and palmitic acids. Therefore, the slow fluorescence decrease up to the free fatty acid/albumin ratio of ~2 and the faster decrease after that could not be explained by the simple competition. Santons and Spector (13, 14) investigated the effects of free fatty acids on the binding of I to human or bovine serum albumin. They found that the fluorescence of I was enhanced when ≤2 mol of palmitate were added to 1 mol of human albumin, but larger amounts of palmitate produced a reduction in the fluorescence of I; with bovine and rabbit albumins, palmitate in all concentrations only reduced the fluorescence of I. The present result with rat albumin is similar to those with bovine and rabbit albumin.

Correlation Between the Free Fraction of I and those of Various Acidic Drugs—Figure 6 shows the correlation between f_I and the free fractions of various acidic drugs. There are highly statistically significant positive correlations in all cases. Warfarin and phenylbutazone were especially well correlated with I, $r \geq 0.9$. Such high correlations are reasonable when considering that warfarin and phenylbutazone reportedly competitively inhibit the binding of I to bovine serum albumin (15). The correlation coefficients between the free fractions of all drugs are listed in Table II; there is a statistically significant positive correlation between the free fraction of any pair of drugs.

This finding suggests that we can probably estimate the degree of plasma binding of such acidic drugs by knowing that of I. However, it must be kept in mind that the free fractions of various drugs obtained in the present study using dilute plasma samples do not necessarily represent the *in vivo* free fraction. To be able to employ this method using I as a clinical test, therefore, it would be necessary to determine the plasma bindings of various drugs using undiluted human plasma and examine the correlations between the free fractions of these drugs and that of I. However, the fluorescence method using I, which we have presented herein, has the advantages that it takes a short time (5–10 min) and needs a very small amount of plasma (<0.1 mL) to measure the free fraction of I.

Note added in proof: Heparin was used to prepare rat plasma in this study. The effect of heparin injection on plasma protein binding of I was subsequently studied and was reported (16).

REFERENCES

(1) G. Levy, "The Effect of Disease States on Drug Pharmacokinetics," L. Z. Benet, Ed., Academy of Pharmaceutical Sciences, American Pharmaceutical Association, Washington, D.C. 1976, pp. 137–151.

(2) A. Yacobi and G. Levy. *J. Pharm. Sci.*, **66**, 567 (1977).

(3) G. Levy and A. Yacobi, *J. Pharm. Sci.*, **63**, 805 (1974).

(4) Y. Sugiyama, J. Sato, and M. Hanano, *J. Pharmacobio. Dyn. (Tokyo)*, **2**, 403 (1979).

(5) R. F. Chen, *J. Biol. Chem.*, **242**, 173 (1967).

(6) Y. Sugiyama, T. Iga, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.*, **26**, 199 (1978).

(7) S. K. Chakrabarti, R. Laliberte, and J. Brodeur. *Biochem. Pharmacol.*, **25**, 2515 (1976).

(8) S. K. Chakrabarti, *Biochem. Pharmacol.*, **27**, 739 (1978).

(9) K. Tsuda and S. Matsunaga, *Yakugaku Zasshi*, **62**, 362 (1942).

(10) R. F. Chen and R. L. Bowman, *Science*, **147**, 729 (1965).

(11) G. Weber and L. B. Young, *J. Biol. Chem.*, **239**, 1415 (1964).

(12) E. Tipping, B. Ketterer, L. Christodoulides, and G. Enderby, *Eur. J. Biochem.*, **67**, 583 (1976).

(13) E. C. Santons and A. A. Spector, *Mol. Pharmacol.*, **10**, 519 (1973).

(14) E. C. Santons and A. A. Spector, *Biochemistry*, **11**, 2299 (1972).

(15) H. W. Jun, L. A. Luzzi, and P. L. Hsu, *J. Pharm. Sci.*, **61**, 1835 (1972).

(16) J. Sato, Y. Sawada, Y. Sugiyama, T. Iga, and M. Hanano, *J. Pharmacobio. Dyn. (Tokyo)*, **3**, 720 (1980).

Interaction of Doxorubicin with Nuclei Isolated from Rat Liver and Kidney

TETSUYA TERASAKI, TATSUJI IGA [x], YUICHI SUGIYAMA, and MANABU HANANO

Abstract □ The interaction of doxorubicin with nuclei isolated from rat liver and kidney was studied by fluorospectrometry. The nuclei had at least two different types of binding sites for the drug. Both Mg^{2+} and Ca^{2+} competitively inhibited the binding of doxorubicin to the nuclei, which showed a remarkable temperature dependency. No significant difference was observed between the numbers of binding sites ($n = 6.70 \times 10^{-2}$ mol/mol of DNA for liver; 6.41×10^{-2} mol/mol of DNA for kidney) or the affinity constants ($K_a = 4.85 \times 10^5$ M^{-1} for liver; 5.41×10^5 M^{-1} for kidney) under quasi-physiological conditions. These results obtained from *in vitro* binding experiments support previous suggestions that the differences in the *in vivo* distribution of doxorubicin among tissues are not due to differences in the nuclear binding of the drug. The amount of nuclei per gram of tissue is the primary determinant of the characteristic tissue distribution of doxorubicin.

Keyphrases □ Doxorubicin—interaction with nuclei isolated from rat liver and kidney, binding, distribution □ Binding—interaction of doxorubicin with nuclei isolated from rat liver and kidney, distribution □ Nuclei, liver and kidney—interaction with doxorubicin, rats, binding, distribution □ Distribution—interaction of doxorubicin with nuclei isolated from rat liver and kidney, binding

Doxorubicin, an anthracycline antibiotic, has cytotoxic and antineoplastic activities and inhibits both enzymatic RNA and DNA synthesis by intercalating with DNA (1). The tissue distribution of doxorubicin has been studied extensively in humans and laboratory animals, but the mechanism of its distribution in such tissues as liver, kidney, and muscle has not

been elucidated (2–6). The tissue distribution of this drug is generally thought to be related to the affinity for the tissue binder, the concentration of binder, and the permeability across the plasma membrane.

The interaction of doxorubicin and other derivatives with DNA or chromatins isolated from tumor cells, calf thymus, and cultured lung cells has been studied by several methods (7–10). Although the binding of the drug to native DNA has been extensively studied, little information has been obtained on its intercalation with nuclei isolated from normal tissues. Moreover, it has been suggested that doxorubicin also interacts strongly with the negatively charged phospholipid, cardiolipin (11). In addition, it was revealed that tumor cells may have a carrier-mediated influx system and an active efflux mechanism (12). Previously, a good correlation between the *in vivo* tissue-to-plasma partition coefficients for doxorubicin in several tissues and the amounts of nuclei per gram of tissue in rats and rabbits was demonstrated, suggesting that there is little or no difference in the nuclear binding of the drug among tissues (13).

The purpose of this study was to determine the *in vitro* binding characteristics of doxorubicin with isolated nuclei from rat liver and kidney in an attempt to elucidate the mechanisms by which the *in vivo* tissue distribution of the drug occurs.
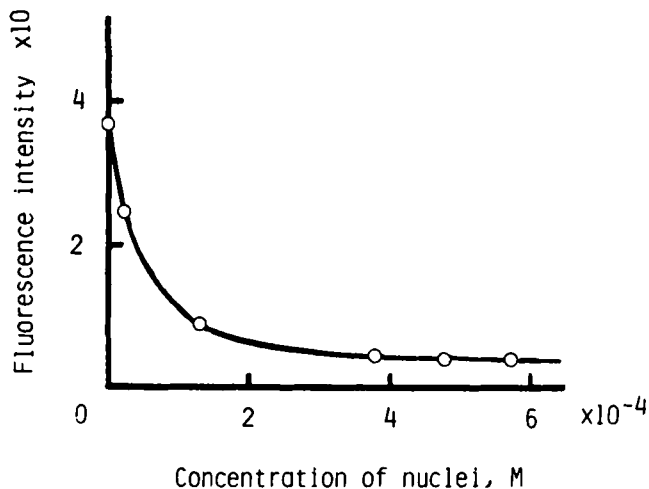
Figure 1—*Effect of nuclear binding on the fluorescence intensity of doxorubicin. The concentration of nuclei is expressed in terms of a nucleotide. A nucleotide molecular weight of 330.9 was used to determine DNA concentration (as nucleotide) as the sodium salt.*

EXPERIMENTAL

Materials—Doxorubicin hydrochloride[1] was stored in the dark in a desiccator at 4°C. Solutions of the drug (2×10^{-6}–5×10^{-4} M) in 405 mM sucrose were freshly prepared immediately before use. Calf thymus DNA[2] and yeast RNA[2] (type XI) were used as standards for the determination of DNA and RNA concentrations in the tissue and nuclei. Micrococcal nuclease[3] was used for the preparation of chromatin. All other reagents were commercial products of analytical grade.

Preparation of Nuclei and Chromatin—Male Wistar rats[4], weighing 250–270 g, were used as liver and kidney donors. Nuclei and chromatin were prepared essentially according to the procedure of Sugano *et al.* (14), except that 1 mM magnesium chloride was added to the isolation buffer to prevent the aggregation of nuclei. No detergent was added to the buffer. The resulting nuclear pellet was suspended in buffer (pH 7.4) containing 340 mM sucrose, 60 mM potassium chloride, 15 mM Tris, 15 mM 2-mercaptoethanol, 1 mM calcium chloride, 1 mM magnesium chloride, and 0.1 mM α-toluenesulfonyl fluoride. The integrity of isolated nuclei was examined microscopically after staining with 1.0% orcein–45% acetic acid solution. The yield of nuclei was 20–30%.

Binding Experiments—The binding studies of doxorubicin to nuclei were

Figure 3—*Scatchard plot of data for the binding of doxorubicin to nuclei isolated from the rat liver. The binding study was performed at 37°C in quasi-physiological buffer. The concentration of nuclei was 4.0×10^{-5}–4.6×10^{-5} M as DNA. The line was calculated for the high-affinity binding site by the SALS method using a digital computer (see text). Key: (●, ◐, ○) observed values from three independent experiments.*

performed by spectrofluorometric titration in a fluorospectrometer[5]. Aliquots (2–10 μL) of freshly prepared doxorubicin stock solution (2×10^{-6}–5×10^{-4} M), up to a 150-μL total sample volume, were added to the cuvette filled with 3 mL of nuclei (2×10^{-5}–5×10^{-5} M as DNA) in a quasi-physiological buffer (pH 7.0) solution containing 135 mM sucrose (which was added to inhibit the swelling of nuclei), 20 mM Tris, 50 mM sodium chloride, 100 mM potassium chloride, 1 mM magnesium chloride, and 1 mM calcium chloride (15, 16). The solution was stirred with a glass rod after each addition, and the fluorescence was measured at 590 nm (excitation at 500 nm) at 10 min (at 37°C) or 30 min (at 7°C) after the addition of the drug. The fluorescence intensity of doxorubicin decreased with increasing amount of nuclei added (Fig. 1), as reported for the interaction of anthracycline antibiotics with DNA and chromatins (7), and the fluorescence intensity (I) of the drug binding to the nuclei can be expressed by:

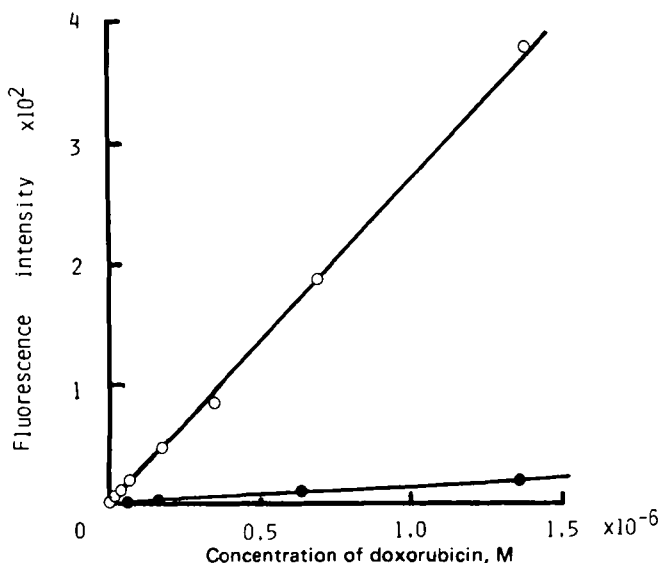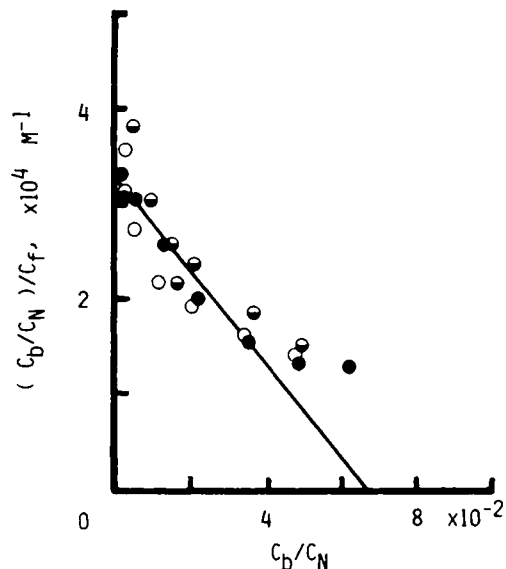$$I = q_f C_f + q_b C_b \qquad \text{(Eq. 1)}$$

Figure 2—*Representative calibration curves for the binding of doxorubicin to nuclei isolated from rat liver. Key: (○) without nuclei; (●) with nuclei.*
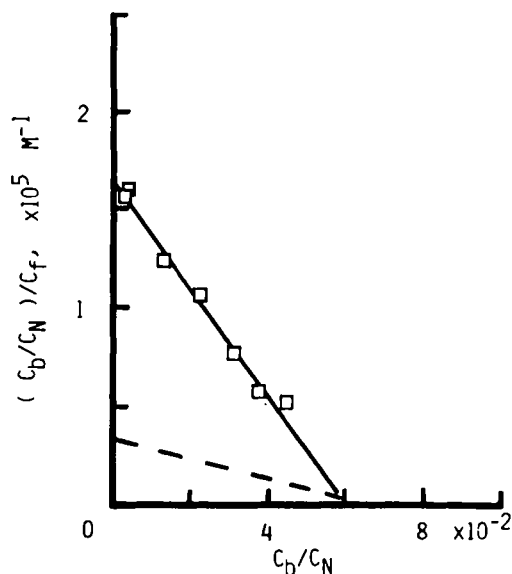

Figure 4—*Scatchard plot for the effect of temperature on the binding of doxorubicin to isolated liver nuclei. The concentration of nuclei was 2.2×10^{-5} M as DNA. Lines were fitted by the SALS method using a digital computer. Key: (□) observed values at 7°C; (---) calculated line at 37°C from Fig. 3 for comparison.*

[1] Adriamycin; Kyowa Hakko Kogyo Co., Ltd., Tokyo.
[2] Sigma Co., Ltd., Chicago, Ill.
[3] Boehringer Mannheim GmbH, Mannheim, West Germany.
[4] Nihon Ikagaku Dobutsu Co., Tokyo.

[5] Hitachi MPF-4.

Table I—Characterization of Nuclei Isolated from Rat Liver and Kidney

	Liver			Kidney		
	Homogenate	Nuclei[a]	Ratio of Nuclei/ Homogenate	Homogenate	Nuclei[a]	Ratio of Nuclei/ Homogenate
RNA[b]/DNA[c]	7.36	0.289	0.039	3.04	0.132	0.043
Protein[d]/DNA[c]	108.0	2.95	0.027	68.0	1.93	0.028
Yield (%)		25.8			21.6	

[a] Obtained from four independent experiments. [b] RNA was extracted by the method of Schneider (17) and determined by the orcinol method (19). [c] DNA was extracted in the same manner as described for RNA and determined by the method of Burton (18). [d] Protein was determined according to Lowry et al. (20) with bovine serum albumin (Fraction V) as the standard.

where q_f and q_b are the quantum yields of free and bound doxorubicin and C_f and C_b are the concentrations of free and bound drug, respectively. The total concentration (C_t) of doxorubicin is given by:

$$C_t = C_f + C_b \qquad \text{(Eq. 2)}$$

The procedure for estimating the quantum yield of drug bound to nuclei is presented in the *Appendix*. Representative calibration curves, determined for each experiment, are shown in Fig. 2.

The apparent binding constants were calculated by means of the following equation using C_f and C_b obtained from Eqs. 1 and 2:

$$(C_b/C_N)/C_f = nK_a - K_a(C_b/C_N) \qquad \text{(Eq. 3)}$$

where C_N is the concentration of nuclei as DNA, n is the number of binding sites, and K_a is the affinity constant. To check the degradation of doxorubicin during the binding experiments, the amount of drug in the nuclear suspension before and after incubation was determined by TLC scanning, as reported previously (13).

Analytical Methods—The concentration of nuclei was expressed as that of nucleotide. The molecular weight of 330.9 (the average molecular weight for a nucleotide) was used to determine DNA concentration as the sodium salt. DNA in the tissue and isolated nuclei was extracted according to the procedure of Schneider (17) and determined colorimetrically by the method of Burton (18). RNA was extracted in the same manner as described for DNA and was determined by the orcinol method (19). Protein was determined according to the procedure of Lowry et al. (20) with bovine serum albumin[6] as the standard.

Data Analysis—The data were analyzed by a least-squares method using a digital computer[7]. The SALS program (21) was used for the calibration curves and the calculation of the binding parameters of the Scatchard plots.

RESULTS

Characterization of Isolated Nuclei—From microscopic observations of nuclei prepared from the liver and kidney, both preparations were confirmed to be unaffected by the isolation procedure, and only slight cytoplasmic contamination was observed. The RNA/DNA and protein/DNA ratios of tissue homogenate and isolated nuclei were compared (Table I). Since no significant metabolism of doxorubicin was observed during the period of the spectrophotometric study, contamination of nuclei with microsomal and/or cytoplasmic enzymes appeared to be negligible.

Scatchard Analysis of Doxorubicin Binding to Liver Nuclei—A Scatchard plot of doxorubicin binding to liver nuclei based on three independent experiments is shown in Fig. 3; reproducibility was good. The curvature of the plot suggests the existence of at least two classes of binding sites. The binding parameters for the high-affinity sites obtained in the lower drug concentration range (9×10^{-8}–4×10^{-6} M) by the least-squares method were $K_a = 4.85 \times 10^5$ M^{-1} and $n = 6.70 \times 10^{-2}$ mol/mol of DNA. However, it was difficult to calculate meaningful binding parameters for the low-affinity sites, since the high inner filter effect of doxorubicin made it difficult to use higher drug concentrations in the binding experiments. The number of high-affinity binding sites is comparable to the value of 8×10^{-2} for the binding of daunomycin to chromatin prepared from Ehrlich ascites tumor cells, as reported by Sabeur et al. (7). However, the affinity constant obtained for nuclei showed a large difference from their value (5×10^6 M^{-1}) (2), which might be due to differences in both temperature and buffer composition.

Effect of Temperature—Figure 4 shows Scatchard plots of the temperature dependency of doxorubicin binding to nuclei. The calculated binding constant at 7°C was $K_a = 2.76 \times 10^6$ M^{-1}, and the number of binding sites was $n = 5.97 \times 10^{-2}$ mol/mol of DNA. This number of binding sites is similar to that

[6] Fraction V; Sigma Chemical Co., St. Louis, Mo.
[7] HITAC M-200H; Hitachi, Tokyo, Japan.

observed at 37°C (6.70×10^{-2}), but there appeared to be a remarkable temperature dependency of the affinity constant, K_a. The calculated enthalpy change was -10 kcal/mol.

Effect of Magnesium and Calcium Ions—To examine the effect of divalent metal ions on doxorubicin binding to liver nuclei, the binding was studied in the absence of 1 mM Mg^{2+} or Ca^{2+} in the buffer. The Scatchard plots of doxorubicin binding to liver nuclei are shown in Fig. 5a for Mg^{2+} and in Fig. 5b for Ca^{2+}. Both divalent metal ions decreased the affinity constant, but did not change the number of binding sites. The affinity constant and the number of binding sites, calculated by the least-squares method, for the high-affinity binding sites in divalent metal ion-free buffer were $K_a = 8.39 \times 10^5$ M^{-1} and $n = 6.03 \times 10^{-2}$ mol/mol of DNA, respectively, without Mg^{2+}, and $K_a = 6.67 \times 10^5$ M^{-1} and $n = 6.44 \times 10^{-2}$ mol/mol of DNA, respectively, without Ca^{2+}. No remarkable decrease in K_a was observed at two- or threefold higher concentrations of these divalent ions.

Effect of pH—Since different intracellular pH values (pH 6.8–7.4) among various tissues have been reported (22), the effect of the buffer pH on doxorubicin binding to liver nuclei was studied over the pH range of 6.4–8.0. As shown in Fig. 6, a small difference appears to exist, but this may not be significant.

Comparison of Doxorubicin Binding to Nuclei and Chromatin Isolated from Liver—Figure 7 shows the Scatchard plots of drug binding to nuclei and chromatin prepared from the liver nuclei. The affinity constant for chromatin in 10 mM Tris buffer at 37°C (pH 7.0) was 2.65×10^6 M^{-1}, and the number of binding sites was 7.59×10^{-2} mol/mol of DNA. The numbers of binding sites to nuclei (Fig. 3) and to chromatin (Fig. 7) are very similar. When doxorubicin binding to chromatin was examined under the same conditions as employed for nuclei (Fig. 3), aggregations of chromatin occurred which might be caused by the high cation concentration (23). Since a homogenous dispersion of chromatin could not be obtained in the physiological buffer, the Tris buffer was used. However, there seemed to be little difference in the binding characteristics of the drug between nuclei and chromatin.

Comparison of Doxorubicin Binding to Nuclei Isolated from Liver and Kidney—To determine whether the binding characteristics of doxorubicin to nuclei are different among different tissues, the binding of the drug to nuclei isolated from kidney, as well as liver, was examined. The results of the antibiotic binding to kidney nuclei are shown in Fig. 8. The calculated affinity constant was 5.41×10^5 M^{-1}, and the number of binding sites was 6.41×10^{-2} mol/mol of DNA. Little difference was observed in these parameters between nuclei isolated from the liver ($K_a = 4.85 \times 10^5$ M^{-1}, $n = 6.70 \times 10^{-2}$ mol/mol of DNA; Fig. 3) and kidney under the same conditions.

DISCUSSION

It is well known that the concentrations of doxorubicin after intravenous administration show large differences among tissues (2–6). We have studied the mechanisms of this characteristic tissue distribution on the basis of the working hypothesis that the amount of nuclei per gram of tissue is the predominant factor and, therefore, the differences in the binding characteristics among tissues are probably negligible (13). In this study, the *in vitro* binding characteristics of doxorubicin to nuclei isolated from the rat liver and kidney were examined in an attempt to prove that these assumptions are valid.

Both the integrity and purity of nuclei are known to be important determinants of the *in vitro* binding of doxorubicin. In this study, the ratios of RNA/DNA and protein/DNA (Table I) are comparable to those reported for liver chromatin by Spelsberg and Hnilica (RNA/DNA ratio = 0.1; protein/DNA ratio = 2.2) (24), and no significant aggregation of the isolated nuclei was observed by microscopic examination. Thus, the nuclei prepared in this study were thought to be suitable for the binding study of doxorubicin. Moreover, the chromosomal proteins, which were suggested to inhibit the interaction of homidium bromide and dactinomycin, bind tightly to DNA (25, 26). Therefore, the release of those important proteins might be negligible in this mild isolation procedure. Since nuclei are the dominant organelles
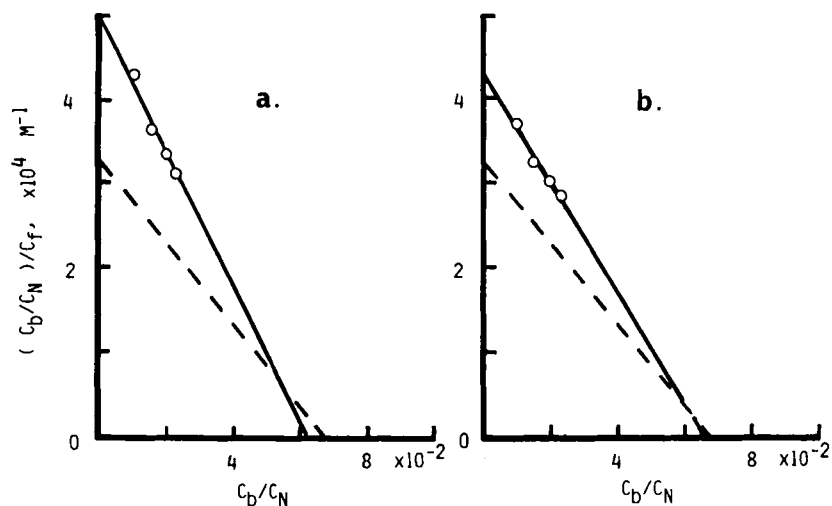
Figure 5—*Scatchard plots for the effects of Mg^{2+} (a) and Ca^{2+} (b) on the binding of doxorubicin to nuclei isolated from the rat liver at 37°C. The concentration of nuclei was 4.3 × 10^{-5} M as DNA in each study. Lines were fitted by the SALS method using a digital computer. Key: (O) in the buffer without Mg^{2+} or Ca^{2+}; (- - -) calculated line from Fig. 3 for comparison.*

associated with the intracellular localization of doxorubicin (27), slight contamination by cytoplasm might not affect the binding parameters.

The results obtained from the nuclei and chromatin binding studies (Figs. 3–8), suggest that both the temperature and buffer contents are more important determinants than is the medium pH. Two binding sites have been reported for daunomycin binding to DNA; one site is a hydrophobic site which binds the antibiotic aglycone with successive base pairs of the double helix, the other site binds daunomycin by an electrostatic interaction involving the DNA phosphate groups and the daunomycin amino group. Data in Fig. 3 also support the existence of at least two binding sites. Another possible interaction by means of hydrogen bonding has also been proposed (28). It has been reported that divalent cations such as Mg^{2+} and Ca^{2+} interact with the phosphate groups of DNA (29). In this study, typical competitive inhibition by both Mg^{2+} and Ca^{2+} was observed in the nuclear binding of doxorubicin (Fig. 5). Accordingly, the present results support the proposed mechanism that the drug binds predominantly to DNA by an electrostatic interaction with phosphate groups.

No significant pH dependency of doxorubicin binding to nuclei was observed over the pH range studied (pH 6.4–8.0) (Fig. 6), which might be due to the complete ionization of the primary phosphate groups of DNA over this pH range. This result is in agreement with the finding that Ca^{2+} showed a constant binding to DNA over the pH range of 5.0–9.5 (29). Consequently, it is considered that doxorubicin binding to nuclei might not be affected by the tissue differences of intracellular pH in the physiological region (22).
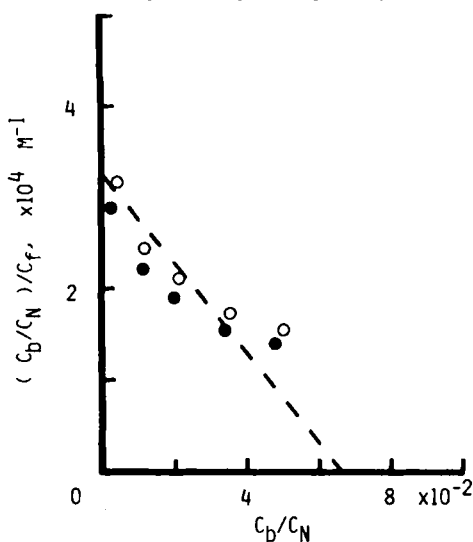
A previous study (13) demonstrated that the *in vivo* tissue-to-plasma partition coefficient of the kidney was 1.5 times greater than that of the liver. However, in the current study no significant difference was observed between the numbers of binding sites (n = 6.70 × 10^{-2} mol/mol of DNA for liver, 6.41 × 10^{-2} mol/mol of DNA for the kidney) or the affinity constants (K_a = 4.85 × 10^5 M^{-1} for the liver, 5.41 × 10^5 M^{-1} for the kidney). Thus, the working hypothesis that the binding of doxorubicin per nuclei is constant among various tissues is supported.

As shown in Fig. 7, the degree of drug binding was similar to nuclei and chromatin. Since chromatin prepared by the procedure employed in this study had a negligible amount of nuclear membrane (14), it is probable that the binding to nuclei reflects the binding characteristics to nuclear DNA rather than to nuclear membranes. Furthermore, the nuclear membrane has numerous nuclear pores (average diameter, 90 nm) (30), so it is unlikely that the membrane permeability is an important determinant of doxorubicin binding either *in vivo* or *in vitro*, in view of the molecular weight of the drug.

A quasi-physiological buffer, which has been proposed to provide the best possible conditions on the basis of morphological examination of isolated nuclei and the intracellular concentrations of ions (15, 16), was used as the buffer for the binding study. Thus, the values of nuclear binding parameters (Figs.
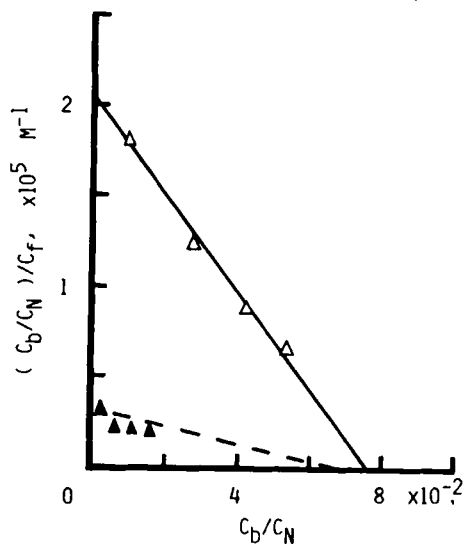

Figure 6—*Scatchard plots of the effect of pH on the binding of doxorubicin to nuclei isolated from the rat liver. The binding study was performed at 37°C in quasi-physiological buffer. The concentration of nuclei was 4.6 × 10^{-5} M as DNA in each study. Key: (O) pH 8.0; (●) pH 6.4; (- - -) pH 7.0, taken from Fig. 3 for comparison.*

Figure 7—*Scatchard plots for the binding of doxorubicin to nuclei and chromatin prepared from rat liver nuclei. The binding was studied in two different concentrations of buffer at 37°C. The solid line was fitted by the SALS method using a digital computer. Key: (△) 10 mM Tris (pH 7.0, concentration of nuclei was 2.2 × 10^{-5} M as DNA); (▲) 135 mM sucrose, 10 mM Tris, 50 mM sodium chloride, 100 mM potassium chloride, 1 mM magnesium chloride, and 1 mM calcium chloride (concentration of nuclei was 6.5 × 10^{-5} as DNA); (- - -) binding curve for liver nuclei, taken from Fig. 3.*
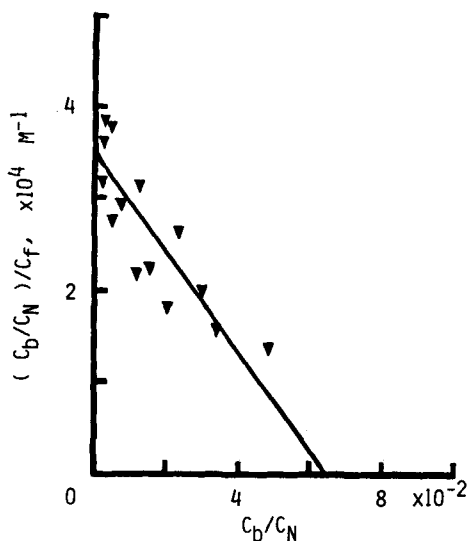
Figure 8—*Scatchard plot of data for the binding of doxorubicin to nuclei isolated from the rat kidney. The concentration of nuclei was 3.7×10^{-5} M as DNA. The line was fitted by the SALS method using a digital computer.*

3 and 8) obtained in this *in vitro* study might be comparable with those under *in vivo* conditions. However, further detailed study would be necessary to elucidate whether these results on *in vitro* nuclei actually reflect the *in vivo* binding.

In conclusion, no difference in the nuclear binding of doxorubicin was demonstrated between the liver and kidney, suggesting that the amount of nuclei per gram of tissue is the primary determinant of the characteristic tissue distribution of this drug.

APPENDIX: Estimation of Quantum Yield of Doxorubicin Binding to Nuclei

Rearranging Eq. 3 gives:

$$(C_b/C_f) = nK_aC_N/(1 + K_aC_f) \qquad \text{(Eq. 4)}$$

According to Eq. 4, in the presence of an excess amount of nuclei doxorubicin is bound completely to nuclei.

Since the fluorescence intensity (I) of doxorubicin decreases with increasing amount of nuclei added, I gives the minimum value (I_{min}) when the drug is bound completely to nuclei, and the maximum value (I_{max}) is obtained in the absence of nuclei. The quantum yields of free (q_f) and bound (q_b) drug are defined as:

$$q_f = I_{max}/C_t \qquad \text{(Eq. 5)}$$

$$q_b = I_{min}/C_t \qquad \text{(Eq. 6)}$$

where C_t is the total concentration of doxorubicin. Substituting Eqs. 5 and 6 into Eq. 1 gives:

$$I = (I_{max}C_f + I_{min}C_b)/C_t \qquad \text{(Eq. 7)}$$

When $K_aC_f \ll 1$, Eq. 4 is reduced to:

$$(C_b/C_f) = nK_aC_N \qquad \text{(Eq. 8)}$$

Substituting Eqs. 2 and 8 into Eq. 7 gives:

$$I = (I_{max} + I_{min}nK_aC_N)/(1 + nK_aC_N) \qquad \text{(Eq. 9)}$$

Thus, rearranging Eq. 9 gives:

$$I = I_{min} + (1/nK_a) \times (I_{max} - I)/C_N \qquad \text{(Eq. 10)}$$

A plot of I versus $(I_{max} - I)/C_N$ should yield a straight line. The intercept of the ordinate gives I_{min}, and the reciprocal of the slope gives nK_a. Accord-

ingly, the quantum yield of bound doxorubicin (q_b) can be calculated from Eq. 6.

REFERENCES

(1) A. D. Marco, F. Arcamone, and F. Zunino, in "Mechanism of Action of Antimicrobial and Antitumor Agents," Vol. 3, (J. W. Corcoran and F. E. Hahn, Eds.), Springer-Verlag, New York, N.Y., 1975, pp. 101–128.

(2) D. W. Yesair, E. Schwartzbach, D. Shuck, E. P. Denine, and M. A. Asbell, *Cancer Res.*, **32**, 1177 (1972).

(3) K. Harrison and H. N. Wagner, Jr., *J. Nucl. Med.*, **19**, 84 (1978).

(4) N. Tavoloni and A. M. Guarino, *Pharmacology*, **21**, 244 (1980).

(5) P. A. Harris and J. F. Gross, *Cancer Chemother. Rep.*, Part 1, **59**, 819 (1975).

(6) K. K. Chan, J. L. Cohen, J. F. Gross, K. J. Himmelstein, J. R. Bateman, Y. Tsu-Lee, and A. S. Marlis, *Cancer Treat. Rep.*, **62**, 1161 (1978).

(7) G. Sabeur, D. Genest, and G. Aubel-Sadron, *Biochem. Biophys. Res. Commun.*, **88**, 722 (1979).

(8) F. Zunino, R. Gambetta, A. D. Marco, and A. Zaccara, *Biochim. Biophys. Acta*, **277**, 489 (1972).

(9) F. Zunino, A. D. Marco, A. Zaccara, and R. A. Gambetta, *Biochim. Biophys. Acta*, **607**, 206 (1980).

(10) H. Waldes and M. S. Center, *Biochem. Biophys. Res. Commun.*, **98**, 95 (1981).

(11) E. Goormaghtigh, P. Chatelain, J. Caspers, and J. M. Ruysschaert, *Biochim. Biophys. Acta*, **597**, 1 (1980).

(12) T. Skovsgaard, *Biochem. Pharmacol.*, **27**, 1221 (1978).

(13) T. Terasaki, T. Iga, Y. Sugiyama, and M. Hanano, *J. Pharm. Pharmacol.*, **34**, 597 (1982).

(14) N. Sugano, Y. Gobou, and K. Shimizu, *J. Biochem.*, **86**, 1651 (1979).

(15) A. I. Lishanskaya and M. I. Mosevitsky, *Biochem. Biophys. Res. Commun.*, **62**, 822 (1975).

(16) D. Veloso, R. W. Guynn, M. Oskarsson, and R. L. Veech, *J. Biol. Chem.*, **248**, 4811 (1973).

(17) W. C. Schneider, *J. Biol. Chem.*, **164**, 747 (1946).

(18) K. Burton, *Biochem. J.*, **62**, 315 (1956).

(19) W. Meybaum, *Z. Physiol. Chem.*, **258**, 117 (1939).

(20) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(21) T. Nakagawa, Y. Koyanagi, and H. Togawa, "SALS, a Computer Program for Statistical Analysis with Least-Squares Fitting," Library Program of the University of Tokyo Computer Center, Tokyo, Japan.

(22) A. Roos and W. F. Boron, *Physiol. Rev.*, **61**, 296 (1981).

(23) A. Jerzmanowski, K. Staron, B. Tyniec-Kroenke, and K. Toczko, *Biochim. Biophys. Acta*, **565**, 356 (1979).

(24) T. C. Spelsberg and L. S. Hnilica, *Biochim. Biophys. Acta*, **228**, 202 (1971).

(25) H. L. Rue and D. Pallotta, *Nucl. Acids Res.*, **3**, 2193 (1976).

(26) L. Kleiman and R. C. C. Huang, *J. Mol. Biol.*, **55**, 503 (1971).

(27) M. J. Egorin, R. C. Hildebrand, E. F. Cimico, and N. R. Bachur, *Cancer Res.*, **34**, 2243 (1974).

(28) W. J. Pigram, W. Fuller, and L. D. Hamilton, *Nature New Biol.*, **235**, 17 (1972).

(29) K. Y. Chang and C. W. Carr, *Biochim. Biophys. Acta*, **157**, 127 (1968).

(30) A. L. Lehninger, in "Principles of Biochemistry," Part I, Worth Publishers, New York, N.Y., 1982, pp. 15–44.