filtered, and evaporated to yield a solid, which was crystallized from $CHCl_3$-hexane to give **31** (2.42 g, 95%): mp 123-124 °C; ^1H NMR ($CDCl_3$-CD_3OD) δ 5.10 (s, 2, $ArCH_2O$), 7.3-8.8 (m, 6, aromatic). Anal. ($C_{11}H_9NO_3$) C, H, N.

3-Nitro-2-naphthaldehyde (32). MnO_2 (5 g, 57.5 mmol) was added to a solution of **31** (2.74 g, 13.5 mmol) in $CHCl_3$ (60 mL), and the solution was stirred at room temperature for 3 h. Additional MnO_2 was added in 5-g portions every 3 h until TLC (SiO_2, 5% MeOH-$CHCl_3$) showed the reaction was complete (a total of 25 g of MnO_2 was used). The reaction mixture was filtered and the solvent evaporated to give a brown solid, which was crystallized from $CHCl_3$-hexane to give **32** (1.80 g, 66%): mp 124 °C; IR ν_{max} ($CHCl_3$) 1695 cm^{-1} (aldehyde C=O); ^1H NMR ($CDCl_3$) δ 7.4-8.8 (m, 6, aromatic), 10.50 (s, 1, CHO). Anal. ($C_{11}H_7NO_3$) C, H, N.

3-Nitro-2-naphthaldehyde Ethylene Acetal (33). A solution of **32** (1.75 g, 8.71 mmol), ethylene glycol (3.5 mL), and *p*-toluenesulfonic acid (20 mg) in toluene (100 mL) was refluxed in a Dean-Stark apparatus for 4 h. The solvent was removed in vacuo and the residue taken up in CH_2Cl_2 (100 mL), washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated to give a brown solid, which was crystallized from $CHCl_3$-hexane to give **33** (2.08 g, 97%): mp 112-114 °C; ^1H NMR ($CDCl_3$) δ 6.68 (s, 1, ArCH), 7.4-8.8 (m, 6, aromatic). Anal. ($C_{13}H_{11}NO_4$) C, H, N.

3-Amino-2-naphthaldehyde Ethylene Acetal (34). A solution of **33** (300 mg, 1.22 mmol) in ethanol (30 mL) was shaken in a Paar shaker in the presence of PtO_2 (200 mg) for 15 min at room temperature under hydrogen (40 psi). The reaction mixture was filtered and the solvent removed in vacuo to give an oil (236 mg, 100%), which was homogeneous by TLC: ^1H NMR ($CDCl_3$) δ 5.90 (s, 1, ArCH), 6.8-7.9 (m, 6, aromatic). Anal. ($C_{13}H_{13}NO_2$) *m/e* 215.095.

***dl*-Benz[*j*]deoxycamptothecin (35).** A solution of **34** (238 mg, 1.11 mmol) and the tricyclic ketone **15a** (211 mg, 0.854 mmol) in anhydrous toluene (50 mL) was refluxed for 30 min in a Dean-Stark apparatus. *p*-Toluenesulfonic acid (10 mg) was then added and the reaction mixture was refluxed for 3 h. The solvent was removed in vacuo and the residue chromatographed (silica gel, 2% MeOH-$CHCl_3$) to give a yellow solid, which was crystallized from 13% MeOH-$CHCl_3$ to give **35** (148 mg, 41%): mp 306 °C dec; IR ν_{max} (KBr) 1735 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR (TFA-d_1) δ 1.19 (t, 3, J = 7 Hz, CH_2CH_3), 2.26 (m, 2, CH_2CH_3), 4.04 (m, 1, ArCH), 5.72 (AB q, 2, J = 18 Hz, $ArCH_2O$), 5.82 (s, 2, CH_2N), 7.8-9.6 (m, 8, aromatic). Anal. ($C_{24}H_{18}N_2O_3$) C, H, N.

***dl*-Benz[*j*]camptothecin (36).** Oxygen was bubbled through a solution of **35** (280 mg, 0.733 mmol), cupric acetate (150 mg), and 25% aqueous dimethylamine (1.5 mL) in DMF (150 mL) until TLC [SiO_2, MeOH-acetone-$CHCl_3$ (5:20:75)] showed the disappearance of starting material (approximately 2 h). The solvent was removed in vacuo and the residue chromatographed (silica gel 60, 4% MeOH-$CHCl_3$) to give a yellow solid, which was crystallized from 13% MeOH-$CHCl_3$ and then HOAc to yield yellow needles (68 mg, 23%): mp 285 °C dec; IR ν_{max} (KBr) 1740 (lactone), 1655 cm^{-1} (pyridinone); ^1H NMR (TFA-d_1) δ 1.14 (t, 3, J = 7 Hz, CH_2CH_3), 2.16 (m, 2, CH_2CH_3), 5.71 (AB q, 2, J = 16 Hz, $ArCH_2O$), 5.81 (s, 2, CH_2N), 7.8-9.6 (m, 8, aromatic). Anal. ($C_{24}H_{18}N_2O_4 \cdot 0.5H_2O$) C, H, N.

18-Methoxydeoxycamptothecin (37). A solution of ketone **15b** (459 mg, 1.66 mmol) and *N*-(*o*-aminobenzylidene)-*p*-toluidine[16] (419 mg, 2.00 mmol) in toluene (40 mL) was refluxed for 30 min. The mixture was cooled, *p*-toluenesulfonic acid (5 mg) was added, and then the mixture was refluxed for an additional 3 h. The solvent was removed in vacuo and the residue chromatographed (silica gel 60, 0.5% MeOH-$CHCl_3$) to give a solid, which was crystallized from $CHCl_3$-EtOAc to yield **37** (477 mg, 79%): mp 260-261 °C; IR ν_{max} ($CHCl_3$), 1740 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$-CD_3OD) δ 2.36 (m, 2, $CHCH_2CH_2$), 3.31 (s, 3, OCH_3), 3.50 (t, 2, J = 6 Hz, CH_2OCH_3), 3.90 (m, 1, $CHCH_2$), 5.28 (s, 2, CH_2N), 5.44 (AB q, 2, J = 6 Hz, $ArCH_2O$), 7.3-8.4 (m, 6, aromatic). Anal. ($C_{21}H_{18}N_2O_4$) C, H, N.

***dl*-18-Methoxycamptothecin (38).** Oxygen was bubbled through a solution of deoxycamptothecin (**37**; 90 mg, 0.249 mmol), cupric nitrate trihydrate (230 mg, 0.954 mmol), and 25% aqueous dimethylamine (65 μL) in DMF (20 mL) until no more starting material remained (approximately 1 h). The reaction was monitored by TLC [silica gel, $CHCl_3$-acetone-methanol (70:20:10)]. The solvent was removed in vacuo, and the residue was taken up in CH_2Cl_2 and washed with H_2O. The organic phase was dried (Na_2SO_4) and evaporated to yield a solid, which was chromatographed (silica gel 60, 1% MeOH-$CHCl_3$) to give a yellow product which was crystallized from $CHCl_3$ to yield **38** (47 mg, 50%): mp 244-245 °C; IR ν_{max} (KBr) 1740 (lactone), 1655 (pyridinone) cm^{-1}; ^1H NMR ($CDCl_3$-CD_3OD) δ 2.16 (m, 2, $CHCH_2CH_2$), 3.36 (s, 3, OCH_3), 3.56 (m, 2, CH_2OCH_3), 5.24 (s, 2, CH_2N), 5.44 (AB q, 2, J = 16 Hz, $ArCH_2O$), 7.5-8.5 (m, 6, aromatic). Anal. ($C_{21}H_{18}N_2O_5$) C, H, N.

Acknowledgment. The support of this investigation by the U.S. Public Health Service Research Grant CA20050-03 from the National Cancer Institute is gratefully acknowledged. The authors also thank Dr. D. Rosenthal and F. Williams for mass spectral data obtained from the Research Triangle Center for Mass Spectrometry.

Accumulation of Drugs by Guinea Pig Isolated Atria. Quantitative Correlations

Heinz Lüllmann, Pieter B. M. W. M. Timmermans,*[1] Gerhard M. Weikert, and Albrecht Ziegler

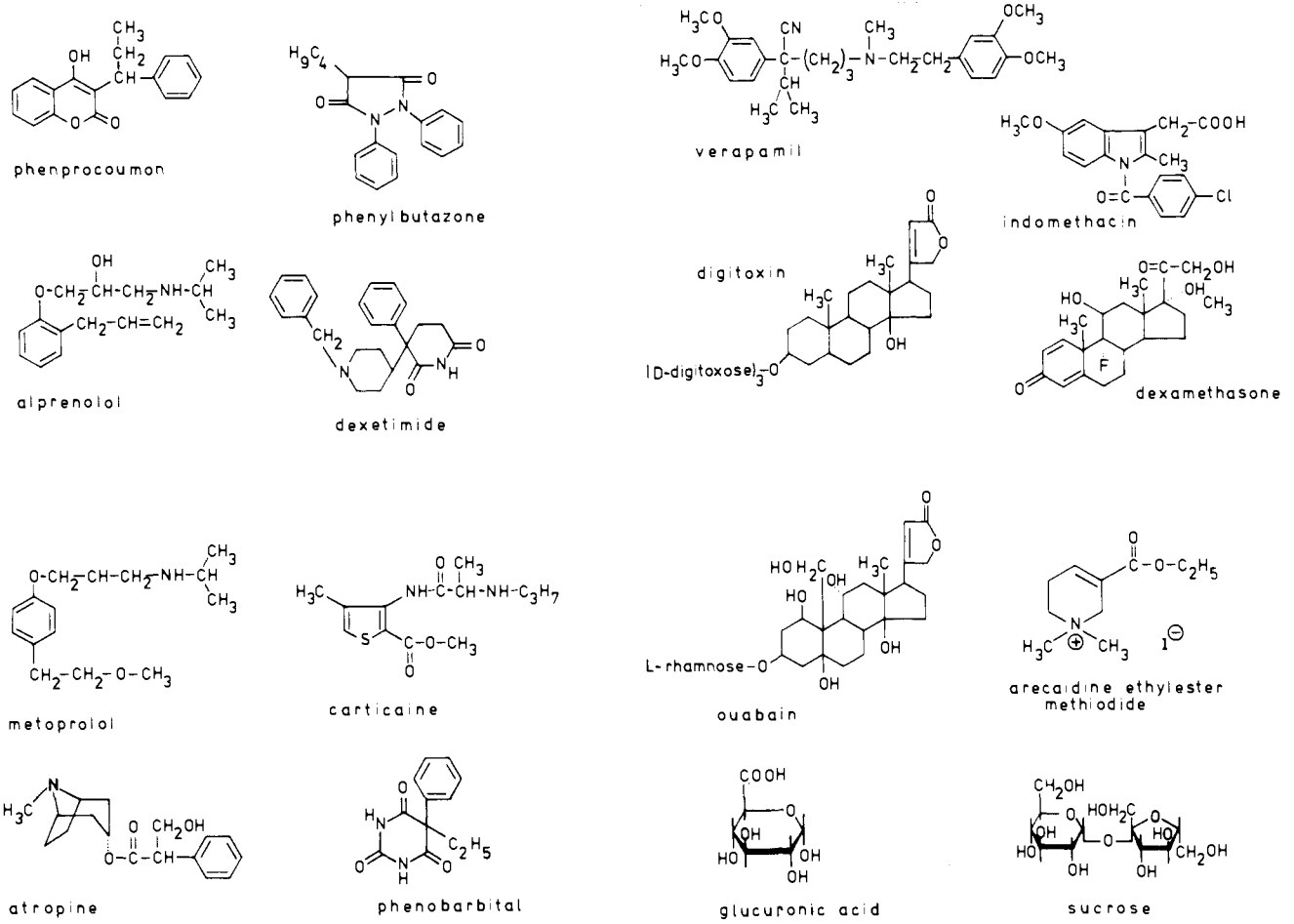
Department of Pharmacology, University of Kiel, Hospitalstrasse 4-6, 2300 Kiel, West Germany. *Received May 18, 1979*

The time course of the tissue accumulation of 16 neutral, cationic, and anionic drugs by resting and 2-Hz stimulated atria of the guinea pig was measured. The accumulation of the substances was quantified by means of their tissue to medium ratios (T/M). Auricles driven with 2 Hz accumulated the drugs faster and during a long period of time to a greater extent than resting atria. By extrapolation of the binding characteristics, the final equilibrium T/M values were estimated. The variance in these accumulation data at equilibrium (log T/M) could be best described by a linear combination of log P (octanol/water) and the ability of the drugs to bind to atrial homogenate (log percent bound/percent free). A parameter calculated from protein binding appeared less significant. Comparable results were obtained for the accumulation data measured in resting and 2-Hz stimulated atrial muscles. It is suggested that the degree of accumulation of drugs into atrial tissue is determined by the facility of their penetration of the plasma membrane and the extent of their intracellular binding.

Uptake studies of drugs by tissues on which they exert their pharmacological effect have frequently been made. These experiments have usually been designed to obtain information on the affinity of drugs for their receptors in the effector organ. Many of these investigations were hampered by a considerable degree of accumulation, which cannot be satisfactorily related to the amount of drug bound by the receptors. The rate of binding of atropine, for instance, appeared to be much slower than the rate of the antagonistic action.[2-4] At equilibrium only a fraction

0022-2623/80/1823-0560$01.00/0

Chart I. Structural Formulas of Compounds Used in This Study



of atropine accumulated seemed to be found at binding sites with the same affinity as could be estimated from pharmacological studies.[5-7] An even greater discrepancy between the time course of drug action and of the rate and degree of drug binding in atrial tissue has been found for the two enantiomers of the atropine-like compound benzetimide.[8,9]

The unusually high tissue to medium ratios found for many drugs suggest the involvement of a large proportion of unspecific binding sites. The physicochemical properties of a drug may be assumed to underly the extent of this binding. In a recent paper, the accumulation of a number of drugs by atrial muscle has been described.[10] Some relationship between the degree of uptake and the lipophilicity of the compound could be recognized. It is the purpose of the present paper to present a quantitative analysis. Attempts have been made to correlate the degree of accumulation of 16 drugs by guinea pig atrial tissue with some physicochemical properties, such as lipophilicity and binding to protein and atrial homogenate.

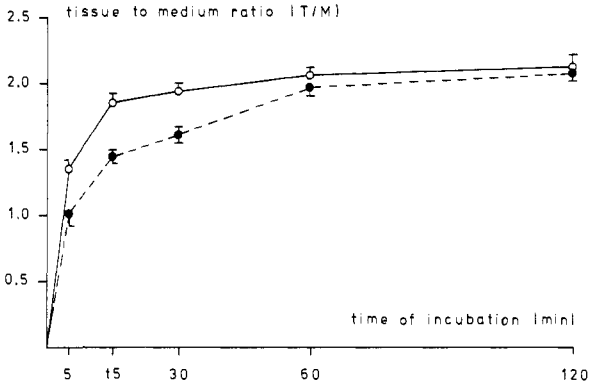


Figure 1. Time course of the accumulation of [^{14}C]phenobarbital (1×10^{-7} M) by isolated guinea pig left auricles. The uptake of the drug is expressed as the tissue to medium ratio (ordinate) determined after different periods of incubation (abscissa) in resting atria (●) and muscles stimulated with 2 Hz (○). The data are presented as mean values ± SEM (n = 6).

Uptake by Resting and 2-Hz Stimulated Guinea Pig Auricles. The structural formulas of the 16 drugs used in the present study have been depicted in Chart I. The uptake experiments with these cationic, neutral, and anionic drugs resulted in great differences in the rate and degree of accumulation by the atrial tissue. For sucrose, glucuronic acid, and arecaidine ethyl ester methiodide, an equilibrium for the uptake was attained within 30–60 min. For ouabain, phenobarbital, atropine, carticaine, and

(1) Recipient of a fellowship from the Alexander von Humboldt Stiftung (Bonn, Bad Godesberg). Corresponding address: Department of Pharmacy, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

(2) H. P. Rang, *Proc. R. Soc. London, Ser. B*, **164**, 488 (1966).
(3) H. P. Rang, *Ann. N.Y. Acad. Sci.*, **144**, 756 (1967).
(4) W. D. M. Paton, *Proc. R. Soc. London, Ser. B*, **154**, 21 (1961).
(5) W. D. M. Paton and H. P. Rang, *Proc. R. Soc. London, Ser. B*, **163**, 1 (1965).
(6) D. R. Waud, *J. Pharmacol. Exp. Ther.*, **158**, 99 (1967).
(7) C. D. Throm and D. R. Waud, *J. Pharmacol. Exp. Ther.*, **160**, 91 (1968).
(8) J. A. Gray, H. Lüllmann, F. Mitchelson, and G.-H. Reil, *Br. J. Pharmacol.*, **56**, 485 (1976).
(9) H. Lüllmann, G.-H. Reil, P. B. M. W. M. Timmermans, and A. Ziegler, in press.
(10) H. Lüllmann, P. B. M. W. M. Timmermans, and A. Ziegler, *Eur. J. Pharmacol.*, **60**, 277 (1979).

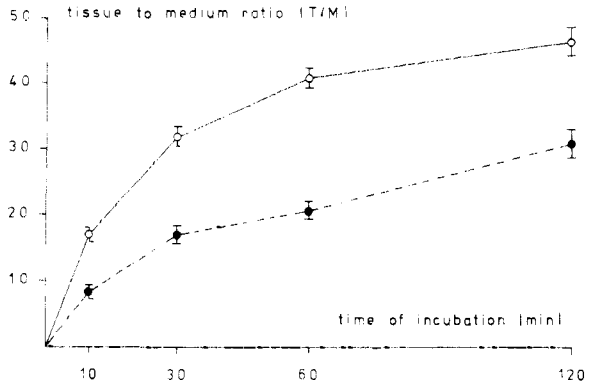


Figure 2. Uptake of [^{3}H]alprenolol (1×10^{-7} M) by isolated left guinea pig atria stimulated with 2 Hz (○) and under resting conditions (●). The accumulation is given as the tissue to medium ratio (means ± SEM; $n = 6$). Note the high tissue accumulation of alprenolol in comparison to phenobarbital (Figure 2).

metoprolol, a final T/M value seemed to be reached after approximately 120 min of incubation. Figure 1 shows the moderate accumulation of phenobarbital in order to illustrate the time course of the uptake process found for these drugs. The remaining compounds were still accumulated by the atrial tissue after 120 min of exposure. However, as has been illustrated for alprenolol (Figure 2), a compound highly accumulated, they eventually will reach a final equilibrium upon prolongation of the incubation time. For all compounds studied, an enhanced rate of accumulation by 2-Hz stimulated muscles compared to quiescent ones was found. With poorly accumulating substances the frequency-induced acceleration of the uptake was only evident in the very first phase of the uptake process. As demonstrated for phenobarbital (Figure 1), identical equilibrium T/M values resulted, irrespective of the stimulation frequency applied. The influence of the driving frequency was more pronounced for drugs yielding higher T/M ratios. As shown for alprenolol (Figure 2), the difference in rate and degree of uptake between resting auricles and those driven with 2 Hz persisted for a long period of time.

The equilibrium T/M ratios of the compounds attained in resting and 2-Hz stimulated auricles are summarized in Table I. For a number of drugs, especially those reaching high T/M ratios, values are reported obtained by extrapolation of the uptake curves. Since the extrapolation of some of these curves to a plateau could only be done with limited accuracy, the values reported represent an approximation of the degree of accumulation. Table I also lists the log T/M values to be used in the correlation studies. These data have been corrected for the amount of drug present in the extracellular space (ECS) of the muscle. The ECS represents approximately 30% of the whole tissue.[11-13] Therefore, an approximated value of 0.3 was substracted from the T/M ratio. It should be noted that this correction yielded very low log T/M values for sucrose and glucuronic acid, indicating that they only occupy the ECS. The cellular uptake of sucrose and glucuronic acid is negligible. Therefore, these values represent the minimal obtainable T/M ratios.

Lipophilicity and Binding to HSA and Atrial Homogenate. Interrelationships. The partition coef-

Table I. Observed and Calculated Tissue to Medium Ratios, T/M and Log T/M, Attained in Resting (0 Hz) and 2-Hz Stimulated Guinea Pig Atria, Partition Coefficients between Octanol and Water, Log P, pK_a Values, Binding to Human Serum Albumin, Log B/F (HSA), and Binding to Atrial Homogenate, Log B/F(Hom), of Various Drugs[o]

no.	compd	T/M (0 Hz)	log T/M (0 Hz)			T/M (2 Hz)	log T/M (2 Hz)			log P	pK_a	log B/F	
			obsd[a]	calcd[b]	Δ log T/M[c]		obsd[a]	calcd[d]	Δ log T/M[c]			HSA	Hom
1	phenprocoumon	110	2.04	1.54	0.50	150	2.18	1.73	0.45	3.62	5[e]	2.128	−0.396
2	phenylbutazone	40	1.60	1.43	0.17	60	1.78	1.60	0.18	3.04	4.4[f]	1.619	−0.100
3	alprenolol	35	1.54	1.42	0.12	47	1.67	1.59	0.08	3.10	9.6[g]	−0.121	−0.158
4	dexetimide	22	1.34	1.52	0.18	27	1.43	1.70	0.27	3.55	*h*	0.087	−0.184
5	verapamil	20	1.30	1.40	0.10	30	1.48	1.55	0.07	2.51	9.2[i]	0.278	0.114
6	indomethacin	15	1.18	1.13	0.05	25	1.40	1.26	0.14	2.00	*j*	0.919	−0.205
7	digitoxin	10	1.00	1.04	0.04	15	1.18	1.17	0.01	1.76		3.222	−0.258
8	dexamethasone	7	0.85	0.99	0.14	9	0.95	1.12	0.17	1.74		0.454	−0.362
9	metoprolol	4.5	0.62	0.56	0.06	8	0.90	0.72	0.18	2.34	9.6[g]	−0.807	−1.619
10	carticaine	3.8	0.54	0.98	0.44	4.8	0.65	1.13	0.48	2.41	7.8[k]	−0.281	−0.739
11	atropine	2.4	0.32	0.50	0.18	4.0	0.57	0.64	0.07	1.76	9.6[l]	−1.004	−1.440
12	phenobarbital	2.2	0.28	0.41	0.13	2.2	0.28	0.54	0.26	1.43	7.5[m]	0.110	−1.444
13	ouabain	1.4	0.04	−0.26	0.30	1.4	0.04	−0.24	0.28	−1.99		−1.066	−1.062
14	arecaidine Et ester CH$_3$I	0.5	−0.70	−0.53	0.17	0.5	−0.70	−0.52	0.18	−2.34		−1.595	−1.464
15	glucuronic acid	0.32	−1.70	−1.76	0.06	0.32	−1.70	−1.73	0.03	−2.57	3.2[n]	−2.000	−4.000
16	sucrose	0.31	−2.00	−2.03	0.03	0.31	−2.00	−2.04	0.04	−3.67		−2.886	−4.000

[a] Corrected for extracellular space. [b] Calculated by using eq 8. [c] Absolute difference between observed and calculated values. [d] Calculated by using eq 10. [e] By analogy with warfarin (pK_a = 5.0; from ref 26). [f] From ref 26. [g] From ref 27. [h] From ref 28. [i] From ref 29. [j] Not available. [k] From ref 25. [l] From ref 30. [m] From ref 31. [n] From ref 32. [o] For experimental details, see Experimental Section.

(11) H. Bauer, H. Lüllmann, and M. Richter, *Pflügers Arch.*, **277**, 48 (1963).
(12) H. Lüllmann and P. A. van Zwieten, *Med. Pharmacol. Exp.*, **16**, 89 (1967).
(13) H. Push, *Experientia*, **34**(8), 1103 (1978).

Table II. Linear Regression Equations Generated by Correlations between the Tissue to Medium Ratios (log T/M) Reached in Resting (0 Hz) and in 2-Hz Stimulated (2 Hz) Guinea Pig Atria and Some Physicochemical Properties for 16 Structurally Dissimilar Drugs

equation	r	s	F	eq no.
log T/M(0 Hz) = 0.446(±0.10)log P − 0.006	0.928	0.440	87.55	4
= 0.835(±0.19)log B/F(Hom) + 1.420	0.929	0.439	88.07	5
= 0.568(±0.25)log B/F(HSA) + 0.549	0.810	0.695	26.75	6
= 0.344(±0.13)log P + 0.218(±0.20)log B/F(HSA) + 0.127	0.951	0.381	61.39	7
= 0.247(±0.10)log P + 0.464(±0.19)log B/F(Hom) + 0.729	0.978	0.254	146.13	8
= 0.010(±0.23)log B/F(HSA) + 0.825(±0.28)log B/F(Hom) + 1.408	0.930	0.451	41.89	9
log T/M(2 Hz) = 0.280(±0.10)log P + 0.452(±0.19)log B/F(Hom) + 0.794	0.979	0.267	146.88	10

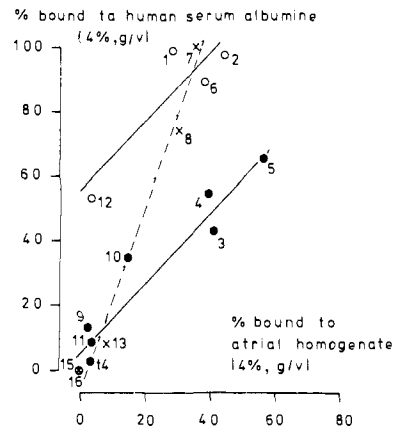


Figure 3. Relationship between the percentage of binding to 4% (g/v) human serum albumin (ordinate) and the percentage of binding to 4% (g/v) atrial homogenate (abscissa) for series of anionic (○), neutral (×), and cationic drugs (●). Binding was determined by incubating 3.3 × 10^{-6} M solutions of the radiolabeled drugs. Numbering refers to Table I.

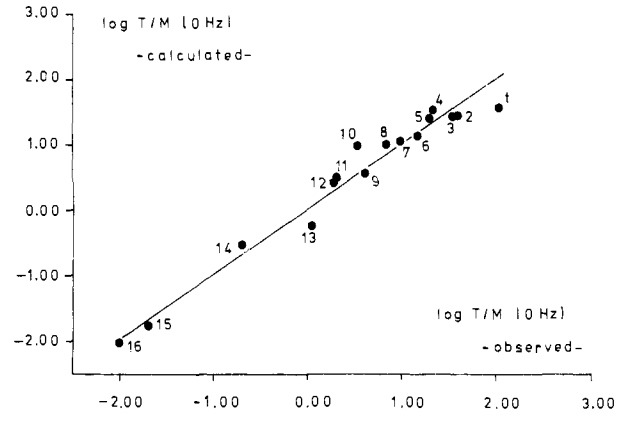


Figure 4. Relationship between the equilibrium log T/M values attained in resting atria of the guinea pig calculated according to eq 8 and the values obtained by extrapolation of the accumulation characteristics. Numbering refers to Table I.

ficients of the uncharged species (log P) between octanol and water (Table I) differ in a wide range (>7 log P units). Consequently, it may be expected that distinct differences in lipophilic behavior will be encountered within this series of compounds. The ability of the drugs to bind HSA and atrial homogenate (4%, g/v, of each) was assessed by incubating 3.3 × 10^{-6} M solutions of the radiolabeled drugs. The results, expressed as log B/F, are also presented in Table I. In order to judge the degree of ionization of the drugs, we also have listed the pK_a values in this table.

The extent of the correlation between the percentage of binding to HSA and atrial homogenate is shown in Figure 3. For the cationic drugs a linear correlation can be recognized with a slope somewhat greater than unity. The neutral and anionic substances are too little in number to allow firm conclusions. However, for the neutral drugs a given increment in binding to atrial homogenate seems to result in a much greater increase in the ability to bind to HSA. Anionic drugs, glucuronic acid excepted, bind to a much greater degree to HSA. The interrelationships between log B/F(HSA), log B/F(Hom), and log P are given by the eq 1–3. As might already be deduced from Figure 3, eq 1 confirms that for all drugs used in this study the ability to bind to atrial homogenate, log B/F(Hom), hardly correlates with the capacity of HSA binding, log B/F(HSA). The octanol/water partition coefficient of the drug, log P, predicts the binding to atrial homogenate better (eq 2) than the binding to HSA (eq 3), although both correlations do not satisfy.

$$\log B/F(\text{Hom}) = 0.622(\pm 0.30)\log B/F(\text{HSA}) - 1.046 \quad (1)$$

$$n = 16;\ r = 0.761;\ s = 0.856;\ F = 19.24$$

$$\log B/F(\text{Hom}) = 0.429(\pm 0.18)\log P - 1.583 \quad (2)$$

$$n = 16;\ r = 0.802;\ s = 0.788;\ F = 25.18$$

$$\log B/F(\text{HSA}) = 0.471(\pm 0.26)\log P - 0.609 \quad (3)$$

$$n = 16;\ r = 0.720;\ s = 1.118;\ F = 15.09$$

Quantitative Correlations. Correlation equations were derived between the final accumulation of the drug in resting and 2-Hz stimulated guinea pig atria, log T/M(0 Hz) and log T/M(2 Hz), respectively, and log B/F(Hom), log B/F(HSA), and log P. The relationships obtained with log T/M(0 Hz) are given in Table II. In correlating log T/M(0 Hz) with either of one parameter, log P and log B/F(Hom) are of equal importance. Both terms explain 86% (= r^2) of the variance in the log T/M(0 Hz) data (eq 4 and 5). The accumulation of the drugs is less satisfactorily described by their tendency to bind to HSA (eq 6). Adding a squared term in log P to eq 4 did not result in a better correlation. All further relationships in two terms (eq 7–9) are statistically better than those formulated in the corresponding single parameters. Equation 8 is the most predictive relationship and is a highly significant improvement over eq 4 ($F_{1,13}$ = 29.09; $F_{1,13;p=0.001}$ = 17.81). It explains about 96% of the variance in the accumulation data. Inclusion of (log P)^2 or log B/F(HSA) or both parameters into eq 8 did not lead to a significant improvement. Other parameter combinations, including pK_a, were also inappropriate. In conclusion, the inspection of the equations given in Table II indicates that the description statistically most significant is given by eq 8 using log P and log B/F(Hom). This relationship provides calculated log T/M(0 Hz) values which agree well with the experimental data (Table I). For all compounds the accumulation is predicted within the limits ±2s. For comparison, the log T/M(0 Hz) values calculated according to eq 8 are plotted in Figure 4 against the data experimentally determined.

The stepwise multiple regression analysis in which log T/M(2 Hz) represented the dependent variable resulted

in virtually identical results as reported above for log T/M(0 Hz). This is not unexpected, since both examined variables are highly correlated ($r^2 = 0.99$). Once more, the linear combination of log P and log B/F(Hom) correlated best with log T/M(2 Hz) (eq 10, Table II).

Discussion

Drugs accumulating into atrial tissue will, first of all, reach the extracellular space (ECS) freely accessible from the surrounding bath. This compartment represents about one-third of the total muscle.[11-13] Consequently, in case the accumulation is solely governed by the filling of the ECS, a tissue to medium ratio of 0.3 will result (sucrose, glucuronic acid). Tissue to medium ratios of more than 0.3 will be obtained for drugs which bind to the cell membrane (plasmalemma) or which are able to cross this lipid boundary. Accumulation within the plasmalemma cannot account for the high T/M values found for many drugs due to its small dimensions. After passage through the cell membrane, the drug will gain access to the cytosol. The concentration in this aqueous phase cannot exceed the bath concentration, since active transport processes are not known for the compounds investigated. Moreover, for dissociable drugs the accumulation cannot be explained by a pH difference between ECS and cytosol.[14] It has to be assumed that the high T/M ratios are caused by the binding of the drugs to structures within the cell (proteins, phospholipids, and more complex structures, e.g., cytomembranes). The physiochemical properties of the drug will determine the rate and the extent of its passage through the plasmalemma and the degree of the successive binding to cellular structures.

In view of the chain of events constituting the accumulation process of drugs in atrial tissue (see preceding paragraph), octanol/water partition coefficients were determined as a measure of lipophilicity, and parameters were obtained describing the ability of the drugs to bind to protein and atrial homogenate. The binding data to HSA and atrial homogenate were expressed as log B/F according to Bird and Marshall.[15] It parallels a concentration ratio in the protein (tissue)–water system, which may be connected to an ordinary partition coefficient and a tissue to medium ratio. In general, this will lead to sharper correlations. The slope relating the HSA to the atrial homogenate binding is close to unity for cationic drugs, indicating that the two processes of binding are quite similar. The same may be applied to anionic drugs with some reservation. Cationic and anionic substances possessing identical atrial homogenate binding differ, however, with respect to their degree of HSA binding.

Octanol/water partition coefficients (log P) can serve as a good reference system for the hydrophobic binding of drugs by proteins.[16-18] However, for our structurally dissimilar substances which also differ in the proportion and the charge of the ionic species upon dissociation, the HSA binding is not well predicted by log P. Scholtan[19] has shown that certain classes of drugs display a specificity for the binding to albumin, which of course octanol lacks. In order to deal with all the drugs in a single equation, at least an additional term for specific electronic interactions is probably needed and, presumably, also one for steric effects. The binding to atrial homogenate may be qualified as more unspecific, since many different binding partners are available, of which proteins only form a part. Consequently, the correlation with log P was found more predictive. It may be added that for structurally closely related barbiturates log P also correlated better with the degree of binding to homogenates of various tissues[20] than to the extent of binding to a single protein system like bovine serum albumin.[16,21]

The degree of accumulation of the drugs could be best described by log P and log B/F(Hom). Comparable results were obtained for the accumulation data measured in resting and 2-Hz stimulated atrial muscles. This finding indicates that the relative differences in the estimated log T/M(0 Hz) values are comparable to those obtained by extrapolation from 2-Hz accumulation characteristics, which are closer to the true equilibrium. This provides further support to the suggestion that an increased mechanical activity of the muscle only results in a more rapid approach of the equilibrium accumulation.[9,10] The appearance of log P in the regression equation may be related to the ability of the drug to cross the plasmalemma. The penetration of a biological membrane is governed by the degree of lipophilicity of the compound involved.[22-24] Although part of the variance in the log B/F(Hom) data is explained by log P, the significant contribution of the former may justify to use the in vitro binding to atrial homogenate as a measure of drug binding to cellular binding sites which become available after the drug has penetrated the plasmalemma. It is not likely that these binding sites are represented by proteins, since protein binding as such did not significantly improve the correlation.

In conclusion, whether the tissue is contracting or left quiescent or whether the drug is neutral, cationic, or anionic, accumulation by atrial muscles can be described by a linear combination of log P and log B/F(Hom). This finding indicates that the extent of accumulation depends on the ability of the drug to pass the plasmalemma and the degree of binding within the cell.

Experimental Section

Drugs. Randomly and specifically radiolabeled drugs were used: [^{3}H]alprenolol and [^{3}H]metoprolol (460 and 233 mCi/mmol, respectively; AB Hässle, Mölndal), [*N-methyl*-^{14}C]arecaidine ethyl ester methiodide and [*thienyl*-2-^{14}C]carticaine (1.1 and 1.72 mCi/mmol, respectively; Hoechst AG, Frankfurt), [^{3}H]dexetimide (17 Ci/mmol; Radioactive Centre, Brussels), [^{3}H]verapamil (10.5 Ci/mmol; Knoll AG, Ludwigshafen/Rhein). The following drugs were purchased from Amersham-Buchler, Braunschweig: [^{3}H]-atropine (426 mCi/mmol), [^{3}H]dexamethasone (19.1 mCi/mmol), [^{3}H]digitoxin (11 Ci/mmol), [^{14}C]glucuronic acid potassium salt (50 mCi/mmol), [^{3}H]indomethacin (19.4 mCi/mmol), [^{3}H]ouabain (15 Ci/mmol), [^{3}H]phenprocoumon (28 mCi/mmol), [*pheno*-2-^{14}C]barbital (20 mCi/mmol), [^{3}H]phenylbutazone (7.7 mCi/mmol), and [^{3}H]sucrose (2 Ci/mmol).

Uptake by Guinea Pig Isolated Atrial Tissue. Left auricles of guinea pigs were dissected and transferred to an organ bath containing Tyrode solution (500 mL) of the following composition

(14) P. A. Poole-Wilson, *J. Mol. Cell. Cardiol.*, 10(6), 511 (1978); also, see references quoted therein.
(15) A. E. Bird and A. C. Marshall, *Biochem. Pharmacol.*, 16, 2275 (1967).
(16) C. Hansch, "Drug Design", Vol. I, E. J. Ariëns, Ed., Academic Press, New York, 1971, p 271.
(17) C. Hansch, "Structure-Activity Relationships", Vol. I, C. J. Cavallito, Ed., Pergamon Press, London, 1973, p 75.
(18) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(19) W. Scholtan, *Arzneim.-Forsch.*, 18, 505 (1968).
(20) L. R. Goldbaum and P. K. Smith, *J. Pharmacol. Exp. Ther.*, 111, 197 (1954).
(21) C. Hansch and S. M. Anderson, *J. Med. Chem.*, 10, 745 (1967).
(22) B. B. Brody, H. Kurz, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, 130, 20 (1960).
(23) L. S. Schanker, *Pharmacol. Rev.*, 14, 501 (1962).
(24) E. J. Lien, Proceedings of the 4th International Symposium on Medicinal Chemistry, J. Maas, Ed., Elsevier, Amsterdam, 1974, p 319.

(mM): NaCl, 137.0; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.0; $NaHCO_3$, 12.0; NaH_2PO_4, 0.21; glucose, 5.6. The solution with a resultant pH of 7.4 was continuously gassed with 95% O_2 and 5% CO_2 at 32 °C. After an equilibration period of 30 min during which all muscles were driven at a frequency of 2 Hz obtained from a Grass S4H device (4 ms, 7-10 V), the tissue was transferred to a Tyrode solution (500 mL) containing the radiolabeled drug and remained resting or was stimulated with 2 Hz. Most compounds were studied at a concentration of 1×10^{-7} M. For dexetimide and ouabain a concentration of 1×10^{-8} M was used, and for metoprolol a concentration of 3×10^{-8} M was used. Due to the limited specific activity, the uptake of arecaidine ethyl ester methiodide and carticaine was investigated at a concentration of 1×10^{-5} M. Sucrose was applied at 8.8×10^{-3} M. After an appropriate incubation time, the atrial preparations were removed, gently blotted between filter paper for 90 s, weighed, transferred to a counting vial, and dissolved in 2 mL of a tissue solvent (Soluene, Packard Instruments). The solubilized tissue was mixed with 10 mL of liquid scintillation fluid (Dimilume, Packard Instruments) and counted (Tri-carb, Packard Model 544, Packard Instruments). Aliquots of the incubation medium were also taken and assayed as described above. The radioactivity was expressed as dpm/g (w/w) of atrial tissue or as dpm/mL of incubation medium, and the tissue to medium ratio (T/M) was calculated.

Partition Coefficients between Octanol and Water. The partition coefficients between octanol and water (P) were determined by preparing 1×10^{-7} M solutions of the drugs either in water saturated with octanol or in water-saturated octanol. Both phases were adjusted in volume so that roughly equal amounts of the compounds were present in both phases after partitioning. This ideal conditions could not be reached for drugs with extremely low or high partition values. For the neutral drugs and arecaidine ethyl ester methiodide, distilled water was used as the aqueous phase. In order to estimate the partition coefficients of the neutral drug species, the partitions were run between octanol and 0.1 N HCl in the case of anionic drugs, whereas the aqueous phase consisted of 0.1 N NaOH in the case of cationic drugs. The partition of carticaine was determined between octanol and Sörensen phosphate buffer (pH 7.0) in order to minimize hydrolysis of the ester. The estimated value was corrected for ionization (pK_a of carticaine = 7.8[25]). The phases were mechanically shaken for 1 h at room temperature, and aliquots were taken of both phases after they had separated completely by standing for 15 h. The radioactive concentration in both phases was determined as described above. The log P value of the drugs reported in Table I have been taken out of the mean P values obtained from four to six experiments (SEM <10%).

Binding to Human Serum Albumin. Human serum albumin (HSA) was purchased from Behringwerke AG (Marburg/Lahn, West Germany) and used without further purification. The concentration of HSA in all binding experiments was 4% (g/v) and the concentration of radiolabeled drug amounted to 3.3×10^{-6} M prepared in 0.067 M Sörensen phosphate buffer at pH 7.4. It has been verified that by using these concentrations of drugs and HSA the binding is examined under the condition of partial saturation. The binding experiments were performed at room temperature in a final volume of 0.6 mL. After incubation for 90 min, a 0.05-mL aliquot was taken and the radioactivity determined as previously described. The residual probe was centrifugated at 145000g for 15 h at 25 °C. A 0.05-mL aliquot was taken from the supernatant and its radioactivity measured. The amount of drug bound to HSA was obtained by subtracting the unbound faction from the amount added initially. The HSA binding is expressed as log B/F, in which B represents percent bound and F represents percent free. The data reported in Table I are the log values calculated from the average B/F values of six separate experiments (SEM <3%).

Binding to Guinea Pig Atrial Homogenate. Left and right atria of guinea pigs were isolated, blotted lightly between filter paper for 90 s, and weighed. The tissue (6%, g/v) was homogenized in ice-cold Sörensen phosphate buffer (0.067 M, pH 7.4) in a Virtris 45 homogenizer for three 20-s periods of maximal speed. Binding was determined by incubating 3.3×10^{-6} M concentrations of the radiolabeled drugs with 4% (g/v) homogenate for 90 min at room temperature. Under these conditions the binding was examined at a level of partial saturation. The procedure followed was identical with the one described above for the binding to HSA. Results are again expressed as log B/F obtained from six separate experiments (SEM <5%) and reported in Table I.

Correlations. Correlations were generated by stepwise multiple regression analysis, fitting the best straight line via the method of least squares. Statistical tests were performed in order to test the relevance of the terms included and to judge the validity of the equations obtained, which involved the correlation coefficient, r, the standard deviation, s, and the significance of the regression, F. Stepwise inclusion of terms was justified by application of the F test. The figures in parentheses are the 95% confidence intervals.

Acknowledgment. The skillful technical assistance of E. Schröder is gratefully acknowledged. [^{14}C]Carticaine was kindly donated by Hoechst AG (Frankfurt), [^{3}H]metoprolol was donated by AB Hässle (Mölndal), and [^{3}H]-verapamil was generously supplied by Knoll AG (Ludwigshafen/Rhein). One of us, P.T., is greatly indebted to the Alexander von Humboldt Stiftung (Bonn, Bad Godesberg) for providing a research fellowship.

(25) Product information by Hoechst AG, Frankfurt, West Germany.
(26) "Martindale: The Extra Pharmacopoeia", 27th ed, Pharmaceutical Press, London, 1977, p XXVII.
(27) G. Johnsson and C.-G. Regårdh, *Clin. Pharmacokinet.*, 1, 233 (1976).
(28) Product information by Janssen Pharmaceutica, Beerse, Belgium.
(29) R. Mannhold, Ph.D. Thesis, Düsseldorf, West Germany, 1978.
(30) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution", Butterworths, London, 1965.
(31) A. J. Biggs, *J. Chem. Soc.*, 2485 (1956).
(32) P. Hirsch, *Recl. Trav. Chim. Pays-Bas*, 71, 999 (1952).