv dose. Each point and vertical bar represent the mean and standard error of four (control) or three (quinidine-treated) guinea pigs. Curves were calculated by the least-squares method (17) using a digital computer. Key: (●) plasma digoxin concentration of control guinea pigs; (○) plasma digoxin concentration of quinidine-treated guinea pigs (digoxin simultaneously administered with 25 mg/kg of quinidine); (□) plasma concentration of quinidine.*

ethylene centrifuge tubes[6]. Bile samples were obtained at 15, 30, or 60 min, while the urine sample was collected for 360 min. The body temperature was kept at 37° using a heat lamp. Plasma was separated by centrifugation for 20 sec[7].

For the tissue distribution study the animals were sacrificed at 1 and 6 hr after digoxin administration by an injection of air into the carotid artery. After removal of blood samples, the brain, heart, liver, and muscle were quickly excised, rinsed with cold saline, blotted, and weighed. All tissues and plasma were stored at −40° until assayed. Tissue samples were homogenized with a three-fold excess of physiological saline[8]. The separation of the metabolites from digoxin was carried out using the method of Harrison and Gibaldi (15). A 100-μl quantity of the plasma sample and 1 ml of the tissue homogenate were shaken for 10 min and then extracted twice with 2 ml of chloroform–methanol (1:1, v/v). The pooled extracts were then streaked on silica gel plates and chromatographed. Glass sheets precoated with silica gel 60[9] of 0.25 mm thickness were developed twice in ethyl acetate–chloroform–acetic acid (90:5:5, v/v/v). Digoxin was visualized by spraying the plate with 3,5-dinitrobenzoic acid and 2 *N* KOH in methanol to develop a violet color. The spots attributable to digoxin from chromatograms of biological samples were scraped into scintillation vials containing 10 ml of scintillation fluid[10]. The extraction coefficients of digoxin from plasma, brain, heart, liver, and muscle were 0.85, 0.45, 0.68, 0.55, and 0.86, respectively. The concentration of ^{3}H-labeled digoxin was determined[11]. Quinidine concentration was determined by the double-extraction method according to Crámer and Isaksson (16).

Serum Protein Binding—Serum was separated from the blood, obtained through the carotid artery, by centrifugation for 10 min at 3000 rpm after standing for 60 min at room temperature. The serum free fraction of digoxin was determined by equilibrium dialysis at 37° for 16 hr using semimicrocells[12] and a semipermeable membrane[13] against

Table I—Digoxin Pharmacokinetics in Guinea Pigs [a]

Parameter	Control (Digoxin)	Digoxin plus Quinidine
P, μg/ml	158.6 ± 13.3	575.3 ± 204.2
π, min^{-1}	0.126 ± 0.042	0.764 ± 0.442
A, μg/ml	68.6 ± 23.6	134.9 ± 5.9
α, min^{-1}	0.022 ± 0.005	0.0308 ± 0.0041
B, μg/ml	11.5 ± 2.5	33.2 ± 11.8
β, min^{-1}	0.00313 ± 0.00067	0.00388 ± 0.00035
Vd_{ss}, liter/kg [b]	5.25 ± 0.78	2.76 ± 0.31 [f]
CL^p_{tot}, ml/(min kg) [c]	31.5 ± 2.6	17.3 ± 3.6 [f]
CL_R, ml/(min kg) [d]	3.4 ± 0.8	0.9 ± 0.5 [f]
CL_B, ml/(min kg) [d]	4.9 ± 0.5	0.9 ± 0.1 [f]
CL_M, ml/(min kg) [e]	23.2	15.5

[a] Results are given as the mean ± *SE* of three or four animals. [b] The volume of distribution at steady state (Vd_{ss}) was calculated by a conventional equation (18) using triexponential equation constants from plasma disappearance curves. [c] The total plasma clearance (CL^p_{tot}) was calculated using CL^p_{tot} = dose/AUC. [d] The biliary clearance (CL_B) and the renal clearance (CL_R) were calculated using the equation in the text. [e] The mean metabolic clearance (CL_M) was calculated by $CL_M = CL_{tot} - CL_B - CL_R$ (see text). [f] Significantly different ($p < 0.05$) from the control guinea pigs.

Krebs–Ringers buffer (pH 7.4), containing 0.6–46.8 ng/ml of [^{3}H]digoxin and 1 and 10 μg/ml of quinidine. The protein binding of digoxin to serum was unchanged between 16 and 20 hr of dialysis at 37°. The quinidine concentration in the protein chamber after dialysis was in the same range as that of the *in vivo* concentration of quinidine (0.5–5 μg/ml) in plasma.

Plasma-to-Blood Concentration Ratio of Digoxin—All procedures were carried out immediately after the blood collection. The blood was incubated with 1 μCi/ml of [^{3}H]digoxin and various amounts of nonlabeled digoxin (1–300 ng/ml as blood concentration) at 37° for 20 min in the presence of quinidine (1 and 10 μg/ml). Preliminary experiments indicated that equilibration was attained within 30 sec between plasma and red blood cells (unpublished data). After centrifugation, an aliquot of the plasma was removed and the concentration of [^{3}H]digoxin was determined as described above. An analytical blank without substrate was determined in the same manner. The hemolysis during the incubation was negligible.

Data Analysis—The digoxin concentration data for individual animals were fitted to the equation $C_t = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$ for the plasma concentration C_t at time t by nonlinear least-squares regression (17). Pharmacokinetic constants (Table I) were determined from the three-exponential equation constants, *i.e.*, P, π, A, α, B, and β, using conventional equations (18). All means are presented with their standard error (the mean ± *SE*). The Student's *t* test was utilized to determine significant differences between the control and the quinidine-treated groups.

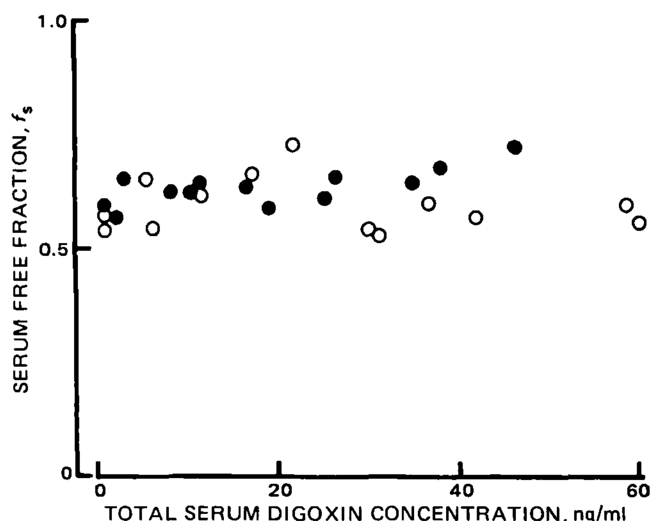

Figure 2—*Serum free fraction as a function of total blood concentration of digoxin with (○) and without (●) quinidine. Equilibrium dialysis was performed at 37° for 16 hr against Krebs–Ringer buffer (pH 7.4) containing 0.61–46.8 ng/ml of [^{3}H]digoxin. The concentration of quinidine was 1 and 10 μg/ml.*

[6] Beckman Instruments, Fullerton, Calif.
[7] Table-top microfuge; Beckman Instruments, Fullerton, Calif.
[8] Teflon glass homogenizer.
[9] Without F; E. Merck, Darmstadt, West Germany.
[10] 0.1 g of 1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene, 4.0 g of 2,5-diphenyloxazole, and 500 ml of Triton X-100/liter of toluene.
[11] Aloka Tri-Carb counter; Aloka Instruments Co., Tokyo.
[12] Kokugo-Gomu Co., Tokyo, Japan.
[13] Type 36/32; Visking Co., Chicago, Ill.

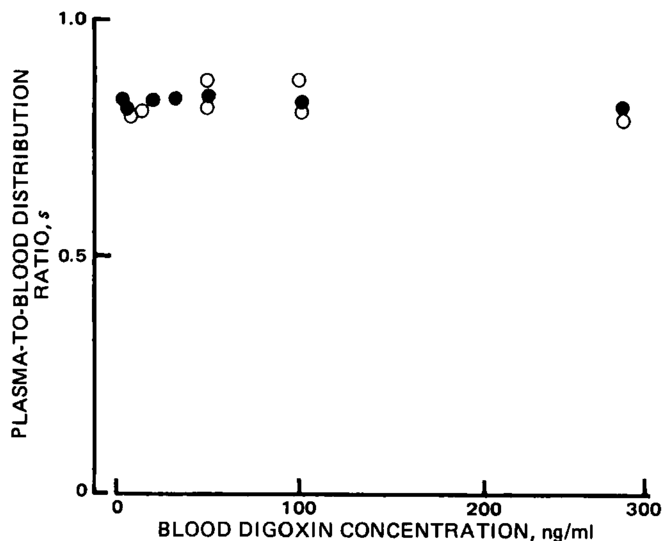

Figure 3—*Plasma-to-blood concentration ratio of digoxin as a function of total plasma concentration with (○) and without (●) quinidine. The blood was incubated with 1–300 ng/ml of [^3H]digoxin (as the blood concentration) at 37° for 20 min. The concentration of quinidine was 1 and 10 µg/ml.*

RESULTS

Effect of Quinidine on Digoxin Elimination from Plasma—The plasma disappearance of digoxin after intravenous administration of 250 µg/kg in the presence and absence of quinidine is shown in Fig. 1. The disappearance of digoxin followed three-exponential curves in both the control and quinidine-treated guinea pigs. The range of the plasma quinidine concentration was 0.5–5 µg/ml during the sampling period of 4 hr. The pharmacokinetic constants were computed by a nonlinear iterative least-squares method (17) and are listed in Table I. In the quinidine-treated guinea pigs, a significant decrease was observed in the total body plasma clearance (CL^{p}_{tot}). The Vd_{ss} was decreased significantly by quinidine.

Effect of Quinidine on Serum Protein Binding of Digoxin—The results from serum protein binding experiments are shown in Fig. 2. In both the experiments, *i.e.*, with and without quinidine, the serum binding of digoxin exhibited apparent linear relationships. The serum free fraction (f_s) did not show a significant alteration in the presence of quinidine.

Plasma-to-Blood Concentration Ratio of Digoxin—The plasma-to-blood concentration ratio of digoxin in the dose range from 1 to 300 ng/ml were determined in the presence and absence of quinidine (Fig. 3). The ratio seems to be constant over the dose range studied and did not show a significant alteration in the presence of quinidine.

Effect of Quinidine on Tissue Distribution of Digoxin—Table II demonstrates the changes in the tissue concentrations of digoxin at 1 and 6 hr after intravenous bolus administration of 250 µg/kg. It is apparent that the tissue concentration of digoxin did not show a significant alteration in the quinidine-treated guinea pigs. There are two exceptions: the liver, with a 50% decrease (significantly different from the control group, $p < 0.05$) in the quinidine-treated group and the brain, where a 370% increase (significantly different from the control, $p < 0.05$) occurred in the same group at 1 hr after intravenous bolus administration of digoxin. The apparent tissue-to-plasma partition coefficients (K_p) at 1 and 6 hr after intravenous bolus administration of digoxin in the presence and absence of quinidine are shown in Table II. The K_p values of most tissues studied significantly decreased in the quinidine-treated group, while the apparent brain-to-plasma concentration ratio at 1 hr significantly ($p < 0.05$) increased in the same group.

Effect of Quinidine on Biliary and Renal Excretion of Digoxin—In the quinidine-treated guinea pigs, no significant difference was observed in the mean bile flow rate for 6 hr when compared with that in the control animals (138.3 ± 16.1 µl/min/kg for the quinidine-treated group, $n = 3$, and 133.8 ± 17.4 for the control, $n = 3$). The biliary excretion rates of digoxin are shown in Fig. 4A; significant decreases ($p < 0.05$) were observed, except at 3 and 4 hr. Cumulative biliary excretion curves of digoxin are shown in Fig. 4B. The amount of digoxin excreted during 6 hr in the control guinea pigs was 13.8 ± 1.1% of the dose ($n = 3$), while that of the quinidine-treated group was 5.6 ± 1.1% ($n = 3$). The cumulative amount of digoxin excreted in urine during 6 hr after intravenous bolus administration in the control guinea pigs was 9.9 ± 2.3% of the dose ($n = 3$), while that of the quinidine-treated group was 5.2 ± 2.9% ($n = 3$). The mean biliary (CL_B) and renal clearances (CL_R) were calculated by:

$$CL_B \text{ or } CL_R = \frac{\text{Cumulative amount of digoxin excreted for 360 min}}{AUC_{0\text{–}360 \text{ min}}}$$

where AUC is the area under the plasma concentration *versus* time curve calculated from pharmacokinetic parameters listed in Table I. The CL_B and CL_R calculated by this equation in the presence and absence of quinidine are also listed in Table I. Both clearances significantly decreased in the presence of quinidine.

From the digoxin content in plasma, liver, and bile, the liver-to-plasma and the bile-to-liver concentration ratios were calculated (Table III). These ratios indicated that the transport of digoxin from plasma to liver and from liver to bile were against a large concentration gradient in the control guinea pigs. But after quinidine treatment, the liver-to-plasma concentration ratio significantly decreased, while the bile-to-liver concentration ratio did not show a significant alteration.

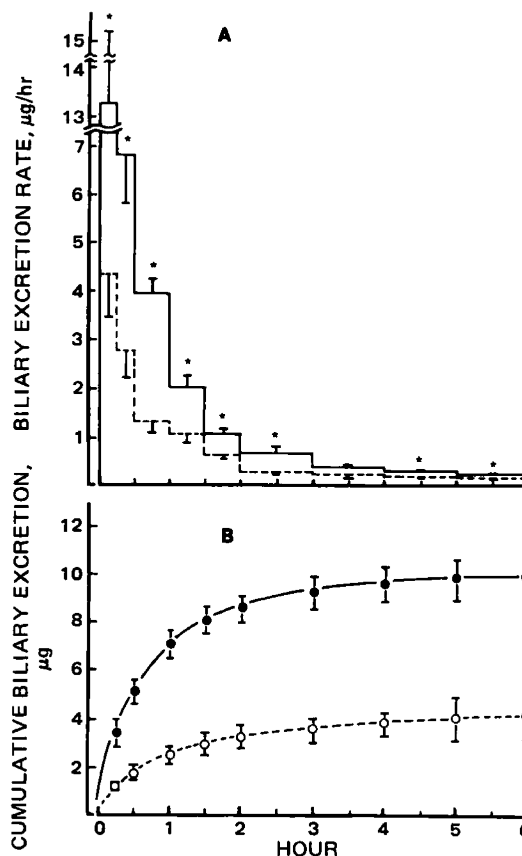

Figure 4—*Biliary excretion profile of digoxin after a 250-µg/kg iv dose. (A) Biliary excretion rate. Each datum and bar represents the mean and standard error of three control (—) quinidine-treated (---) guinea pigs; (*) statistically significant at p = 0.05 when compared with the biliary excretion rate of the control guinea pigs. (B) Cumulative biliary excretion curves. Each point and bar represents the mean and standard error of three control (●) or quinidine-treated (○) guinea pigs.*

DISCUSSION

The results of this study in guinea pigs are in agreement with previous studies, in which the increases in the serum (plasma) concentrations of digoxin were observed in the presence of quinidine in humans (1–5), dogs (6), and guinea pigs (7). Many investigators have reported that quinidine decreased Vd and CL_{tot} of digoxin in humans (5) and dogs (6). In this study the decreases of both parameters were also observed in guinea pigs (Table I). The lack of a substantial change in the elimination half-life ($t_{1/2}$) of digoxin suggested that the parallel changes in Vd and CL_{tot} tend to counterbalance each other. In plasma protein binding, no significant difference was observed in the serum free fraction of digoxin with or without quinidine (Fig. 2). This finding suggests that quinidine does not

Table II—Tissue Distribution of Digoxin in Guinea Pigs [a]

		1 hr [b]		6 hr [b]	
		Concentration, ng/g	K_p [c]	Concentration, ng/g	K_p [c]
Control (Digoxin)	Brain	2.3 ± 1.2	0.09 ± 0.05	12.5 ± 1.1	3.42 ± 0.31
	Muscle	168.3 ± 4.1	6.63 ± 0.16	108.7 ± 6.0	29.81 ± 1.65
	Heart	170.0 ± 6.6	6.69 ± 0.26	34.1 ± 5.3	9.36 ± 1.47
	Liver	94.9 ± 12.8	3.74 ± 0.50	36.0 ± 1.5	9.88 ± 0.40
Digoxin + Quinidine	Brain	8.5 ± 1.0 [d]	0.17 ± 0.02 [d]	15.0 ± 0.6	1.76 ± 0.20 [d]
	Muscle	208.7 ± 31.2	4.14 ± 0.62 [d]	157.0 ± 25.5	18.38 ± 2.98 [d]
	Heart	212.0 ± 15.3	4.21 ± 0.30 [d]	42.5 ± 7.5	4.98 ± 0.87 [d]
	Liver	— [e]	— [e]	16.6 ± 5.2 [d]	1.95 ± 0.61 [d]

[a] Results are given as the mean ± *SE* of three guinea pigs. [b] At 1 and 6 hr after bolus intravenous administration of 250 μg/kg of digoxin. [c] The apparent tissue-to-plasma partition coefficient. [d] Significantly different ($p < 0.05$) from the control guinea pigs. [e] — Not determined.

alter the binding of digoxin to serum (plasma) proteins. A possible reason for this effect might be that digoxin binds mainly to albumin in serum (plasma), but the basic drug quinidine binds not only to albumin but also to lipoprotein or α_1-acid glycoprotein (19).

The plasma-to-blood concentration ratio (s) of digoxin did not show a significant change when coadministered with quinidine in guinea pigs (Fig. 3). Using ^{86}Rb-labeled human erythrocytes, Doering *et al.* (20) recently showed that quinidine did not interfere with the glycoside receptor. Thus, changes in s in the presence of quinidine cannot be explained by this mechanism. The decrease in Vd at steady state may be compatible with the hypothesis that quinidine displaces digoxin from the tissue binding sites (14). As shown in Table II, digoxin extensively binds to tissues such as skeletal muscle, liver, and heart, and the K_p values significantly decreased in the presence of quinidine. Straub *et al.* (13) recently reported that high concentrations of quinidine displace digoxin from bovine heart ATPase preparations. However, similar studies by Doering (2) failed to demonstrate the displacement of ouabain by quinidine from the sarcolemma fraction of lamb ventricular myocardium. Recent *in vitro* studies by Ball *et al.* (14) showed that quinidine competes for the binding site on Na^+,K^+-ATPase with digoxin. The effect of quinidine on Na^+,K^+-ATPase, however, occurred at considerably higher concentrations of quinidine than those in the therapeutic range found in humans. Thus, it is uncertain whether quinidine displaces digoxin from Na^+,K^+-ATPase in therapeutic serum concentrations. It has also been reported that another cinchona alkaloid, quinine, decreases the initial uptake rate of ouabain, a cardiac glycoside, by isolated rat hepatocytes (21). In a similar way, digoxin uptake may be inhibited by quinidine.

The renal clearance (CL_R) significantly decreased in the presence of quinidine (Table I). This suggested that quinidine may reduce digoxin secretion or increase digoxin reabsorption as shown in dogs (11), but it is difficult to determine which is the predominant factor from the restricted findings to date.

Koup *et al.* (22) reported the mean value of CL_{tot} for digoxin, which was significantly higher than that of the CL_R of digoxin in the human, and Fenster *et al.* (23) also reported a similar result. Furthermore, considerable biliary excretion of digoxin was evident in guinea pigs (24) and rats (25). It has been suggested that the CL_{tot} of digoxin in rats essentially reflected the sum of CL_R and the hepatic clearance (CL_H) which involves the biliary and metabolic clearance (25). Therefore, the mean metabolic clearance (CL_M) was estimated by the difference calculated by $CL_M = CL_{tot} - CL_B - CL_R$ (Table I). The decrease of CL_M in the quinidine-treated guinea pigs was not so remarkable when compared with the decreases in CL_B or CL_R.

The hepatic transport of digoxin may occur in at least two discernible steps: the uptake from plasma into liver and the transport from liver into bile. As shown in Table III, quinidine may suppress the carrier-mediated transport of digoxin from plasma to liver or the tissue binding, but the transport from liver to bile may not be affected by quinidine.

Table III—Liver-to-Plasma and Bile-to-Liver Concentration Ratios for Digoxin in Control and Quinidine-Treated Guinea Pigs [a]

	Control (Digoxin)	Digoxin plus Quinidine
Liver-to-Plasma	9.88 ± 0.40	1.95 ± 0.61 [b]
Bile-to-Liver	4.25 ± 0.53	5.49 ± 3.10

[a] Results are given as the mean ± *SE* of three guinea pigs. The mean digoxin concentrations in the liver and plasma at 6 hr, and in the bile from 5 to 6 hr after bolus intravenous administration of 250 μg/kg of digoxin were used for the calculation of the ratios. [b] Significantly different ($p < 0.05$) from the control guinea pigs.

The distribution of digoxin to the CNS is of interest in view of the suggestion that some of the effects of cardiac glycosides may occur as a result of the effect on the CNS (26). As shown in Table II, the concentration of digoxin measured in the brain was low in guinea pigs (this study), and in humans (27) digoxin seems to gradually enter the spinal fluid. But the concentration of digoxin in the choroid plexus of the human has been found to be at least as high as that in the ventricular myocardium (27). The slow entry of digoxin into the brain may be due to an efflux mechanism through the choroid plexus. Thus, the increase of digoxin distribution to the brain at 1 hr after bolus intravenous administration in the presence of quinidine might be due to the inhibition of the transport system from the cerebrospinal fluid to blood through the choroid plexus for digoxin. On the contrary, the decrease of the apparent brain-to-plasma concentration ratio (K_p) at 6 hr after bolus intravenous administration of digoxin might be due mainly to the displacement of the glycosides from the binding sites (Na^+,K^+-ATPase) in the brain cells.

In conclusion, the quinidine–digoxin interaction was demonstrated in guinea pigs. Quinidine caused an inhibition in the tissue distribution of digoxin, which was shown in the significant decrease of the K_p values of digoxin, and this result may explain the significant decrease of Vd_{ss}. Furthermore, quinidine may cause a reduction of biliary, renal, and metabolic clearances, which significantly decrease the CL_{tot} of digoxin.

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Pharmacokinetics of Heparin V: *In Vivo* and *In Vitro* Factors Affecting the Relationship Between Concentration and Anticoagulant Effect of Heparin in Rat Plasma

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Abstract □ There are appreciable interindividual variations in rats of baseline activated partial thromboplastin time (APTT) and of the anticoagulant effect of heparin added to plasma (as reflected by the slope of the regression line describing the essentially linear relationship between ln APTT and heparin concentration). Determination of baseline APTT and slope value on two occasions, 7 days apart, in the same rats revealed that (unlike in humans) these characteristics were subject also to considerable intraindividual variation. To explore the possible reasons for the observed variability, the effect of citrate concentration (acid citrate solution is used as a blood anticoagulant in the collection of plasma), calcium concentration (in the recalcifying solution used to initiate coagulation), and plasma incubation time (for activating the coagulation system) was determined. All three variables had pronounced effects on the anticoagulant response to heparin. Since rat erythrocytes are almost totally impermeable to citrate, hematocrit is a determinant of plasma citrate concentration when acid citrate solution is added in constant proportion to rat blood. Accordingly, inter- and intraindividual differences in baseline APTT and slope values were measured in another experiment in which the citrate solution to plasma (rather than blood) volume ratio was held constant and blood samples were obtained 30 days apart to permit the return of hematocrit values to normal. Intraindividual variation of the coagulation characteristics was appreciably decreased under these conditions. There are important differences between rats and humans with respect to the effect of citrate concentration and plasma incubation time on baseline APTT and on the anticoagulant action of heparin, as well as with respect to the relationship between these two characteristics.

Keyphrases □ Heparin—pharmacokinetics, concentration and anticoagulant effect, *in vivo* and *in vitro* factors □ Pharmacokinetics—heparin, concentration and anticoagulant effect, *in vivo* and *in vitro* factors □ Anticoagulants—heparin, effect of concentration, *in vivo* and *in vitro* factors, pharmacokinetics

Safe and effective anticoagulant therapy with heparin is complicated by the chemical and pharmacological heterogeneity of this natural product (1–3), by inter- and intraindividual differences in anticoagulant response (4–8) that necessitate individualization and frequent changes of the dosing rate of this drug, and by questions concerning the suitability of the various *in vitro* clotting tests used as intermediate therapeutic end points to serve as indices of therapeutic efficacy (prevention of thrombosis) and safety (absence of hemorrhagic episodes due to excessive anticoagulation) (9, 10). An individual's anticoagulant response to a given dose or dosing rate of heparin is subject to two sources of considerable variation, one pharmacokinetic and the other pharmacodynamic: the disposition (systemic clearance and biological half-life) of the drug and the relationship between heparin concentration in plasma (the site of anticoagulant action) and the magnitude of anticoagulant effect (6). Practical and ethical considerations impose limitations on exploration of these problems in humans and make it desirable to use animal models for certain pharmacokinetic and pharmacodynamic studies of heparin. The rat appears to be promising for this purpose. Like humans, rats exhibit dose-dependent elimination kinetics of heparin (11). The anticoagulant response to this drug as reflected by the activated partial thromboplastin time (APTT) is log-linearly related to the concentration of added heparin in plasma over a wide concentration range in both humans and rats (4, 11).

Studies in normal human adults (4) have shown a significant correlation between hematocrit and an index of the anticoagulant response of plasma to added heparin (the slope of the essentially linear relationship between ln APTT and the concentration of heparin added to plasma, to be referred to in this article as the slope or slope value). It has also been observed that there are pronounced interindividual differences in both baseline APTT (*i.e.*, APTT of plasma without added heparin) and slope value, but that intraindividual differences are relatively small in humans (4).

Contrary to these findings, it was found in the initial phase of the present study that baseline APTT and slope values in individual rats, while exhibiting similar interindividual differences as in humans, were poorly reproducible when measured again 7 days later. To explore the reasons for these intraindividual differences in rats, the relationship of hematocrit, citrate concentration (acid citrate solution is added to blood to prevent coagulation),

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