2-[1(*R*)-Methyl-2-oxo-4(*R*)-isopropenyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (VIII*b*)—The iodo lactone(s) VII*b* (3.8 g) in 100 ml of dry benzene was reduced with triphenyltin hydride (6.72 g, 0.02 mole). Distillation (150–154° and 0.1 mm) provided VIII*b* (975 mg, 72% based on monoiodo starting material, 91% based on triiodo starting material): IR ($CHCl_3$) 1795 and 1720; NMR ($CDCl_3$) 1.35 (3, s, CH_3), 1.75 (3, s, CH_3), 4.68 (1, t, J = 2 Hz, O—C—H), and 4.82 ppm (2, m, $=CH_2$); $M^{\oplus}$, m/z 208; $[\alpha]_D^{26}$ ($CHCl_3$) +114.2°.

Anal.—Calc. for $C_{12}H_{16}O_3$: C, 69.20; H, 7.68. Found: C, 68.95; H, 7.79.

2-[1(*R*)-Methyl -2- oxo-4(*R*)-isopropyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (VIII*a*)—The iodo lactone VII*a* (1.0 g, 3 mmoles) in 30 ml of dry benzene was reduced with triphenyltin hydride (0.98 g, 2.8 mmoles). The benzene was removed, and the resulting gum was fractionally sublimed [67° (0.05 mm)] to yield VIII*a* (200 mg, 32%): IR ($CHCl_3$) 1785 and 1715; NMR ($CDCl_3$) 0.95 (6, d, J = 6 Hz, 2 CH_3), 1.35 (3, s, CH_3), and 4.67 ppm (1, t, J = 2 Hz, O—C—H); $M^{\oplus}$, m/z 210; $[\alpha]_D^{26}$ ($CHCl_3$) +127°; mp 84–85°.

Anal.—Calc. for $C_{12}H_{18}O_3$: C, 68.55; H, 8.57. Found: C, 68.33; H, 8.52.

Sodium Borohydride Reductions—The procedure used was that of Elisberg *et al.* (12) modified by length of reaction time and solvent. To 0.5 mmole of the keto lactone in 30 ml of 95% ethanol stirring in an ice bath was added 1 mmole of sodium borohydride. Ice water was added to the mixture, the solution was extracted with ether three times, and the organic layer was dried over sodium sulfate.

2-[1(*S*)-Methyl-2(*R*)-hydroxy -4(*S*)- isopropenyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (III*b*)—The keto lactone (VIII*b*) (560 mg, 2.69 mmoles) in 40 ml of 95% ethanol was reduced with sodium borohydride (200 mg, 5.38 mmoles). Workup after a 6-hr reaction time and distillation produced III*b* (270 mg, 48%): IR (neat) 3500 and 1770; NMR ($CDCl_3$) 1.1 (3, s, CH_3), 1.74 (3, s, CH_3), 4.35 (1, t, J = 3 Hz, O—C—H), and 4.73 ppm (2, s, $=CH_2$); $M^{\oplus}$, m/z 210.

Anal.—Calc. for $C_{12}H_{18}O_3$: C, 68.59; H, 8.57. Found: C, 68.34; H, 8.63.

2-[1(*S*)-Methyl-2(*R*)-hydroxy-4(*S*)-isopropyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (III*a*)—The keto lactone, VIII*a* (315 mg, 1.5 mmoles) in 30 ml of 95% ethanol was reduced with sodium borohydride (113 mg, 3.0 mmoles). Workup after a period of 7 hr and distillation gave III*a* (130 mg, 41%): IR (neat) 3500 and 1775; NMR ($CDCl_3$) 0.85 (3, d, J = 5 Hz, CH_3), 1.1 (3, d, J = 4 Hz, CH_3), 1.22 (3, s, CH_3), and 4.3 ppm (1, s, O—C—H); $M^{\oplus}$, m/z 212.

Anal.—Calc. for $C_{12}H_{20}O_3$: C, 67.94; H, 9.43. Found: C, 67.85; H, 9.49.

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Influence of Ionic Strength on Rectal Absorption of Gentamicin Sulfate in the Presence and Absence of Sodium Salicylate

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Abstract □ The rectal absorption of gentamicin sulfate in rats, both in the presence and absence of sodium salicylate, was facilitated by the use of high ionic strength aqueous formulations. The relative order of effectiveness in promoting gentamicin absorption was sodium dihydrogen phosphate ≃ sodium chloride ≫ potassium chloride, indicating a preferential effect of sodium ions. The increased gentamicin bioavailability in response to sodium salicylate adjuvant activity appeared to be independent of and additive to the increased gentamicin absorption due to high ionic strength conditions. The inability of sorbitol to increase gentamicin bioavailability above control levels indicated that elevated osmotic pressure was not a major determinant of rectal gentamicin absorption.

Keyphrases □ Gentamicin—rectal absorption, effect of ionic strength and specificity, sodium salicylate adjuvant □ Sodium salicylate—as adjuvant, rectal absorption of gentamicin, effect of ionic strength and specificity □ Absorption, rectal—gentamicin, sodium salicylate adjuvant, effect of ionic strength and specificity

Sodium salicylate has been reported to enhance the rectal absorption of water-soluble compounds (1, 2). Two types of formulations were used in the salicylate studies, an aqueous microenema and a fatty-base suppository. The influence of variations in ionic strength and ionic specificity on salicylate-enhanced rectal absorption has not been thoroughly examined in either formulation.

The effect of sodium concentration on absorption of fluid, as classically illustrated by the active transport of sodium from the large intestine and the concomitant water

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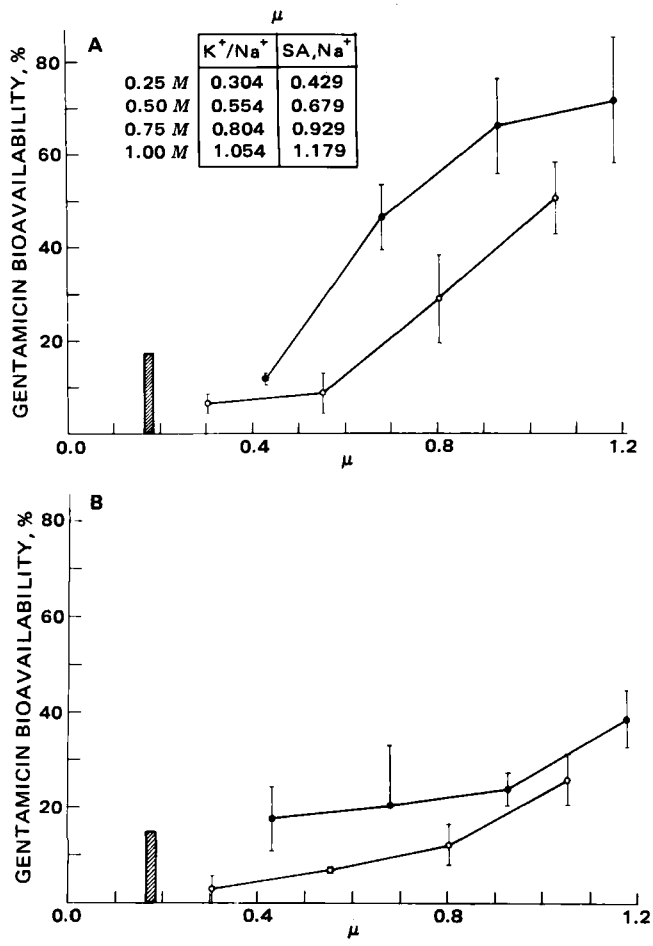

Figure 1—*Gentamicin bioavailability in the presence (●) or absence (○) of sodium salicylate following administration of aqueous microenemas of varying ionic strength (μ) to the unligated rectal compartment. Sodium chloride (A) or potassium chloride (B) at 0.25, 0.50, 0.75, and 1.00* M *was the major determinant of ionic strength, with minor contributions from sodium salicylate (SA) and gentamicin sulfate. Bars (▨) represent ionic strength of standard physiological solutions. Error bars represent standard deviations for* n = 3–6.

absorption, is well documented (3, 4). Recent studies have demonstrated a direct link between the transport of sodium ions and macromolecules in the rat small intestine (5, 6). Sodium ions have also been shown to influence calcium transport in the small intestine (7). However, the influence of ionic strength on adjuvant-assisted rectal absorption of water-soluble compounds has not been reported.

In the present study, the effect of various ionic species on the rectal absorption of gentamicin, a model water-soluble compound, in the presence and absence of sodium salicylate are examined. The purpose of the study is threefold: (*a*) to determine the effect, if any, of ionic strength on rectal absorption of a water-soluble compound; (*b*) to examine possible ionic specificity with regard to promoting rectal absorption; and (*c*) to determine if the absorption-promoting activity of sodium salicylate is influenced by alterations in ionic species or total ionic strength.

EXPERIMENTAL

Adult male Sprague-Dawley rats (200–250 g) were fasted, with free access to water, for 18–24 hr prior to the experiments. Animals were anesthetized by intramuscular injections of urethane (0.1 ml of 43% ethylcarbamate[1] in distilled water/100 g of body weight).

Experimental formulations were administered as aqueous microenemas, given with a 1-ml syringe at an intrarectal depth of 2.5 cm. Each animal received a 250-μl microenema, pH 5.0, containing 2.5 mg of gentamicin sulfate[1] with or without 5 mg of sodium salicylate. Ionic strength was controlled by the addition of various salts, as indicated in each individual set of experiments. Total ionic strength included contributions from the drug and adjuvant, as well as from the added salts, and was determined by the following formula:

$$I = \tfrac{1}{2}\sum m_i z_i^2 \qquad \text{(Eq. 1)}$$

where I is the ionic strength, m_i is the molality of i, and z_i is the charge of i.

In some experiments, designed to minimize effects due to possible movement of the microenema solution away from the site of administration, the rectum was ligated 4 cm from the anal opening. With care taken not to restrict the vasculature, this procedure ensured retention of the microenema in the distal 4 cm of the rectal compartment.

Blood samples (0.5 ml) were taken from the external jugular vein at 15, 30, 60, and 90 min following rectal administration of the microenema. Serum was isolated and frozen until assayed within 1–2 weeks. For determination of intravenous serum profiles, each animal received 2.5 mg of gentamicin sulfate in 250 μl of 0.1 *M* Tris-HCl buffer, pH 7.5. Blood samples (0.5 ml) were collected at 5, 10, 20, 30, 60, and 90 min and processed as described for rectal administration. Serum gentamicin was determined by microbiological assay using *Bacillus subtilis* (ATCC #6633) as the target organism (8). Results are expressed as percent bioavailability, calculated as:

$$\%\ \text{Bioavailability} = \frac{(\text{AUC})_{\text{rectal}}(\text{Dose})_{\text{iv}}}{(\text{Dose})_{\text{rectal}}(\text{AUC})_{\text{iv}}} \times 100 \qquad \text{(Eq. 2)}$$

The area under the serum concentration–time curve from 0 to 90 min, AUC_0^{90}, was calculated by a summation of trapezoidal areas. For the calculation of the intravenous AUC_0^{90}, the linear portion of the log serum concentration *versus* time profile was extrapolated to $t = 0$ min to correct for the drug distribution phase. Three to six animals were used for determining mean AUC_0^{90} values for both rectal and intravenous serum profiles. Following rectal administration, the absorption of gentamicin sulfate was virtually complete by 30–60 min, and the serum $t_{1/2}$ value (220–240 min) was the same as that observed after intravenous administration.

RESULTS

The effect of sodium chloride on unligated rectal delivery of gentamicin, with and without sodium salicylate as the adjuvant, is shown in Fig. 1A. Without adjuvant, no apparent sodium chloride effect is observed until the ionic strength (μ) in the microenema exceeds 0.55. A linear increase in percent bioavailability occurred from $\mu = 0.544$ to $\mu = 1.054$ ($r = 0.9998$ by regression analysis). At 1.0 *M* NaCl ($\mu = 1.054$), gentamicin bioavailability was 51 ± 7.9%. Solutions of greater sodium chloride ionic strength were not examined, so it is not known whether the sodium chloride effect on bioavailability would plateau prior to reaching 100% gentamicin delivery. At all ionic strengths examined, gentamicin bioavailability was greater in the presence of sodium salicylate. At higher ionic strengths with sodium salicylate, the effect of sodium chloride was no longer linear and appeared to plateau around 70–80% bioavailability.

Figure 1B shows the results of a similar set of experiments using unligated rectum in which potassium chloride was substituted for sodium chloride as the major contributor to total ionic strength. The effect of potassium chloride alone (26 ± 5.6% at $\mu = 1.054$) on gentamicin delivery was significantly less than the corresponding response observed with sodium chloride (51 ± 7.9% at $\mu = 1.054$). In the presence of sodium salicylate, the gentamicin bioavailability using potassium chloride was 39 ± 5.9% at $\mu = 1.179$, whereas with sodium chloride the bioavailability was 72 ± 13.4%.

In the absence of adjuvant and added salts, gentamicin sulfate bioavailability was 12 ± 4.5%. The inclusion of 2–10% sorbitol in the formulation caused a slight decrease in gentamicin sulfate absorption (Table I). Including 30% sorbitol in microenemas containing gentamicin sulfate and adjuvant did not significantly increase bioavailability (36 ± 14.8%) above that seen with sorbitol-free microenemas containing adjuvant and drug along (29 ± 7.4%).

[1] Sigma Chemical Co., St. Louis, Mo.

Table I—Effect of Sorbitol on Rectal Gentamicin Sulfate Absorption from Aqueous Formulations

Formulation[a]		Gentamicin Sulfate
Adjuvant, %	Sorbitol, %	Bioavailability, %[b]
0	0	12 ± 4.5
0	2	5 ± 3.4
0	5	3 ± 0.3
0	10	4 ± 2.3
2	0	29 ± 7.4
2	30	36 ± 14.8

[a] All formulations contained 1% gentamicin sulfate. [b]Based on $n = 3$ animals.

The use of potassium chloride or potassium dihydrogen phosphate with ligated tissue produced somewhat equivocal results (Fig. 2A). There was no apparent difference between the gentamicin bioavailability after the administration of potassium chloride alone compared with that of potassium chloride administered with sodium salicylate, except at very high ionic strengths ($\mu > 1.0$). Potassium dihydrogen phosphate, in conjunction with sodium salicylate, yielded the highest gentamicin bioavailability in the potassium series (63 ± 9.4% at $\mu = 1.179$), although this value was significantly less than the bioavailability observed with sodium dihydrogen phosphate and sodium salicylate (88 ± 13.2% at $\mu = 0.929$) (Fig. 2B). As seen in the experiments with unligated rectal tissue, bioavailabilities attained with sodium chloride and sodium salicylate were greater than those observed with sodium chloride alone.

DISCUSSION

The ability of salicylate-type adjuvants to enhance the GI absorption of water-soluble compounds has been previously established (1, 2). In this study, the ionic contributions of sodium and potassium salts to rectal absorption of a model water-soluble compound, gentamicin, were examined. Ionic strength and specificity were found to significantly affect the rectal absorption of gentamicin.

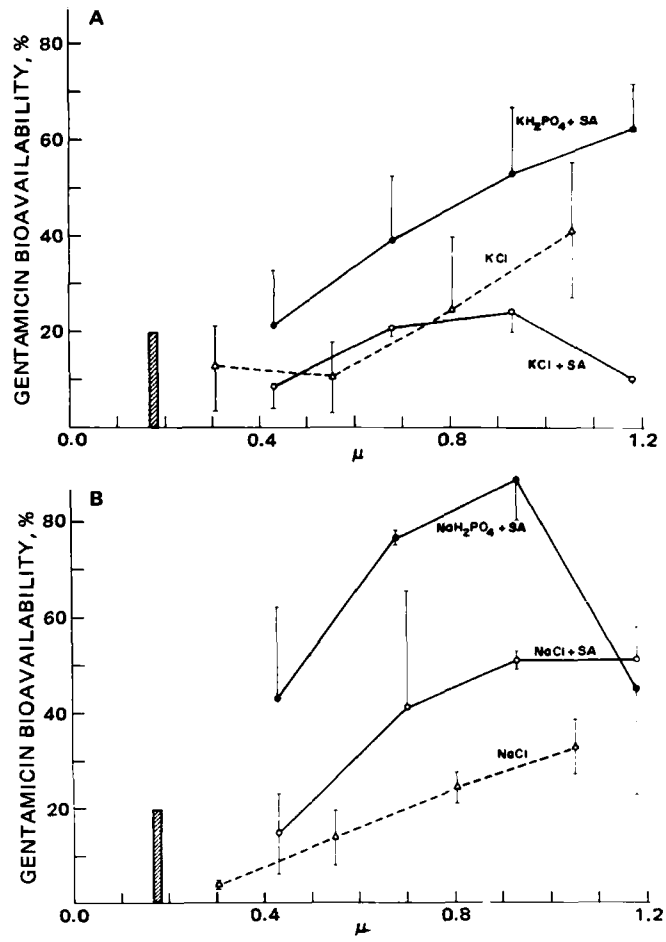

Figure 2—*Gentamicin bioavailability in the presence (—) or absence (- - -) of sodium salicylate (SA) following administration of aqueous microenemas of varying ionic strength (μ) to the ligated rectal compartment. The potassium (A) or sodium (B) salts were the major determinants of ionic strength, with minor contributions from sodium salicylate and gentamicin sulfate. Bars (▨) represent ionic strength of standard physiological solutions. Error bars represent standard deviations for* n = *3–6.*

Sodium chloride significantly increased gentamicin absorption, even in the absence of sodium salicylate. It is not possible to determine, from the present data, the precise mechanism involved in the sodium chloride enhancement of drug permeability across the rectal mucosa. However, several possibilities exist: (*a*) high sodium or chloride concentrations may activate some undefined transport mechanism for gentamicin; (*b*) active transport of sodium out of the rectal lumen, with concomitant water movement, may establish mass transfer conditions favoring absorption of gentamicin; or (*c*) high ionic strength solutions may damage or disrupt the normal epithelial barrier and allow concentration-dependent movement of gentamicin through the disrupted barrier.

The mucosal layer in the rectum and lower portion of the large intestine is not classically viewed as an absorptive area except for the absorption of water and small ions (*e.g.*, sodium). This does not preclude the possible existence of specific transport mechanisms for water-soluble compounds (*e.g.*, gentamicin), although it is unlikely that such a system exists. The apparent inability of the rectal tissue to absorb gentamicin in the absence of the salicylate adjuvant further refutes the existence of a specific transport system which could be affected by the sodium or chloride ion.

Possible tissue damage at very high concentrations of sodium chloride may account for part of the observed increase in gentamicin absorption, although comparable gentamicin serum levels were not observed at higher concentrations of potassium chloride or sorbitol. The inability of sorbitol to increase gentamicin bioavailability indicates that the osmotic pressures generated by high sodium chloride or potassium chloride solutions are unlikely causes of the increased delivery. The fact that sodium chloride was twice as effective as potassium chloride in both the presence and absence of sodium salicylate (at comparable ionic strengths) suggests certain ionic specificities, possibly implicating the involvement of the membrane sodium–potassium pump.

Sodium dihydrogen phosphate and potassium dihydrogen phosphate were tested in the ligated rectum to determine if the absorption promoting potential of the salts was influenced by the anionic species. Maximal effects in the ligated rectum were observed at lower ionic strengths than in the unligated rectum. This is probably due to the increased effective concentration of drug and salt in the limited exposed surface area of the ligated rectum. With both sodium and potassium, the dihydrogen phosphate salts appeared to be more effective than the chloride salts in promoting gentamicin delivery. While this increased absorption using dihydrogen phosphate salts may be a real effect, it is also possible that alterations in rectal pH may have caused ionic strengh changes that were responsible for promoting absorption. All microenemas were administered as buffered solutions (pH 5) with sodium dihydrogen phosphate predominately existing in solution as sodium and dihydrogen phosphate. The rectal compartment in the rat is capable of some buffering activity and tends to adjust the pH of aqueous solutions to a range of pH 7–8 (unpublished observations). If the pH of the microenema is buffered *in vivo* to the pH 7–8 range, a significant portion of the dihydrogen phosphate may be ionized to monohydrogen phosphate. Since the ionic strength is a function of charge squared, this would significantly increase the total ionic strength of the solution and could account for the greater effect of sodium dihydrogen phosphate as compared with sodium chloride. Sodium chloride ionic strength is unaffected by pH. Since the buffering effect of the rectal compartment is not instantaneous, the relative concentrations of the two ionized species of the phosphate salts will be continually changing. To determine if the buffering capacity of the rectum changes the ionic strength of the formulation, and therefore the absorption of gentamicin sulfate, controlled pH perfusion studies will be required. At this point, the possibility that phosphate has a greater effect on gentamicin sulfate absorption than chloride cannot be precluded.

An important deduction that can be made from this study is that the absorption-promoting activity of sodium salicylate is not solely attributed to ionic strength. In the data presented in the figures, ionic contributions from sodium salicylate were included in the calculations of total ionic strength in the microenemas. The increase in gentamicin bioavailability due to the adjuvant activity of sodium salicylate is significantly greater than the bioavailability achieved with adjuvant-free microenemas at the same total ionic strength (adjusted by sodium chloride). Sodium salicylate, therefore, possesses an absorption-enhancing potential above that which can be attributed to sodium ionic strength alone.

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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