5.81. Found: C, 62.15; H, 6.70; Cl, 21.80; N, 5.67.

An identical product was obtained by treating 10 g of Id-HCl with 40 ml of a 1 *M* solution of sodium methoxide in 1000 ml of dry methanol.

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Binding of Quinidine to a Red Blood Cell Hemolysate Preparation

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Abstract □ A constant degree of binding of quinidine to a red blood cell hemolysate preparation was found for a clinically significant range of concentrations at 37° using the methods of equilibrium dialysis and ultracentrifugation. By repeating the binding experiments at 37° but over a much wider range of quinidine concentrations, it was possible to calculate an association constant (252 liters/mole) and the apparent number of binding sites (1.54). Quinidine appeared to be bound to a single binding site. In another series of experiments performed at therapeutic levels and 37°, the competitive binding of quinidine was studied in the presence of red blood cell hemolysate and serum, each placed in individual compartments of a dialysis cell but separated by a semipermeable membrane. Following attainment of equilibrium, free drug was separated from bound drug by ultracentrifugation. Calculations indicated that slightly more than half of the drug was contained within the serum compartment.

Keyphrases □ Quinidine—binding to a red blood cell hemolysate preparation, influence on protein binding of quinidine, binding parameters □ Binding—quinidine to a red blood cell hemolysate preparation, influence on protein binding of quinidine □ Erythrocyte binding—quinidine to a red blood cell hemolysate preparation, binding parameters

Quinidine has been shown to interact with various proteins contained within the body's vascular pool. For example, elucidation of a quantitative quinidine–albumin relationship at pH 7.4 established the existence of one receptor location on an albumin molecule and an association constant of 7.7×10^3 (1). There is also extensive binding of quinidine to human platelets as evidenced by its large accumulation within the platelet, much greater than would be expected on the basis of pH partitioning (2). Moreover, quinidine has been found to penetrate red blood cells (3), which might be expected to occur for an organic base with a pKa of 8.6 (4) whose unionized form is lipid soluble (5). Because of its potential for binding, the purposes of this study were to determine the binding affinity of quinidine to an erythrocytic preparation obtained from freshly drawn human blood samples and to determine its competitive influence on the protein binding of quinidine in human serum.

EXPERIMENTAL

Blood Collection—Venous blood samples used in all experiments were drawn from the cephalic veins of two male Caucasians. Samples of 50–100 ml were collected from each donor and placed in a 500-ml erlenmeyer flask containing enough glass beads to cover about two-thirds of the bottom of the flask. The flask was gently swirled for about 10 min to remove the clotting fraction. The suspension of cells was then decanted into an appropriate container and centrifuged. The plasma and buffy layer were discarded and the packed red cells were washed two times with an isotonic pH 7.2 phosphate buffer preparation. The washed cells were resuspended with the isotonic buffer preparation to about 80% of their original volume and pooled.

Preparation of Red Blood Cell Hemolysate—The pooled resuspended cells were hemolyzed with an ultrasonic vibrator[1] and centrifuged at 5000 rpm to remove ghosts. The resulting solution was assayed for hemoglobin content. The volume of the hemolysate preparation was determined and enough additional buffer was added so that the final dilution contained the desired concentration of hemoglobin. The buffer preparation used to adjust the hemoglobin concentration as well as to resuspend the washed red blood cells was formulated to approximate the intracellular pH and ionic strength of the red blood cell. All chemicals used were reagent grade quality, and distilled water was used to prepare all solutions. The pH[2] of the buffer measured 7.2 and the ionic strength was calculated to be 0.18 *M*; the quantities of chemicals used per liter were: $Na_2HPO_4 \cdot 7H_2O$, 1.940 g; KH_2PO_4, 0.312 g; $MgCl_2 \cdot 6H_2O$, 1.017 g; NaCl, 0.512 g; and KCl, 9.76 g. This particular chemical composition yielded 16, 131, 10, and 167 mEq/liter of Na^+, K^+, Mg^{+2}, and Cl^-, respectively, which compared to 16, 150, 10, and 80 or more mEq/liter, respectively, for the same ionic species present in a red blood cell (6). The purpose of studying binding to a red blood cell hemolysate preparation instead of a relatively pure solution of crystallized hemoglobin was to mimic more closely the *in vivo* conditions and, thus, come closer to estimating drug affinity for protein under clinical conditions.

Equilibrium Dialysis—Protein binding to hemolysate components for two different hemoglobin concentrations was determined using a dialyzing system which consisted of a cylindrical Plexiglas cell separated into two compartments by a dialyzing membrane[3]. The cells were similar to those described by Patel and Foss (7), with the exception that a different compartment capacity (depth 5.6 mm, diameter 20.05 mm) was used. Each cell compartment had a capacity of 5.0 ml; however, solutions were added in volumes of 4.7 ml, thus leaving a small volume of air after the plastic screws were inserted into the sampling ports and producing a closed system. The membrane was cut to the proper size, soaked in 70% ethanol for approximately 3 hr to remove impurities, and stored in isotonic buffer under refrigeration until needed; the membranes were thoroughly rinsed with fresh buffer before use. Varying drug concentrations were dissolved in isotonic buffer and pipeted into one compartment of the cell designated the external compartment. The internal compartment contained red blood cell hemolysate. Equilibrium dialysis was carried out at 37° for 20–22 hr in a water bath equipped with a shaker[4]. Additional cells, containing isotonic buffer and drug in one compartment and buffer in the other, were incubated with shaking at 37° and used to determine the time of equilibrium. The extent of protein binding was calculated from the concentration of free quinidine on the external side.

The dialysis cells were also used to determine the binding of quinidine to the red blood cell hemolysate in competition with serum by placing each preparation in compartments separated by a dialysis membrane. The study was performed in a manner simulating *in vivo* conditions in that the initial serum and red blood cell hemolysate were adjusted to pH 7.35 by bubbling a 5% carbon dioxide–95% oxygen mixture through the solutions and were incubated in a 37° shaker water bath. The red blood cell hemolysate was prepared as previously described. The serum was obtained from separate samples of whole blood from the same donors and allowed to stand for approximately 10 min before centrifuging to facilitate removal of the clotting fraction as well as the red blood cells. The serum was decanted into an appropriate container and stored overnight under refrigeration before use. The study was performed at two concentrations of hemoglobin to simulate *in vivo* conditions of binding at normal and anemic hemoglobin concentrations, 0.002218 and 0.001478 *M*, respectively. The volume in each compartment was 4.7 ml. Aliquots of 0.5 ml were removed from each side following attainment of equilibrium and measured for total quinidine concentration, whereas free drug was determined by removing samples of 4.0 ml from each side and separating the free and bound forms by ultracentrifugal separation. In addition, 0.1 ml was removed from each side for sodium-ion determination, which was used to correct for the Gibbs-Donnan effect.

Ultracentrifugation—Buffered isotonic solutions of the hemolysate preparation containing both hemoglobin and quinidine in known concentrations and a volume of 4 ml were placed into 8-ml plastic centrifuge tubes. The tubes[5] were then capped and spun at 50,000 rpm in the preheated preparative rotor of the ultracentrifuge[6]. Centrifugation was carried out for 2.5–3 hr at 37°, and the rotor was permitted to stop without use of the brake. Samples of 0.25–1.0 ml containing only free drug were removed from the clear supernatant upper layer and used to determine the extent of binding.

Analytical Methods—Although quinidine sulfate[7] USP was used throughout the study, all concentrations are expressed in molarity of the base, quinidine. Quinidine concentrations were determined by the method of Cramer and Isaksson (5) in which quinidine is first extracted into benzene and finally transferred to sulfuric acid before fluorometric determinations[8] are made. A calibration curve was prepared from solutions that were treated identically to the unknown drug-protein samples but contained only known amounts of quinidine; blank samples were similarly prepared but from solutions devoid of quinidine and protein.

The concentration of hemoglobin present in the hemolysate preparation was determined from the iron content according to the relationship (8):

$$\frac{\text{g hemoglobin/}}{\text{100 ml solution}} = \frac{\text{mg iron/100 ml}}{3.40} \quad \text{(Eq. 1)}$$

The total iron content was determined in triplicate by a spectrophotometric method (9) and averaged. The hemoglobin concentrations were expressed in molarity by using a molecular weight of 67,000 (10). The pooled plasma samples were assayed for total protein to confirm the presence of a normal protein concentration[9].

Since quinidine exists predominately as the cation at pH values of 7.2–7.4, it will be distributed across the dialysis membrane in accordance with the Gibbs-Donnan equilibrium (11). To assess the Gibbs-Donnan effect, sodium ion was measured on the external and internal sides of the membrane by flame photometry[10].

All necessary calculations were performed by a digital computer[11]. A nonweighted least-squares fit of the experimental data (Fig. 1) was obtained by the use of the BMDX 85 FORTRAN IV computer program, which improves upon initial estimates by means of stepwise Gauss-Newton iterations (12).

RESULTS AND DISCUSSION

Corrections for Free Quinidine Concentration—For the equilibrium dialysis studies, which included a concentration range of 2.2–78 μM, it was necessary to correct for the space occupied by protein, of which 97.5% (w/v) was hemoglobin (6), for binding of

[1] Model LS75 sonifier equipped with a model S75 probe, Branson Instruments, Inc., Stanford, Conn.
[2] Model 28B Metrion III pH meter, Coleman Instruments, Maywood, Ill.
[3] Dialyzing cellophane, Union Carbide Corp., New York, N.Y.
[4] Model G-76 water bath and gyrotory shaker, New Brunswick Scientific Co., New Brunswick, N.J.

[5] High-speed polycarbonate tubes with Noryl caps, Beckman Instruments, Palo Alto, Calif.
[6] Model L2-65B preparative ultracentrifuge equipped with type Ti50 rotor, Beckman Instruments, Palo Alto, Calif.
[7] Merck and Co., Rahway, N.J.
[8] Fluorospec, SF-100, Baird Atomic Inc., Bedford, Mass.
[9] Kindly performed by the Clinical Laboratory, Harborview Hospital, Seattle, Wash.; serum protein content, 8.0 g %, and albumin content, 5.2 g %, were within normal limits.
[10] Model 403, atomic absorption spectrophotometer, Perkin-Elmer Corp., Norwalk, Conn.
[11] CDC 6400, Computer Science Center, University of Washington, Seattle, Wash.

Table I—Binding of Quinidine to Human Red Blood Cells Hemolysate[a] Studied over a Clinically Significant Range of Molar Concentrations at 37° Using Ultracentrifugation and Equilibrium Dialysis

Method Used	H_b[b], $\times 10^3$	D_t[c], $\times 10^5$	D_f[d], $\times 10^5$	D_b[e], $\times 10^5$	β[f]	nK_a[g], liters/mole
Ultracentrifugation	0.505	0.311	0.238	0.0726	0.234	—
		0.932	0.768	0.163	0.175	—
		1.55	1.31	0.239	0.154	—
		4.66	4.07	0.593	0.127	—
		7.76	6.45	1.32	0.170	—
Average (*SD*)					0.172 (0.0392)	411.3
Ultracentrifugation	1.011	0.311	0.191	0.0120	0.386	—
		1.55	1.03	0.0519	0.334	—
		3.11	2.04	0.107	0.343	—
		4.66	4.19	0.0472	0.101	—
		7.76	6.04	0.172	0.222	—
Average (*SD*)					0.277 (0.116)	379.0
Equilibrium dialysis[h]	1.33	0.216	0.120	0.0957	0.444	—
		0.647	0.360	0.287	0.444	—
		1.05	0.640	0.407	0.389	—
		2.13	1.24	0.889	0.418	—
		3.14	1.92	1.21	0.386	—
		5.42	2.97	2.44	0.451	—
Average (*SD*)					0.422 (0.0288)	549.0
Equilibrium dialysis[h]	1.33	0.223	0.112	0.109	0.493	—
		0.647	0.360	0.287	0.444	—
		1.05	0.640	0.407	0.389	—
		2.08	1.30	0.787	0.378	—
		3.12	1.94	1.18	0.378	—
		5.34	3.06	2.29	0.428	—
Average (*SD*)					0.418 (0.0458)	540.1
Equilibrium dialysis[h]	1.33	0.220	0.114	0.106	0.481	—
		0.655	0.351	0.304	0.465	—
		1.038	0.652	0.387	0.372	—
		2.11	1.26	0.855	0.450	—
		3.45	1.66	1.69	0.505	—
		5.28	3.14	2.14	0.405	—
Average (*SD*)					0.439 (0.0521)	588.5
Ultracentrifugation	1.77	1.08	0.591	0.493	0.455	—
		5.71	3.31	2.41	0.421	—
Average (*SD*)					0.438 (0.0239)	440.8
Equilibrium dialysis[i]	1.96	2.20	1.26	0.939	0.427	—
		3.38	1.77	1.61	0.476	—
		5.44	3.23	2.21	0.406	—
Average (*SD*)					0.436 (0.0362)	226.9
Equilibrium dialysis[i]	1.96	0.569	0.504	0.0652	0.115	—
		0.949	0.840	0.109	0.115	—
		2.05	1.47	0.575	0.281	—
		3.74	1.28	2.46	0.659	—
		5.21	3.56	1.65	0.317	—
Average (*SD*)					0.297 (0.223)	215.3
Equilibrium dialysis[i]	1.96	0.575	0.496	0.0790	0.137	—
		0.960	0.824	0.136	0.142	—
		2.04	1.48	0.554	0.272	—
		3.19	2.04	1.15	0.361	—
		5.15	3.63	1.52	0.295	—
Average (*SD*)					0.242 (0.0986)	162.7

[a] Represents erythrocytic contents with ghosts removed and diluted to appropriate hemoglobin concentrations using a pH 7.2 phosphate buffer containing approximate cell concentrations of K^+, Na^+, Mg^{+2}, and Cl^-. [b] Molar hemoglobin concentration, experimentally determined. [c] Total molar quinidine concentration. [d] Free molar quinidine concentration. [e] Bound molar quinidine concentration. [f] Fraction bound. [g] n = number of binding sites on hemoglobin; K_a = association constant. [h] Gibbs–Donan correction, Na^+ (internal)/Na^+ (external) = 1.032 ± 0.0362. [i] Gibbs–Donnan correction, Na^+ (internal)/Na^+ (external) = 1.080 ± 0.0389.

drug to the dialysis cell and/or membrane, and for the Gibbs–Donnan effect. Because of the space occupied by protein, a correction was made based upon the fractional water volume of a normal red blood cell, 0.7142 (13). Since the concentration of free quinidine in erythrocyte water exceeds the experimentally determined concentration based upon the total volume of the red blood cell hemolysate, the measured free molar drug concentration in the external compartment, D_f (external), is divided by 0.7142 to relate this quantity to the molar free drug concentration in the internal compartment. However, for hemoglobin concentrations [Hb] other than average for adult males, *i.e.*, 0.002388 M (12), the correction for D_f (internal) was achieved by use of Eq. 2:

$$D_f \text{ (internal) corrected for hemoglobin space} = 1 - (1 - 0.7142)\frac{[\text{Hb}]}{0.002388} \quad \text{(Eq. 2)}$$

Preliminary experiments showed that binding of quinidine to the plastic dialysis cells and/or membrane was on the order of 6.08 ± 1.10% (n = 10) over the concentration range studied and, therefore, necessitated a correction.

Because the ionic strength was not inordinately large and the pH of the red blood cell hemolysate, 7.2, was not too distant from the isoelectric point of hemoglobin, 7.07 (14), a small but numerically significant Gibbs-Donnan effect was measured (Table I). The correction for the Gibbs-Donnan effect was based upon the assumption that sodium is a freely diffusible ion whose asymmetric distribution is a direct consequence of the concentration of negatively charged hemoglobin present in the internal compartment of the dialysis cell. Thus, the requirements of the Gibbs-Donnan effect states that the ratio of the sodium-ion concentration in the internal chamber to the concentration of sodium ion in

Table II—Competitive Binding of Quinidine to Red Blood Cell Hemolysate[a] and Serum Proteins Studied over a Clinically Significant Range of Molar Concentrations at 37° Using Equilibrium Dialysis

Cell Number	Red Blood Cell Hemolysate Compartment					Serum Compartment				
	H_b[b], ×10³	D_t[c], ×10⁵	D_f[d], ×10⁵	D_b[e], ×10⁵	β[f]	P_t[g], ×10³	D_t[c], ×10⁵	D_f[d], ×10⁵	D_b[e], ×10⁵	β[f]
1	1.48	0.128	0.050	0.080	0.609	1.16	0.106	0.0408	0.0652	0.615
2	1.48	0.324	0.109	0.215	0.664	1.16	0.381	0.0766	0.304	0.800
3	1.48	0.505	0.238	0.267	0.529	1.16	0.858	0.160	0.698	0.813
4	1.48	1.00	0.415	0.588	0.586	1.16	1.66	0.300	1.36	0.819
5	1.48	1.62	0.632	0.988	0.610	1.16	2.32	0.709	1.61	0.694
6	1.48	3.24	2.15	1.09	0.335	1.16	3.94	1.30	2.64	0.670
Average (SD)[h]					0.551 (0.106)					0.735 (0.0868)
7	2.22	0.154	0.0781	0.0761	0.493	1.16	0.168	0.0351	0.133	0.791
8	2.22	0.241	0.0594	0.182	0.754	1.16	0.307	0.140	0.166	0.542
9	2.22	0.490	0.364	0.126	0.257	1.16	0.876	0.193	0.683	0.779
10	2.22	1.28	0.499	0.778	0.609	1.16	1.27	0.282	0.988	0.778
11	2.22	1.97	0.618	1.35	0.686	1.16	1.75	0.427	1.32	0.756
12	2.22	3.68	2.31	1.37	0.372	1.16	3.25	0.422	2.82	0.870
Average (SD)[i]					0.583 (0.152)					0.753 (0.110)

[a] Represents erythrocytic contents with ghosts removed and diluted to appropriate hemoglobin concentrations using a pH 7.35 phosphate buffer containing approximate cell concentrations of Na^+, K^+, Mg^{+2}, and Cl^-. [b] Molar hemoglobin concentration, experimentally determined. [c] Total molar quinidine concentration, experimentally determined. [d] Free molar quinidine concentration, experimentally determined by ultracentrifugation. [e] Bound molar quinidine concentration, calculated. [f] Fraction bound. [g] Total molar protein concentration; albumin concentration was 0.000754 M. [h] Gibbs–Donnan correction, Na^+ (serum)/Na^+ (red blood cell hemolysate) = 1.077 ± 0.0636. [i] Gibbs–Donnan correction, Na^+ (serum)/Na^+ (red blood cell hemolysate) = 1.121 ± 0.0603.

the external chamber is equal to a similar ratio of any membrane-permeable cations present in solution including the free quinidine cation. As a result, the following relationship is valid:

$$D_f \text{ (internal) corrected for Gibbs–Donnan effect} = \frac{[D_f \text{ (external)}][\text{Na}^+ \text{ (internal)}]}{\text{Na}^+ \text{ (external)}} \quad \text{(Eq. 3)}$$

The terms on the right-hand side of Eq. 3 are experimentally determined and permit the correction to be made.

The remaining parameters, D_b and D_t, the molar concentrations of bound drug and total drug present in the internal compartment, respectively, were calculated according to Eqs. 4 and 5:

$$D_t = D_i - D_f \text{(external)} \quad \text{(Eq. 4)}$$

$$D_b = D_t - D_f \text{(internal)} \quad \text{(Eq. 5)}$$

where D_i refers to the initial molar concentration placed into the external compartment of the dialysis cell.

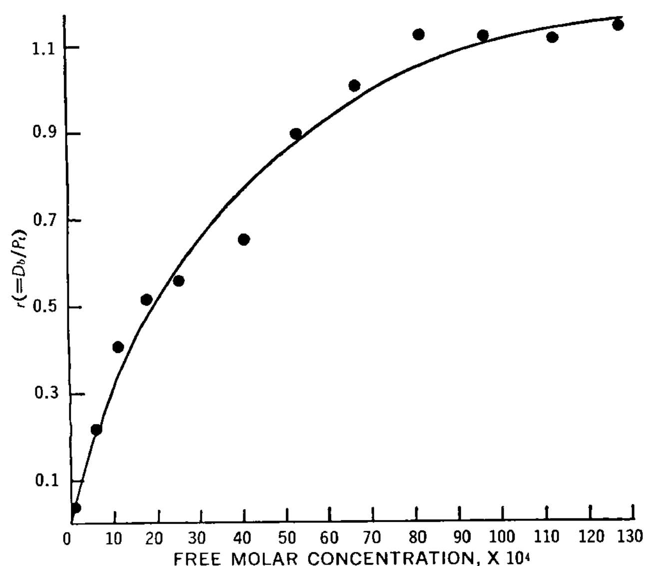

Figure 1—*Binding of quinidine to red blood cell hemolysate, of which 97.5% of the total proteins is hemoglobin* (P_t *is taken as hemoglobin concentration, 9.93* × *10⁻⁴* M). *Binding was studied at 37° and evaluated by ultracentrifugation. The computer-generated line of best fit yielded an association constant of 251.6 liters/mole.*

Analysis of binding utilizing the technique of ultracentrifugation obviated the need for correcting for the Gibbs–Donnan effect. In addition, no binding of quinidine to the centrifuge tubes could be detected. A correction for the space occupied by hemoglobin in the red blood cell hemolysate was made by use of Eq. 2.

Characterization of Binding—The relative binding of quinidine to red blood cell hemolysate was studied over a range of D_t values of approximately 30-fold (0.0466–1.40 μM) including therapeutic levels. It can be seen from Table I that a constant fraction of quinidine was bound to the red blood cell hemolysate when the concentration of hemoglobin was kept constant; however, as the concentration of hemoglobin was reduced, the fraction bound likewise was reduced. It was assumed that hemoglobin was primarily responsible for the protein binding of quinidine in human red blood cell hemolysate since it represents 97.5% of the total protein content within a red blood cell.

The interaction of quinidine, presumably with hemoglobin, was studied by ultracentrifugation at 37° over a concentration range of quinidine sufficient to characterize the binding parameters, n and K_a, which are the number of binding sites on the hemoglobin molecule and the association constant for the interaction, respectively. The binding parameters are related by Eq. 6:

$$r = \frac{D_b}{P_t} = \frac{nK_aD_f}{1 + K_aD_f} \quad \text{(Eq. 6)}$$

where r describes the ratio of D_b to total moles of protein P_t, and n, K_a, and D_f are as previously defined. Equation 6 makes the assumptions that binding involves only one protein in which each binding site is uninfluenced by the state of occupancy of other sites and that the affinity of each site for drug is identical (15). A linear transformation of Eq. 6 can be expressed as Eq. 7, which is the basis for the well-known Scatchard plot (16):

$$\frac{r}{D_f} = nK_a - rK_a \quad \text{(Eq. 7)}$$

the linearity of which indicates that only one class of sites needs to be considered mathematically. The experimental data conformed to Eq. 7, yielding n and K_a values from the slope and intercept of a linear (Pearson r = 0.914) plot. These parameter values were used as initial estimates in the nonlinear curve-fitting routine. When assuming quinidine to interact exclusively with hemoglobin, the data could be fitted according to Eq. 6 as shown by Fig. 1. The n and K_a values obtained from the fit are 1.54 and 252 liters/mole, indicating relatively weak binding.

The results of the binding experiments performed over a relatively small range of quinidine concentrations and at five different hemoglobin concentrations (Table I) can be described by Eq. 8 (17):

$$\beta = \frac{D_b}{D_t} = \frac{1}{1 + D_f/nP_t + 1/(nK_aP_t)} \quad \text{(Eq. 8)}$$

which is derived from mass balance considerations and Eq. 6 where β represents the fraction of total drug bound to hemoglobin. When D_f is small in comparison to nP_t, the second term in the denominator of the right-hand side of Eq. 8 becomes negligible and Eq. 8 can be expressed in Eq. 9 for the purposes of calculating nK_a:

$$nK_a = \frac{\beta}{(1 - \beta)P_t} \quad \text{(Eq. 9)}$$

where P_t is taken as the molar concentration of hemoglobin. Values of nK_a were calculated from Eq. 9 and are listed in Table I. The average of these values, 390.4 liters/mole, very closely agrees with the value of 389.4 liters/mole obtained from Fig. 1 using Eq. 6. When using Eq. 9 and an nK_a of 390 liters/mole, it was found that β varies from 0.449 to 0.511 for the normal range of hemoglobin for males and from 0.411 to 0.482 for females.

Competitive Binding—The competitive binding of quinidine in the presence of red blood cell hemolysate and serum was studied at two hemoglobin concentrations to determine the significance of hemoglobin variation to the fraction of quinidine bound in normal human serum (Table II). From the D_t values determined experimentally for each cell compartment, ratios of drug concentrations in serum to that in red blood cell hemolysate were calculated for each compartment and averaged, yielding values of 1.34 ± 0.329 and 1.15 ± 0.344 for the compartments containing normal and anemic hemoglobin concentrations, respectively. Expressed as a percentage, the same systems contained 57.3 and 53.5% of quinidine in the serum compartments.

Although the nK_a value for the quinidine–albumin system (1) is about 10 times larger than the nK_a determined for the quinidine–hemoglobin system, an almost equal percentage of quinidine equilibrates between each compartment because of the much larger concentration of hemoglobin than serum proteins. When both compartments were considered as a single system composed of serum separated from erythrocytic components by a semipermeable membrane, then the average percentage of total quinidine bound to proteins was calculated as 66.1 ± 0.0842 for the system containing normal serum and normal hemoglobin concentrations and 64.9 ± 0.0494 for the system containing normal serum and anemic hemoglobin concentrations.

It has been suggested (15, 17, 18) that elimination is a first-order process depending on free drug concentration. The differences in D_f in normal serum as a consequence of varying hemoglobin concentration is near 1% and, therefore, of doubtful significance to the elimination kinetics of quinidine. However, the binding of quinidine to hemoglobin as well as serum proteins would be important to the pharmacokinetics of quinidine, depending upon how rapidly an equilibrium is reestablished (protein binding as well as partitioning across red blood cells[12]) as drug is eliminated from the body. Under the conditions of a rapid readjustment of equilibrium, the instantaneous rate of drug loss would be directly proportional to the concentration of total drug in the circulating fluids and not to the concentration of free drug. As a consequence, assays currently in use (5, 19) that measure quinidine concentrations in plasma and not blood would overestimate the volume of distribution and, hence, total body clearance. However, if the process of elimination can be demonstrated as first order, either plasma or whole blood concentrations of quinidine measured as a function of time could be utilized in calculating the first-order rate constants characterizing drug transfer between the compartments. This is a result of the concentration of drug bound to hemoglobin as well as of plasma proteins remaining constant over a therapeutic range and, therefore, directly proportional to either D_f or D_t. Moreover, if elimination were a more rapid process than the binding and debinding of quinidine to hemoglobin and albumin, the elimination rate would become dependent upon the rate of binding and debinding. That is, the rates associated with the protein binding process would become rate determining to elimination, but the overall process of elimination would remain first order as long as the fraction bound was essentially constant over therapeutic drug levels (18, 20).

[12] It was estimated from a preliminary experiment that the partitioning of quinidine into red blood cells reaches an equilibrium between 2 and 6 min. Defibrinated whole blood (10 ml) was spiked with 100 μg of quinidine, and aliquots were placed into Wintrobe tubes and centrifuged for approximately 1 min. A volume of 50 μl of serum was removed and assayed. Timing was begun with the spike and ended when the serum was removed from the Wintrobe tube. Equilibration was two-thirds complete after 2 min. The experiment was performed at room temperature.

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