Pharmacokinetic Study on the Mechanism of Tissue Distribution of Doxorubicin: Interorgan and Interspecies Variation of Tissue-to-Plasma Partition Coefficients in Rats, Rabbits, and Guinea Pigs

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Abstract □ The mechanism of interorgan and interspecies variations in the tissue distribution of doxorubicin was studied in rats, rabbits, and guinea pigs. The permeation properties of doxorubicin were investigated by using isolated rat erythrocytes as a model membrane. A significant dependency of the uptake rate constant on medium pH was observed, suggesting that only un-ionized doxorubicin is diffusible through the plasma membrane. The values of plasma unbound fraction (f_p) of doxorubicin were 0.344 for rats, 0.415 for rabbits, and 0.529 for guinea pigs. The tissue DNA concentrations of rats were larger than those of rabbits and smaller than those of guinea pigs in corresponding organs or tissues. An *in vitro* organ model that described the distribution behavior of doxorubicin in the whole body was constructed, and an equation was derived to estimate the tissue-to-plasma partition coefficients, (K_p) from *in vitro* experiments. The *in vitro* K_p values showed comparatively good agreement with the *in vivo* K_p values for the various organs or tissues in all three species. Doxorubicin is exclusively bound to the nuclei in the cells. The variation of the K_p values in different organs depended on the amount of nuclei per gram of tissue. The primary determinant of the interspecies variation in the K_p values was the difference in tissue DNA concentrations among rats, rabbits, and guinea pigs, and a secondary determinant was the difference in f_p values.

Keyphrases □ Doxorubicin—pharmacokinetics, mechanism of tissue distribution, tissue-to-plasma partition coefficient □ Pharmacokinetics—doxorubicin, mechanism of distribution, tissue-to-plasma partition coefficients □ Partition coefficients—tissue to plasma, mechanism of distribution of doxorubicin

The tissue distribution of doxorubicin has been studied in several animal species (1–5). The characteristic feature of the tissue distribution of doxorubicin is that the tissue-to-plasma partition coefficient (K_p), a commonly used physiological pharmacokinetic parameter, is extraordinarily large and shows clear differences among tissues. Although it has been demonstrated that doxorubicin and its derivatives interact with DNA, chromatin, and nuclei (6–8), the mechanism of tissue distribution of doxorubicin has not been quantitatively elucidated.

In general, the physiological distribution of most drugs is governed by two factors: (*a*) their reversible binding equilibrium with proteins and/or other constituents in blood and tissues and (*b*) the concentration difference of unbound drug, which depends on the membrane permeability (9). Therefore, much has been done on the changes in plasma protein binding as a possible determining factor of interspecies, individual, or disease-state differences in drug distribution (10–12). However, it has not yet been possible to explain organ and interspecies differences in drug distribution in terms of the K_p values for any drug, despite various studies of drug interactions with intracellular organelles, e.g., nuclei, mitochondria, and microsomes.

In previous studies (13, 14), a comparatively good correlation was found between the K_p value of doxorubicin and the tissue DNA concentration, *i.e.*, the amount of nuclei per gram of tissue in rats and rabbits, and there was no significant difference in the characteristics of doxorubicin binding to the nuclei isolated from the rat liver and kidney tissues. The purpose of this study was to elucidate the mechanism of organ and interspecies variation in the tissue distribution of doxorubicin by means of measurements of tissue DNA concentrations and *in vitro* binding studies with plasma and nuclei.

THEORETICAL SECTION

Organ Model—A diagrammatic representation of an anatomical compartment in an organism is presented in Fig. 1. In estimating the K_p value of doxorubicin in the compartment from the results of *in vitro* experiments, the following assumptions were made:

1. The nucleus is the dominant organelle in tissue binding.
2. The variations in tissue distribution depend on the amount of nuclei per gram of tissue, *i.e.*, tissue DNA concentration, and the differences in nuclear binding parameters among tissues and species are negligible.
3. Only the un-ionized form which is free from plasma and nuclear binding can diffuse through the plasma membrane.

It has been reported on the basis of fluorescence microscopy in hamsters (15) that anthracycline antibiotics are localized in the cell nucleus. Furthermore, >85% of doxorubicin was also localized in the 600×*g* subcellular fraction of each tissue after intravenous administration of 10 mg/kg in rats[1]. In a previous study (13), a good correlation was found between *in vivo* K_p value and the tissue DNA concentration. Therefore, it was suggested that the dominant factor in organ differences in the tissue distribution is the amount of nuclei per gram of tissue. Moreover, there was no difference in tissue nuclear binding characteristics of doxorubicin between the liver and kidney (14). Although it was reported that a resistant type of tumor cell has a carrier-mediated influx system and active efflux mechanisms (16, 17), there is little information regarding the permeation of doxorubicin through the plasma membrane into the normal cell. Therefore, the membrane permeability to doxorubicin was examined by using isolated rat erythrocytes as a model membrane in the present study.

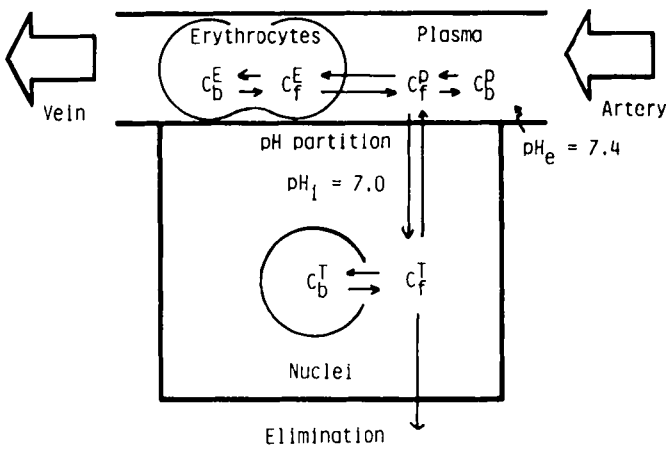

Figure 1—*Diagrammatic representation of the organ model.*

[1] Unpublished results.

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Table I—Comparison of Tissue DNA Concentrations Among Three Species

Tissue	Tissue DNA Concentration[a]		
	Rat, M[b]	Rabbit, M[b]	Guinea Pig, M
Adipose tissue	$1.35 \times 10^{-3} \pm 0.15 \times 10^{-3}$	$4.89 \times 10^{-4} \pm 0.88 \times 10^{-4}$	$1.74 \times 10^{-3} \pm 0.22 \times 10^{-3}$
Muscle	$9.15 \times 10^{-4} \pm 1.59 \times 10^{-4}$	$2.60 \times 10^{-4} \pm 0.53 \times 10^{-4}$	$8.23 \times 10^{-4} \pm 0.52 \times 10^{-4}$
Liver	$6.43 \times 10^{-3} \pm 0.38 \times 10^{-3}$	$3.81 \times 10^{-3} \pm 0.51 \times 10^{-3}$	$8.43 \times 10^{-3} \pm 0.62 \times 10^{-3}$
Gut	$2.05 \times 10^{-2} \pm 0.08 \times 10^{-2}$	$1.77 \times 10^{-2} \pm 0.10 \times 10^{-2}$	$2.57 \times 10^{-2} \pm 0.25 \times 10^{-2}$
Heart	$4.59 \times 10^{-3} \pm 0.38 \times 10^{-3}$	$2.24 \times 10^{-3} \pm 0.08 \times 10^{-3}$	$5.96 \times 10^{-3} \pm 0.37 \times 10^{-2}$
Stomach	$1.11 \times 10^{-2} \pm 0.04 \times 10^{-2}$	$7.77 \times 10^{-3} \pm 0.47 \times 10^{-3}$	$1.81 \times 10^{-2} \pm 0.05 \times 10^{-2}$
Kidney	$1.25 \times 10^{-2} \pm 0.09 \times 10^{-2}$	$1.28 \times 10^{-2} \pm 0.12 \times 10^{-2}$	$1.71 \times 10^{-2} \pm 0.06 \times 10^{-2}$
Lung	$2.35 \times 10^{-2} \pm 0.04 \times 10^{-2}$	$2.00 \times 10^{-2} \pm 0.08 \times 10^{-2}$	$3.25 \times 10^{-2} \pm 0.09 \times 10^{-2}$
Spleen	$5.41 \times 10^{-2} \pm 0.38 \times 10^{-2}$	$5.13 \times 10^{-2} \pm 0.27 \times 10^{-2}$	$5.99 \times 10^{-2} \pm 0.13 \times 10^{-2}$

[a] Mean ± *SE* of each experiment; $n = 3$–7. Calculated with calf thymus DNA (mol. wt. 330.9 per unit nucleotide as sodium salt) as the standard. [b] Obtained from a previous report (13).

From the aforementioned assumptions regarding the distribution of doxorubicin in the organs, an equation can be derived to estimate the K_p value as follows. K_p is defined as the ratio of the tissue concentration to the concentration in the leaving venous plasma, *i.e.*:

$$K_p = \frac{C_f^T + C_b^T}{C_f^P + C_b^P} \quad \text{(Eq. 1)}$$

where C^T and C^P are the drug concentrations in the tissue and venous plasma, respectively, and the subscripts f and b denote the unbound and bound drug species, respectively. Appropriate rearrangement of Eq. 1 gives:

$$K_p = \frac{C_f^T}{C_f^P} \times \frac{C_f^P}{C_f^P + C_b^P} \times \left(1 + \frac{C_b^T}{C_f^T}\right) \quad \text{(Eq. 2)}$$

It has been reported by several investigators that the intracellular pH is slightly lower than that in the extracellular fluid and the blood plasma (18). Since doxorubicin is a basic drug which is more highly ionized at lower than higher pH values, the antibiotic diffuses out less rapidly than it enters, and the concentration of unbound doxorubicin may be somewhat higher inside than outside the tissue cells. Therefore, the ratio of the unbound drug in the tissue to that in plasma can be expressed by:

$$\frac{C_f^T}{C_f^P} = \frac{1 + 10^{pK_a - pH_i}}{1 + 10^{pK_a - pH_e}} \quad \text{(Eq. 3)}$$

The concentration ratio of unbound to bound doxorubicin in the tissue can be described by a Langmuir-type equation:

$$\frac{C_b^T}{C_f^T} = \frac{nK_aC_N}{1 + K_aC_f^T} \quad \text{(Eq. 4)}$$

where n and K_a are the number of binding sites and the affinity constant to nuclei, respectively, and C_N is the tissue DNA concentration. If the relation $K_aC_f^T \ll 1$ holds, Eq. 5 is obtained:

$$\frac{C_b^T}{C_f^T} = nK_aC_N \quad \text{(Eq. 5)}$$

Substituting Eqs. 3 and 5 into Eq. 2 gives:

$$K_p = \frac{1 + 10^{pK_a - pH_i}}{1 + 10^{pK_a - pH_e}} \cdot f_p \cdot (1 + nK_aC_N) \quad \text{(Eq. 6)}$$

where f_p is the unbound fraction in plasma, and pH_i and pH_e are the intra- and extracellular pH values, respectively. The value used for f_p (0.344) was determined in the present study, whereas those for n and K_a were obtained from previously reported data (14) and are 6.70×10^{-2} and 4.85×10^5 M^{-1}, respectively. The values used for C_N were also obtained from previously reported data (13) and are listed in Table I. The values for pK_a, pH_e, and pH_i were obtained from the literature, and are 8.15 (8), 7.4 (18), and 7.0 (18), respectively.

Table II—Physiological Constants for Doxorubicin Tissue Distribution Modeling and the Apparent Tissue-to-Plasma Concentration Ratios at the Terminal Phase after Bolus Intravenous-Injections to Guinea Pigs[a]

Tissue	Volume, mL[b]	Blood flow, mL/min[c]	$K_{p,app}$[d]
Arterial blood	7.6	35.3	—
Adipose tissue	11.6	0.5	64 ± 6
Muscle	145.0	10.2	178 ± 24
Liver	11.6	13.5	273 ± 12
Gut	9.0	9.2	351 ± 60
Heart	0.9	2.7	585 ± 50
Stomach	2.4	3.0	436 ± 43
Kidney	2.1	8.4	595 ± 41
Lung	2.7	35.3	656 ± 91
Spleen	0.3	0.8	1964 ± 311

[a] Body weight, 290 ± 4 g ($n = 4$). [b] Determined experimentally from the wet tissue weight by assuming a density of 1.0 for each tissue except for muscle and arterial blood volume. The muscle volume was assumed to be one-half the body weight, as in rats (22), and the arterial blood volume was calculated from the body weight by previously reported methods (23). [c] Obtained from previously reported data (24, 25). [d] Mean ± *SE* of four guinea pigs.

EXPERIMENTAL SECTION

Materials—Doxorubicin hydrochloride[2] was used. The antibiotic was kept in the dark in a desiccator at 4°C. Solutions of doxorubicin (2×10^{-6}–5×10^{-4} M in 405 mM sucrose) were freshly prepared before use. All other reagents were commercial products of analytical grade.

Animals—Male Wistar rats[3] (weight, 250–270 g), male albino rabbits[4] (weight, 2.6–2.8 kg), and male Hartley guinea pigs[3] (weight, 280–300 g) were used.

Blood-to-Plasma Concentration Ratio—The blood-to-plasma concentration ratio (R_B) was determined as described previously (13).

***In Vivo* Apparent Tissue-to-Plasma Concentration Ratio ($K_{p,app}$) in Guinea Pigs**—Doxorubicin was dissolved in saline and administered to guinea pigs *via* a jugular vein at a dose of 10 mg/kg 2 h after the operation. Food and water were given *ad libitum*. At 6, 12, 24, and 48 h after administration, blood samples were collected *via* a jugular artery, and then the guinea pig was killed by exsanguination. Each tissue was immediately excised, rinsed with saline, and stored at −40°C until use. Doxorubicin contents in plasma and tissues were determined by a TLC scanning method as described previously (13).

Hepatic and Renal Clearances in Guinea Pigs—The urinary excretion of doxorubicin was determined from the total amount excreted through the urinary bladder until 48 h after injection of 10 mg/kg iv. The apparent renal blood clearance ($CL_{B,app}^{KD}$) and the apparent hepatic blood clearance ($CL_{B,app}^{LV}$) were determined, and then the organ intrinsic clearance (CL_{int}) was calculated by:

$$f_p \cdot CL_{int} = \frac{CL_{B,app} \cdot Q_B \cdot R_B}{Q_B - CL_{B,app}} \quad \text{(Eq. 7)}$$

where Q_B is the organ blood flow rate.

***In Vivo* K_p Value**—The K_p value for guinea pigs was calculated from $K_{p,app}$ by a previously described method (13).

Plasma Binding Experiment—The rat plasma was first dialyzed for 16 h at 4°C with a semipermeable membrane[5] against pH 7.4 isotonic buffer (30 mM Tris, 131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, and 1.0 mM $CaCl_2$). The plasma unbound fraction was determined by equilibrium dialysis at 37°C for 15 h against the same buffer as used for predialysis in semimicro cells[6]. No denaturation of proteins after dialysis was observed by polarization analysis (19) with 1-anilino-8-naphthalenesulfonate, as reported previously (20). The degradation of doxorubicin during the equilibrium dialysis and the adsorption of doxorubicin on the dialysis membrane (~30% of the initial content) were corrected for in the calculation of the binding parameters. The concentrations of doxorubicin in both plasma and buffer were determined by a fluorospectrometric TLC scanning method (13).

Tissue DNA Concentration—The tissue DNA concentration of guinea pigs was determined by a previously described method (13). The molecular weight of the DNA used was 330.9 (per unit of nucleotide as the sodium salt).

Preparation of Rat Erythrocytes—After injection of heparin at a dose of 0.1 mL/100 g of body weight (100 U), whole blood was collected *via* a jugular

[2] Adriamycin; Kyowa Hakko Kogyo Co., Ltd., Tokyo.
[3] Nihon Ikagaku Dobutsu Co., Tokyo.
[4] Ichikawaya, Tokyo.
[5] Type 36/32; Visking Co., Chicago, Ill.
[6] Kokugogomu Co., Tokyo.

Table III—Pharmacokinetic Parameters of Doxorubicin in Guinea Pigs [a]

Parameter[b]	Value[c]
Dose, μg	$2.88 \times 10^3 \pm 0.001 \times 10^3$
AUC_p, $\mu g \cdot min/mL$	$2.24 \times 10^2 \pm 0.55 \times 10^2$
$CL^{tot}_{B,app}$, mL/min	5.35 ± 0.73
X_u, μg	$6.52 \times 10^2 \pm 0.35 \times 10^2$
$CL^{KD}_{B,app}$, mL/min	1.22 ± 0.19
$CL^{LV}_{B,app}$, mL/min	4.13 ± 0.57
$f_p CL^{KD}_{int}$, mL/min	3.42 ± 0.37
$f_p CL^{LV}_{int}$, mL/min	$1.43 \times 10 \pm 0.10 \times 10$
β, min^{-1}	$2.94 \times 10^{-4} \pm 1.61 \times 10^{-4}$

[a] Body weight, 290 ± 4 g (n = 4), except for X_u and $CL^{KD}_{B,app}$ (300 g; n = 2). [b] AUC_p, area under the plasma concentration *versus* time curve; $CL^{tot}_{B,app}$, apparent total blood clearance; $CL^{KD}_{B,app}$, apparent renal blood clearance; $CL^{KD}_{B,app}$, apparent hepatic blood clearance; X_u, amount excreted in urine; f_p, plasma unbound fraction; CL_{int}, renal intrinsic clearance; CL^{LV}_{int}, hepatic intrinsic clearance; β, slope of the plasma concentration—time curve at the terminal phase. [c] Mean ± *SE* of four guinea pigs.

artery at 30 min. Erythrocytes were prepared essentially by the procedure of Horie *et al.* (21), except that buffer (pH 7.4) containing 131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 0.12 mM $CaCl_2$, 3 mM dibasic sodium phosphate, and 10 mM Tris was used in the preparation.

Uptake Experiment—To determine the initial velocity of uptake of doxorubicin, 1.5 mL of the erythrocyte suspension (10% v/v) was incubated in medium containing 20 mM Tris, 131 mM NaCl, 5.2 mM KCl, 0.9 mM $MgSO_4$, 1.12 mM $CaCl_2$, 3 mM dibasic sodium phosphate, and 5 mM glucose. After preincubation for 3 min with shaking (2 Hz) at 37°C, 0.01 mL of an isotonic solution containing various amounts of doxorubicin (1.81–2.59 μg) was added. Triplicate samples of 1.0 mL of the medium were removed and layered on 200 μL of silicone oil with a density of 1.05[7] in a 2.0-mL polyethylene tube[8]. The samples were then centrifuged for 30 s in a tabletop microcentrifuge[8]. The supernatant was discarded, the oil surface was washed with 0.6 mL of distilled water, 0.6 mL of distilled water was again added, and the erythrocytes were completely hemolyzed with vigorous mixing. Then 0.6 mL of 50 mM Tris-HCl buffer (pH 7.4) was added, and the polyethylene tube was centrifuged for 30 s in a tabletop microcentrifuge[8]. To determine the amount of doxorubicin taken into the erythrocytes, doxorubicin in the supernatant was determined by fluorospectrometric TLC scanning method (13).

RESULTS

Distribution to Erythrocytes, Plasma Protein Binding, and Tissue DNA Concentration—No concentration dependency in doxorubicin distribution into erythrocytes in guinea pigs was observed over the tested plasma concentration range of doxorubicin (0.00947–1.549 μg/mL), which corresponded to the *in vivo* plasma concentration range after injection of 10 mg/kg iv. The value of the blood-to-plasma concentration ratio (R_B) was 2.40 ± 0.09 (n = 12).

Furthermore, no concentration dependency of plasma protein binding of doxorubicin was observed over the *in vivo* plasma concentration range of doxorubicin (0.0131–1.884 μg/mL for rats, 0.0108–1.992 μg/mL for rabbits, and 0.00733–2.183 μg/mL for guinea pigs). The values of plasma unbound fraction were 0.344 ± 0.014 (n = 15) for rats, 0.415 ± 0.019 (n = 11) for rabbits, and 0.529 ± 0.021 (n = 12) for guinea pigs. The tissue DNA concentrations in various tissues of guinea pigs are listed in Table I, along with those of rats and rabbits [determined previously (13)].

***In vivo* K_p Value**—The physiological parameters for guinea pigs and the $K_{p,app}$ at the terminal phase after bolus intravenous injection of doxorubicin are summarized in Table II. The pharmacokinetic parameters determined in guinea pigs are listed in Table III. By using the values listed in Tables II and III, the *in vivo* K_p values of doxorubicin of guinea pigs were calculated and are summarized inTable IV, along with those of rats [determined previously (13)] and rabbits [estimated from the literature values (3)].

Membrane Permeability—The uptake of doxorubicin into isolated erythrocytes was measured at pH 7.05, 7.42, 7.85, and 8.07. The plots of cell-to-medium ratio (C/M ratio) *versus* time were linear for 80 s at all pH values studied. When extrapolated, these lines passed through the origin, which suggested that there was no adsorption of doxorubicin on the cell surface. The slope of each line, which represents the uptake rate constant, k (min^{-1}), was plotted against the pH value of the medium (Fig. 2). The good correspondence

[7] Aldrich Chemical Co., Milwaukee, Wis.
[8] Beckman Instrument Co., Felton, Calif.

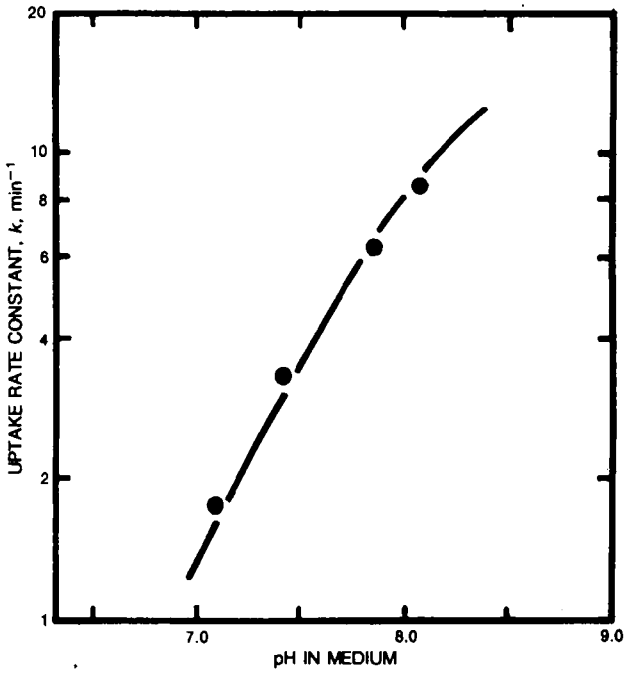

Figure 2—*Log* k-*pH profile for the uptake of doxorubicin into rat erythrocytes. The theoretical curve was calculated by a nonlinear iterative least-squares method (26) with a digital computer by* $k_{pH} = k_0(1/1 + 10^{pK_a - pH})$, *where* k_{pH} *and* k_0 *are the apparent uptake rate constant and the uptake rate constant of undissociated species, respectively;* $k_0 = 2.065 \times 10\ min^{-1}$; $pK_a = 8.15$. *Key: (●) observed values; (—) calculated curve.*

between the calculated and observed values suggests that only un-ionized doxorubicin can permeate through the cell membrane.

Estimation of the K_p value from *In Vitro* Experiments—*In vitro* K_p values for rats, rabbits, and guinea pigs were calculated by Eq. 6 and are listed in Table IV with the *in vivo* K_p values. The *in vivo* and *in vitro* K_p values for rats, rabbits, and guinea pigs are compared in Fig. 3.

DISCUSSION

In this study, the mechanism of *in vivo* tissue distribution of doxorubicin was examined in rats, rabbits, and guinea pigs. The goal of the study was to elucidate the characteristic tissue distribution mechanism to account for interorgan and interspecies variation in drug levels and to determine whether the K_p values obtained from *in vitro* studies coincide with those obtained from *in vivo* studies. The *in vivo* K_p values were characteristically large, and there were fairly large differences among tissues and animal species (Table IV). The K_p values of doxorubicin were also determined from the *in vitro* organ model (Fig. 1), on the basis of the three assumptions described in the *Theoretical Section*, for comparison with *in vivo* values to check the validity of the model. As far as we are aware, the mechanism of tissue distribution of a drug has never been elucidated in detail.

Since there was insufficient information on the plasma membrane permeability of doxorubicin in normal cells, we examined this permeability in isolated intact cells. In a preliminary study, the uptake of doxorubicin into isolated rat hepatocytes, prepared by the method of Berry and Friend (27) as modified by Bauer *et al.* (28), was examined, but the uptake rate of doxorubicin could not be exactly determined due to the rapid metabolism at the incubation temperature of 37°C. Therefore, rat erythrocytes, which can be easily prepared, were utilized as a model membrane. Since it appeared that only the un-ionized molecule could permeate through plasma membranes (Fig. 2), assumption 3 (see *Theoretical Section*) may be applicable to the tissue distribution of doxorubicin. The number of binding sites and the affinity constant for isolated nuclei, which were used for the calculation of the *in vitro* K_p values, were determined previously in quasi-physiological buffer (pH 7.0) containing 135 mM sucrose, 20 mM Tris, 50 mM NaCl, 100 mM KCl, 1 mM $MgSO_4$, and 1 mM $CaCl_2$ at 37°C, and values of $n = 6.70 \times 10^{-2}$ and $K_a = 4.85 \times 10^5\ M^{-1}$ were obtained for rat liver nuclei (14). Since the conditions used for measuring doxorubicin binding were considered to be close to physiological on the basis of both morphological examination of isolated nuclei and the concentrations of the intracellular metal ions (29, 30), the parameters of nuclear binding determined in the *in vitro* studies might be comparable with those determined for the *in vivo* tissue distribution of doxorubicin.

Table IV—*In Vivo* and *In Vitro* K_p Values in Three Species[a]

Tissue	Rat		Rabbit		Guinea Pig	
	In Vivo[b]	*In Vitro*	*In Vivo*[c]	*In Vitro*	*In Vivo*	*In Vitro*
Adipose tissue	33 ± 4.4	36 ± 1.7	19 ± 3.5	16 ± 1.0	54 ± 4.3	70 ± 3.4
Muscle	54 ± 4.2	24 ± 1.9	27 ± 3.9	9 ± 0.6	135 ± 14	34 ± 1.2
Liver	243 ± 39	166 ± 6.4	56 ± 10.3	119 ± 6.2	365 ± 16	335 ± 12
Gut	161 ± 23	528 ± 18	50 ± 7.5	551 ± 20	336 ± 55	1019 ± 43
Heart	230 ± 17	119 ± 5.4	57 ± 5.0	71 ± 2.4	571 ± 48	237 ± 8.4
Stomach	258 ± 29	286 ± 9.6	—	242 ± 9.1	417 ± 40	718 ± 22
Kidney	349 ± 27	322 ± 13	494 ± 43	399 ± 17	684 ± 47	679 ± 22
Lung	354 ± 54	605 ± 19	155 ± 24	623 ± 22	656 ± 91	1289 ± 40
Spleen	404 ± 72	1391 ± 56	746 ± 82	1596 ± 58	1795 ± 259	2374 ± 73

[a] Mean ± *SE* of each experiment. [b] Data obtained from a previous report (13). [c] Values of $K_{p,app}$, except for the spleen, were obtained from previously published data (3), K_p values were then calculated by assuming that R_B was equal to the R_B of the rat, determined previously (13). The value of $K_{p,app}$ for spleens was estimated from the observed values (at 8, 24, and 48 h) in Fig. 6 of Ref. 3, and then the K_p was calculated.

Since. Eq. 6 holds under the condition $K_a C_f^T \ll 1$, its applicability was examined as follows. The plasma concentrations of doxorubicin at the terminal phase after intravenous administration were $<2 \times 10^{-7}$, 8.2×10^{-8}, 1.3×10^{-7}, and 1.2×10^{-7} M for rats, rabbits, guinea pigs, and humans, respectively. Based on the assumption of pH partition, the value of C_f^T was estimated by:

$$C_f^T = \frac{1 + 10^{pK_a - pH_i}}{1 + 10^{pK_a - pH_e}} \cdot f_p C_p \qquad \text{(Eq. 8)}$$

Values for pK_a (8.15), pH_i (7.0), pH_e (7.4), K_a (4.85×10^5 M^{-1}), and f_p (0.344 for rats, 0.415 for rabbits, 0.529 for guinea pigs, and 0.5 for humans) were substituted into the equation; the f_p value for humans was obtained from the study of Chan *et al.* (4). The values of $K_a C_f^T$ were 7.8×10^{-2}, 4.0×10^{-2}, 6.1×10^{-2}, and 5.7×10^{-2} for rats, rabbits, guinea pigs, and humans, respectively. Therefore, Eq. 6 should be applicable to these four species.

To account for interorgan variations of tissue distribution of doxorubicin, four possibilities can be considered: (*a*) organ or tissue difference in the amount of nuclei per organ or tissue wet weight (C_N), (*b*) organ or tissue difference in binding parameters to nuclei (n and K_a), (*c*) larger binding capacity of doxorubicin to other organ or tissue constituents than to nuclei, and (*d*) a specific membrane transport mechanism. In view of the results that doxorubicin was exclusively localized in the cell nucleus (15) and that there was little difference of tissue nuclear binding parameters between liver and kidney (14), but a good correlation between the *in vivo* K_p values and the tissue DNA concentrations was observed (13), the first possibility might primarily account for the differences in doxorubicin distribution among tissues, although the evidence is insufficient to rule out some contribution of the other mechanisms, if they operate. On the assumption that there is no difference in nuclear drug-binding parameters of various tissues among rats, rabbits, and guinea pigs the *in vitro* K_p values were estimated. In Figs. 3A–C, the *in vitro* and *in vivo* K_p values for rats, rabbits, and guinea pigs listed in Table IV, respectively, are compared; comparatively good agreements can be seen. These results suggest that Eq. 6 is applicable to describe the tissue distribution of doxorubicin in rabbits and guinea pigs as well as in rats. Although slight discrepancies are apparent, the *in vitro* K_p values show a relatively good correlation with *in vivo* K_p values. The reason for the discrepancies are probably (*a*) a shift of the intracellular pH from 7.0, (*b*) operation of a specific transport system, (*c*) involvement of other tissue constituents which affect the intracellular binding, and (*d*) slight differences in the nuclear binding parameters among tissues.

Furthermore, the following factors may be involved in the interspecies variations in K_p values: (*a*) differences in tissue DNA concentrations (C_N), (*b*) differences in plasma unbound fraction (f_p), (*c*) differences in nuclear binding parameters (n and K_a), and (*d*) differences in the rate of specific transport. The tissue DNA concentrations of rabbits were smaller than those of rats in all tissues studied, except the kidney and spleen, and those of guinea pigs were larger than those of rats in most tissues except the muscle (Table I). These differences in tissue DNA concentration among rats, rabbits, and guinea pigs are similar to those among the *in vivo* K_p values for these animal species (Table IV). Thus, it was suggested that differences in tissue DNA concentration are an important factor in the interspecies variation in K_p values. Based on the results of plasma protein binding, the difference in f_p values between rats ($f_p = 0.344$) and guinea pigs ($f_p = 0.529$) may be one of the important factors in the variation of *in vivo* K_p values between rats and guinea pigs. However, the difference in f_p values between rabbits ($f_p = 0.415$) and rats ($f_p = 0.344$) may not be a determinant of the variation in K_p values between these two animal species, since the f_p value of rabbits was larger than that of rats, whereas the *in vivo* K_p values of rabbits were smaller than those of rats in most tissues (Table IV). Consequently, it was concluded that the primary determinant of the interspecies variation in K_p values among rats, rabbits, and guinea pigs is the interspecies differences in tissue DNA concentration and that the difference in f_p values is a secondary determinant.

Plasma binding, tissue binding, and membrane permeability have been considered to be factors that affect the tissue distribution of drugs (10), but little quantitative evidence has been obtained up to the present regarding interorgan and/or interspecies variation in the tissue distribution of drugs. To obtain the *in vitro* K_p value, it is important to estimate accurately the *in vitro* tissue binding under physiological conditions. A usual method is to use tissue homogenates, but this leads to several experimental problems such as disruption and degeneration of drug-binding components, requirement for binding cofactors, and drug metabolism. Moreover, the actual drug-binding component often cannot be distinguished from other intracellular constituents. A useful method for physiological estimation of the tissue binding would be to determine the drug binding by using isolated organelles or macromolecules known to be the primary drug binder in the tissues, as shown in the present study with doxorubicin in rats.

In conclusion, the mechanism of the organ or tissue distribution of doxorubicin in rats, rabbits, and guinea pigs may be summarized as follows. Doxorubicin is strongly and exclusively bound to the nuclei in the cells. The variations in the organ or tissue distribution of doxorubicin are due to dif-

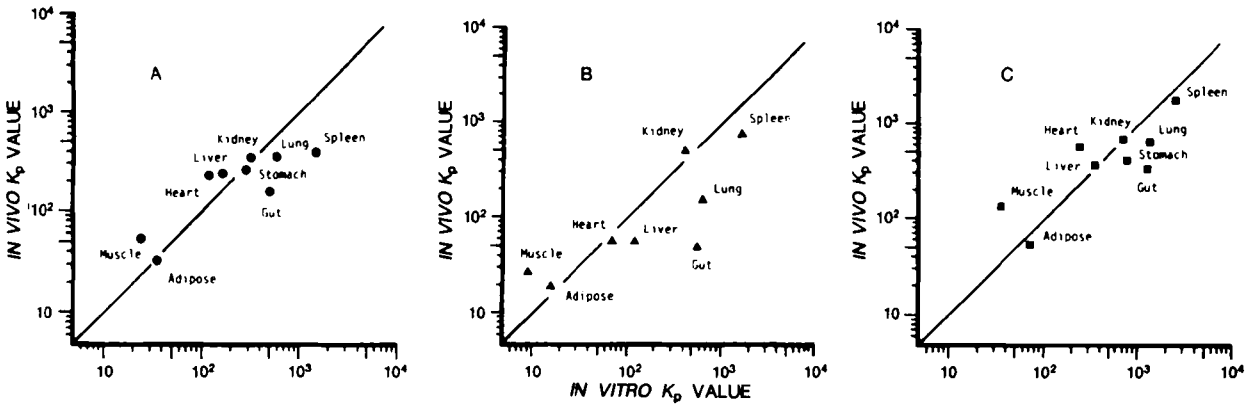

Figure 3—*Comparison between* in vivo *and* in vitro *tissue-to-plasma partition coefficients (K_p values) in rats (A), rabbits (B), and guinea pigs (C). Solid lines represent a 1:1 relationship.*

ferences in the amount of nuclei per gram of organ or tissue. The unbound concentration of doxorubicin in the cytosol must be ~2.3 times higher than that in plasma according to the pH partition hypothesis.

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Preparation and Biodistribution of ^{99m}Tc-Labeled Tyramine Iminodiacetic Acid

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Abstract □ The synthesis and biodistribution properties of ^{99m}Tc-labeled *N*-substituted tyramine, [*N*-(4-hydroxyphenethyl)iminodiacetic acid] are described. Tissue distribution studies in rats were indicative of high hepatic and kidney extraction, accompanied by rapid plasma and urinary clearance and minimal biliary excretion. These findings were substantiated by organ image analysis. The preliminary data indicate that this labeled material may represent a new class of radiopharmaceuticals for the evaluation of hepatic and renal functions.

Keyphrases □ Biodistribution—^{99m}Tc-labeled tyramine iminodiacetic acid, preparation □ Tyramine iminodiacetic acid—biodistribution and preparation □ Radiopharmaceuticals—preparation and biodistribution of ^{99m}Tc-labeled tyramine iminodiacetic acid

Tyramine (I), a noncatecholic phenethylamine found in a variety of plants and animal tissues (1), is produced by decarboxylation of its parent amino acid tyrosine (2). Normally, the physiological effects of tyramine are minimal because >90% of the tyramine is rendered metabolically inactive by mitochondrial monoamine oxidase during its first passage through the liver after absorption (3, 4). The rapid rate of elimination and its inability to cross the blood–brain barrier (5) protect the body from the adverse peripheral and neurotoxic effects of tyramine.

Abnormal metabolism of tyramine has been implicated in a variety of clinical disorders. Abnormal urinary excretion of the amine occurs in patients suffering from Parkinson's disease (6), schizophrenia, induced hypertensive crisis (7), cystic fibrosis (8), epilepsy (9), hypertyrosinemia (10), depression, and migration (11). We have recently demonstrated that the tyramine concentration is abnormally elevated in the plasma and urine of patients with cirrhosis (12, 13), hepatitis (14), and Reye's syndrome (15), as well as in experimental animals with hepatic insufficiency or portacaval shunt (16).

In view of its clinical importance, we have taken the initiative to develop a radiopharmaceutical from tyramine. In this report, we describe the synthesis, biological distribution, and image characteristics of ^{99m}Tc-labeled *N*-(4-hydroxyphen-

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