Pharmacokinetic and Clinical Implications of Quinidine Protein Binding

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Abstract □ Equilibrium dialysis was used to assess factors influencing quinidine binding to serum proteins. Binding was not influenced by prolonged storage at −20° or by variation in total serum quinidine concentrations over a clinically relevant range. Protein binding decreased with increasing temperature between 24 and 37° ($p < 0.001$) and when total serum protein concentrations were decreased from 6.0 to 2.6 g/100 ml by dilution with phosphate buffer. The addition of therapeutic concentrations of salicylic acid, phenylbutazone, and tolbutamide significantly reduced quinidine binding ($p < 0.025$), but 10 other commonly coadministered drugs did not. Displacement of quinidine by salicylic acid was nonlinear; the extent of displacement increased with increasing total quinidine concentrations. The unbound quinidine fraction among 12 healthy subjects varied almost twofold and was partly explained by differences in serum albumin concentrations within the usual range of normal (3.9–4.8 g/100 ml). Pharmacokinetic analysis was performed on simultaneous total and unbound serum quinidine concentrations in 12 volunteers who received single intravenous doses of quinidine lactate. The mean elimination half-lives of total and unbound quinidine were not significantly different. However, the mean volume of distribution and total metabolic clearance of unbound quinidine were considerably greater than those determined using total drug concentrations. Renal clearance of free quinidine exceeded creatinine clearance, consistent with tubular secretion of the unbound fraction. Between-subject variability in elimination half-life and metabolic clearance of quinidine was less when free rather than total serum concentrations were analyzed. Acute ECG changes due to intravenous quinidine correlated better with unbound than with total drug concentrations. Thus, measurement of unbound as well as total serum quinidine concentrations may provide additional understanding of variations between individuals in pharmacokinetics and clinical effects of quinidine.

Keyphrases □ Quinidine—pharmacokinetic and clinical implications of protein binding, equilibrium dialysis □ Protein binding—quinidine, clinical implications □ Pharmacokinetics—quinidine, clinical implications of protein binding, equilibrium dialysis □ Cardiac depressants—quinidine, pharmacokinetic and clinical implications of protein binding

The measurement of plasma or serum levels of drugs as a means of monitoring and titrating clinical response is widely accepted (1); but for many drugs, serum concentrations and clinical effects are not well correlated. A major factor may be variability in protein binding of the drug in serum. The degree of protein binding of extensively bound drugs is a major determinant of the intensity and duration of pharmacological action (2–7). Only unbound or free drug can diffuse across membrane barriers to the sites of pharmacological activity or of metabolic alteration or excretion from the body. Since quinidine is approximately 70–80% bound to serum proteins (8, 9), alterations in quinidine binding may contribute to the variability in clinical response (10, 11).

An equilibrium dialysis technique was used to assess protein binding of quinidine. Some factors that contribute to alterations in binding are identified. The potential clinical significance of individual differences in protein binding was assessed in a study relating the acute ECG effects of intravenous quinidine to the pharmacokinetics of total and unbound drug.

EXPERIMENTAL

Determination of Total and Unbound Quinidine—*Dialysis*—Equilibrium dialysis as described by Hughes *et al.* (12) was used with modifications. Plasma or serum was incubated with quinidine sulfate and other drugs at room temperature for at least 30 min prior to dialysis. Serum specimens from volunteers receiving quinidine were kept frozen at −20° and thawed just prior to dialysis.

Equilibrium dialysis of 2 ml of plasma or serum was carried out with 15-cm strips of cellulose dialysis tubing[1] that were treated with distilled water at 100° for 4 hr and then rinsed with cold distilled water. The integrity of all bags was checked, and they were used immediately after preparation. The tubing ends were sealed by double knots, and the bags were then suspended in 7.5 ml of pH 7.4 phosphate buffer (14.11 g of K_2HPO_4/liter, 2.59 g of KH_2PO_4/liter, and 1.99 g of NaCl/liter) and dialyzed for 20 hr at 37° with gentle agitation.

Quantitation of Quinidine—A modification of the method of Cramer and Isaksson (13) was used to assay quinidine in all specimens. Serum or dialysate (0.5–1.0 ml) was alkalinized with 1 ml of 0.1 *N* NaOH and extracted with 7.5 ml of toluene (containing 1.5% isoamyl alcohol). After shaking (5 min) and centrifugation (10 min at 3000 rpm), 5 ml of the organic layer was removed and back-extracted with 5 ml of 0.1 *N* H_2SO_4. The toluene layer was discarded, and the fluorescence of an aliquot of the acidic aqueous phase was measured using a spectrophotofluorometer[2] at an excitation of 350 nm and an emission of 450 nm. This method has a sensitivity limit of 0.03 μg/ml and is linear through concentrations of at least 20 μg/ml. The coefficient of variation of replicate samples was less than 3%.

Recent studies assessed the specificity of the extraction-fluorescence technique. In a number of reports, serum quinidine concentrations determined by extraction-fluorescence were essentially identical to those determined by more specific chromatographic methods (14–18). In two other studies, the fluorescence assay yielded concentrations 5–20% higher than chromatographically determined levels (19, 20). The difference is apparently due to fluorescent quinidine metabolites that are not entirely excluded by the extraction. Additional alkaline washing of extracts prior to quantitation by fluorescence improves specificity (21).

In any case, overestimation of quinidine concentrations by the assay method used in the present study, if it did occur, would be relatively small and unlikely to introduce important errors in pharmacokinetic calculations. This is particularly true for plasma concentration determinations following single doses of quinidine since accumulation of metabolites would be minimal.

***In Vitro* Studies**—*Method Assessment*—The following studies employed blood bank plasma to which indicated amounts of quinidine sulfate and other drugs were added.

1. Recovery was assessed in replicate samples containing quinidine sulfate, 0.5–10 μg/ml ($n = 12$).
2. Reproducibility was determined in multiple specimens containing quinidine sulfate, 0.5–20 μg/ml, and the coefficient of variation (CV = standard deviation divided by the mean, expressed in percent) at each drug concentration was calculated.
3. The time necessary to reach equilibrium was determined by dialyzing replicate plasma specimens containing 5 μg of quinidine sulfate/ml at 37° for 6, 12, 18, 20, 22, and 26 hr.
4. Dialysis of replicate specimens at both room temperature (24°) and at 37° was compared.
5. Concentration dependence of protein binding of quinidine was determined by dialyzing replicate specimens containing 0.5, 1.0, 5.0, and 10 μg of quinidine sulfate/ml.
6. Protein concentration was varied by diluting plasma with phosphate

[1] Size 20, 25225-226, VWR Scientific Inc., Newton Upper Falls, Mass.
[2] American Instrument Co., Silver Spring, Md.

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Table I—Effects of Drugs on Quinidine Protein Binding

Added Drug	Concentration, μg/ml	Quinidine Protein Binding[a], Mean (±SD) Percent Unbound		Value of Student's Independent *t*	Level of Significance (Two-Tailed) Test)
		Without Added Drug	With Added Drug		
Propranolol	0.1	28.9 (±1.3)	29.0 (±3.1)	0.07	NS[b]
Warfarin	5.0	27.2 (±1.2)	28.6 (±1.5)	1.81	NS
Salicylic acid	200.0	27.8 (±1.5)	30.5 (±0.9)	3.86	$p < 0.005$
Phenylbutazone	100.0	28.8 (±2.1)	31.7 (±1.4)	2.85	$p < 0.025$
Phenytoin	20.0	28.7 (±1.4)	30.0 (±2.0)	1.28	NS
Tolbutamide	75.0	29.4 (±2.6)	34.0 (±1.1)	3.97	$p < 0.005$
Diazepam	1.0	31.4 (±2.7)	29.8 (±1.1)	1.35	NS
Sulfisoxazole	100.0	30.6 (±0.5)	29.4 (±3.0)	0.99	NS
Pentobarbital	3.0	30.2 (±0.9)	29.9 (±1.7)	0.38	NS
Trichloroacetic acid	100.0	30.4 (±2.1)	29.4 (±1.4)	0.93	NS
Digoxin	0.002	29.3 (±1.8)	29.9 (±1.5)	0.59	NS
Acetaminophen	10.0	30.7 (±2.3)	31.0 (±1.0)	0.32	NS
Chlordiazepoxide	1.0	28.8 (±0.8)	28.5 (±0.5)	0.73	NS

[a] Quinidine sulfate concentration in all samples was 5 μg/ml; $n = 6$ except for chlordiazepoxide where $n = 5$. [b] Not significant.

buffer at pH 7.4. Replicate specimens (with a quinidine sulfate concentration of 5 μg/ml) at each dilution were dialyzed.

7. The effect of storage of serum samples at −20° on protein binding of quinidine was examined by comparing binding in a fresh specimen of plasma to binding after freezing overnight and thawing to room temperature.

8. The effect of blood collection methods on quinidine protein binding was determined by transferring aliquots of a freshly drawn blood sample into: (*a*) a glass tube, (*b*) an additive-free (red-top) evacuated blood collecting tube[3] without contact with the rubber stopper, and (*c*) an evacuated tube with the sample contacting the rubber stopper.

In Vitro Drug Interactions—The effect of the following drugs, prepared in the therapeutically relevant concentrations (Table I) using absolute ethanol and distilled water, on quinidine protein binding was tested in replicate blood bank plasma samples containing 5 μg of quinidine sulfate/ml: phenylbutazone, digoxin, pentobarbital, phenytoin, tolbutamide, salicylic acid, acetaminophen, propranolol, warfarin, sulfisoxazole, chlordiazepoxide, diazepam, and trichloroacetic acid.

All drugs were added to plasma specimens and incubated at room temperature for at least 30 min prior to dialysis. The diluate for the added drugs (absolute ethanol and water) did not influence quinidine binding. Control samples containing 5 μg of quinidine sulfate/ml alone were analyzed in parallel.

The interaction of salicylic acid and quinidine was studied in detail in freshly obtained human plasma. A series of samples containing 5 μg of quinidine sulfate/ml with salicylic acid concentrations varying from 20 to 400 μg/ml was dialyzed. A series of replicate samples containing 200 μg of salicylic acid/ml with quinidine sulfate concentrations varying from 0.5 to 20 μg/ml also was dialyzed.

Clinical Studies—Intravenous (5 mg/kg) quinidine base as the lactate salt was administered by 15-min infusion to 12 healthy young volunteers who gave written informed consent (22). Free and total quinidine concentrations in multiple serum samples drawn in the 30 hr after each dose were measured as already described and analyzed by iterative weighted nonlinear least-squares regression techniques (22). All urine was collected in divided samples for 48 hr. Urinary excretion of quinidine over 48 hr and the apparent excretion rate constant were used to determine the projected cumulative urinary excretion of quinidine. The following pharmacokinetic variables were then determined for total and free quinidine: elimination half-life, total volume of distribution, total metabolic clearance, and renal clearance.

Acute ECG effects immediately following the intravenous infusion were analyzed by a blind observer. Changes in ventricular rate, corrected QT, and QRS intervals were quantitated. The relation of these changes to free and total drug levels was assessed by linear regression analysis.

Within-subject variability in quinidine protein binding was assessed in seven patients who received quinidine on more than one occasion separated by at least 2 weeks.

RESULTS

Method Evaluation—Mean (±*SD*) recovery was 99.4 ± 1.6% ($n = 12$) when dialysis bags were used immediately after preparation.

[3] Vacutainer, Becton-Dickinson and Co., Rutherford, N.J.

The coefficient of variation for replicate specimens of quinidine sulfate concentrations ranging from 0.5 to 20 μg/ml was always less than 5%. Protein binding was not altered by freezing and storage of serum samples. Contact with rubber stoppers resulted in a small increase in the unbound quinidine fraction (19.0 *versus* 22.4% unbound). Equilibrium was complete by 18 hr of dialysis, and no further changes in protein binding were seen through 26 hr of dialysis. At 24°, quinidine was 25.1 ± 0.8% unbound ($n = 7$) as compared to 29.6 ± 0.6% ($n = 5$) at 37° ($t = 10.18$, $p < 0.001$).

Serial dilution of plasma to 40% of the original concentration increased the unbound fraction from 32 to 44% (Fig. 1). Total protein concentrations of progressively diluted samples ranged from 6.0 to 2.6 g/100 ml, and albumin concentrations ranged from 3.9 to 1.5 g/100 ml.

At normal concentrations of serum proteins, there was no saturability in protein binding as quinidine sulfate concentrations were increased through the therapeutically relevant range from 0.5 to 10.0 μg/ml.

Drug Interactions—Salicylic acid, phenylbutazone, and tolbutamide significantly reduced quinidine protein binding (Table I). Other drugs had no significant effect.

Binding of quinidine in the presence of salicylic acid (200 μg/ml) decreased from 82.5 to 69.0% as the quinidine concentration was increased from 0.25 to 20 μg/ml. Quinidine binding at 5.0 μg/ml decreased from 83.0 to 72.5% as the salicylic acid concentration was increased from 20 to 400 μg/ml (Fig. 2).

Clinical Studies—The mean percent of unbound quinidine among the 12 volunteers ranged from 18.7 to 30.3% (Fig. 3). This variability was partly related to differences in the serum albumin concentrations ($r = -0.569$, $p < 0.05$) (Fig. 3). Pharmacokinetic data for free and total quinidine are summarized in Fig. 4. Use of total rather than unbound quinidine concentrations in pharmacokinetic analyses does not influence the half-life estimation but results in substantially lower values for the volume of distribution and total clearance.

Renal clearance of quinidine based upon total serum levels (1.44 ml/min/kg) averaged slightly less than creatinine clearance (1.58 ml/min/kg), but renal clearance of unbound quinidine—the fraction available

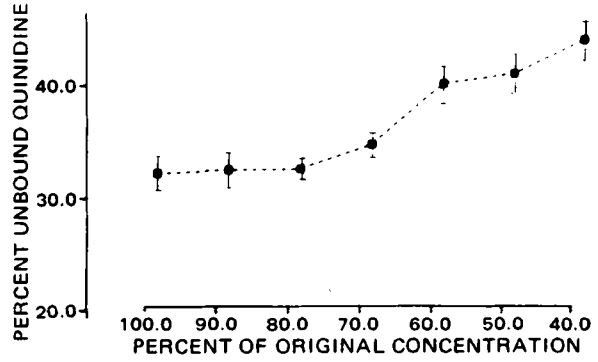

Figure 1—*Effect of plasma dilution on quinidine protein binding. Blood bank plasma was diluted with phosphate buffer, decreasing total protein concentration from 6.0 to 2.6 g/100 ml. The quinidine sulfate concentration was 5 μg/ml in all samples, and each point is the mean* (±SE) *of five determinations.*

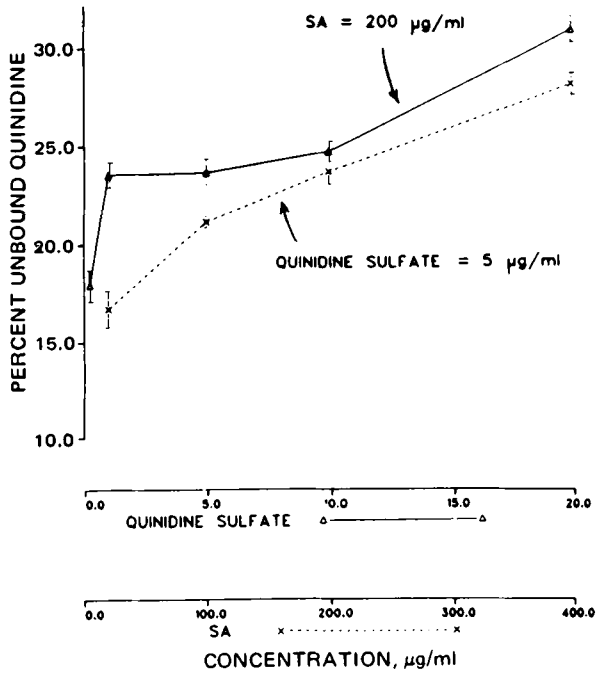

Figure 2—*Effect of salicylic acid (SA) on quinidine binding. The solid line represents the effect of a therapeutic serum concentration of salicylic acid (200 μg/ml) on the saturability of quinidine protein binding where the quinidine sulfate concentration was increased from 0.5 to 20 μg/ml. The dashed line shows the progressive increase in the unbound fraction of quinidine sulfate (5 μg/ml) as the salicylic acid concentration was increased from 20 to 400 μg/ml. Each point is the mean (±SE) of three or four determinations.*

for filtration—averaged 5.97 ml/min/kg. This amount exceeds creatinine clearance and suggests that the unbound fraction undergoes tubular secretion. Figure 5 shows representative serum concentration curves for free and total quinidine.

Individual differences in protein binding partly explained the differences between subjects in pharmacokinetic variables and in acute clinical effects as determined by acute ECG changes. The percent unbound quinidine was significantly correlated with total clearance ($r = 0.68$, $p < 0.05$). Correlations of percent unbound with elimination half-life ($r = 0.48$) and volume of distribution ($r = 0.24$) did not reach significance. Two of the three ECG changes correlated better with free than with total quinidine concentration (Table II).

In contrast to the substantial between-subject differences in protein binding, within-subject variation from time to time was minimal in six of seven subjects and varied by less than 20% in the seventh. Differences were not related to minor changes over time in serum albumin concentration.

DISCUSSION

Equilibrium dialysis for determination of quinidine protein binding utilizes a well-validated approach (23) and has proved to be reliable and reproducible. The method has a sensitivity limit of 0.25 μg of total quinidine/ml of the original serum sample, which incorporates the lower

Table II—Relation of Total and Unbound Serum Quinidine Concentrations to Acute ECG Effects

ECG Change[b]	Number of Data Pairs	Correlation Coefficients for ECG Changes *versus* Peak Serum Quinidine Concentration[a] Total	Unbound
QRS duration	12	0.279	0.536[c]
Corrected QT interval	10[d]	0.626[c]	0.581[c]
Ventricular rate	12	0.050	0.648[c]

[a] Peak concentrations reached at the end of the 15-min infusion. [b] Changes over prequinidine baseline values. [c] $p < 0.05$. [d] Corrected QT interval could not be determined for two subjects.

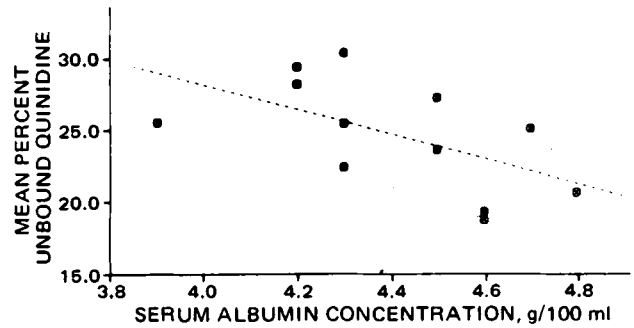

Figure 3—*Variation of quinidine protein binding with serum albumin in 12 healthy young volunteers. Each point is the mean of two or more determinations. The dashed line was determined from least-squares regression analysis ($r = -0.57$, $p < 0.05$).*

limit of the therapeutic range for this drug. When dialysis bags are prepared and used immediately, recovery is essentially 100% complete. Delay in using the dialysis bags increases variability and decreases recovery, presumably due to binding of quinidine by the cellulose tubing. Although some investigators added bactericidal agents to their dialysis solutions (12), this step is generally not necessary. Some turbidity of the phosphate buffer dialysate is observed after 24 hr of dialysis, but protein binding is unchanged between 18 hr of dialysis, when the solution is clear, and 24 hr.

Since quinidine protein binding may be influenced by collection of blood into heparinized tubes (24), all of the blood samples were collected into nonheparinized tubes. Contact of the sample with the red rubber stopper of collection tubes slightly reduced the extent of binding, but the difference was small and unlikely to be of clinical importance.

Increased temperature, decreased protein concentration, and the presence of certain drugs that are highly protein bound can increase the unbound or free fraction of quinidine within the therapeutic range. With salicylic acid, the displacement effect was nonlinear. Salicylic acid not only decreased quinidine protein binding at any given quinidine concentration, but the displacement effect was greater at higher quinidine concentrations. This interaction might cause a disparity between total blood level and expected clinical effect in the therapeutic range and disproportionately greater toxicity at higher blood levels.

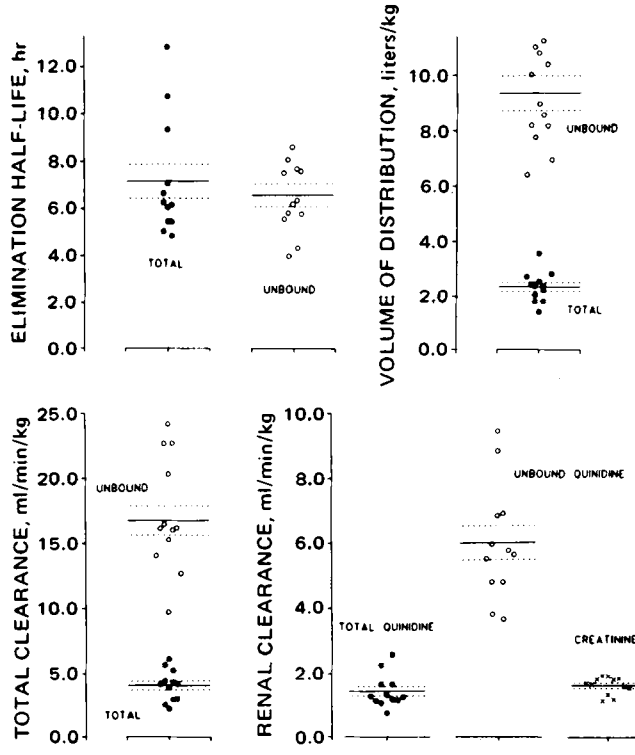

Figure 4—*Comparison of pharmacokinetics of unbound and total quinidine. Individual and mean (±SE) values of pharmacokinetic variables are shown for all 12 healthy volunteers.*

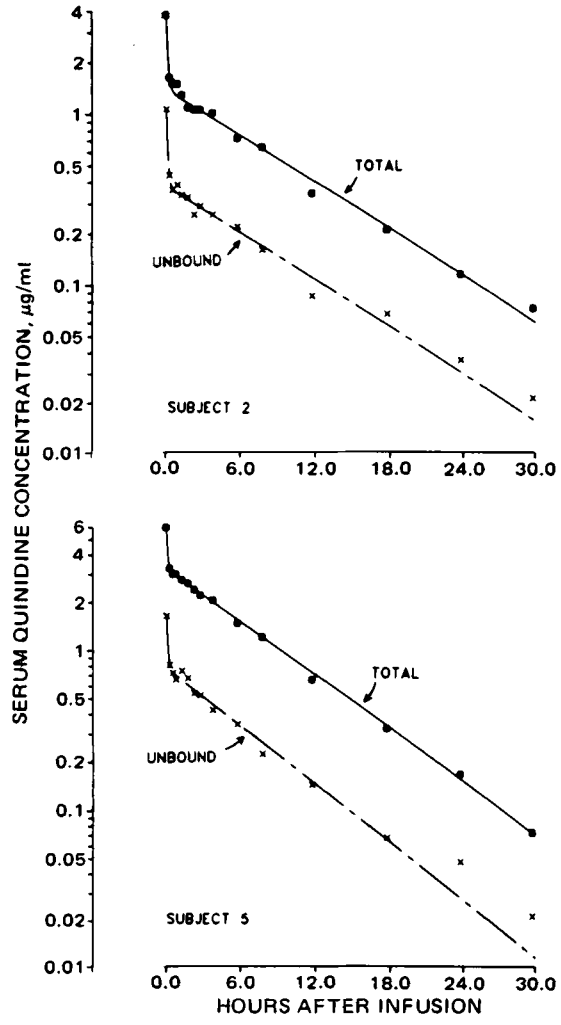

Figure 5—*Serum concentration curves for free and unbound quinidine in two volunteers who received a single intravenous dose of 5 mg of quinidine base/kg. Also shown are pharmacokinetic functions determined by iterative least-squares regression analysis.*

Quinidine binding by blood bank plasma was frequently less extensive than with fresh serum or plasma. Preservative and/or anticoagulants added to bank specimens may explain this result. Protein concentrations of donors also may vary. Since some of the *in vitro* studies required large volumes of plasma, use of bank specimens was unavoidable. Although the same plasma pool was used for any given set of studies, the use of bank plasma with its higher baseline unbound fraction might minimize observed displacement effects. For example, the use of a freshly obtained plasma specimen during the detailed quinidine–salicylic acid interaction study resulted in a much larger displacement effect (from 17 to 24% unbound) than did the same concentrations of both drugs in bank plasma (from 27 to 30% unbound). Thus, the observations of protein-binding displacement interactions in the *in vitro* study probably are a conservative reflection of such interactions during actual drug therapy in humans.

Quinidine protein binding among the healthy volunteers on no other medication varied almost twofold. Some of this variability was related to differences in serum albumin concentrations, even though all albumin concentrations were in the normal range and did not vary greatly (3.9–4.8 g/100 ml). α-Lipoprotein concentrations were not measured but may also contribute to differences in binding. These clinical findings confirm the *in vitro* data and suggest that hypoalbuminemic patients are at greater risk from quinidine toxicity even at total serum quinidine levels within the therapeutic range.

Pharmacokinetic studies of free quinidine yield values of volume of distribution and total clearance that are much larger than those determined using total (free plus bound) quinidine levels. Although these calculations do not identify the precise sites of drug distribution (25), the findings suggest that quinidine distribution is more extensive than that predicted by total drug concentrations. In addition, renal clearance of unbound quinidine exceeds creatinine clearance, consistent with renal tubular secretion of the unbound drug.

Measurement of free quinidine decreased the between-subject variability in some pharmacokinetic variables. The coefficient of variation for elimination half-life was 35% when total drug data were used and 24% when free drug data were used. Likewise, the coefficient of variation of total metabolic clearance decreased from 30 to 23% when free drug data were used. Variability in protein binding appears to contribute to variability in both clearance and elimination half-life values.

Differences in protein binding also contributed to variability in the clinical response. Peak free drug levels ranged from 0.64 to 1.6 μg/ml following a 5-mg/kg iv infusion, and these levels correlated significantly with changes in the heart rate, QRS duration, and corrected QT interval. Peak levels of total quinidine ranged from 1.7 to 5.0 μg/ml, and only the corrected QT interval was significantly related to total serum levels.

Many factors can influence protein binding of quinidine. Two such factors analyzed in the present study—hypoalbuminemia and coadministration of other drugs—commonly occur among patients receiving quinidine. Other potential sources of variability need to be identified. These alterations in protein binding contribute importantly to variability in critical pharmacokinetic parameters as well as to the clinical response to this drug.

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Fourier Transform Carbon-13 NMR Spectra of Ampyrone and Aminopyrine

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Abstract □ The natural abundance ^{13}C-NMR spectra of ampyrone and aminopyrine were obtained using the pulse Fourier transform technique. The chemical shifts were assigned with the help of the chemical shift theory, multiplicity generated in single-frequency off-resonance decoupled spectra, relaxation time, and comparison with structurally related compounds.

Keyphrases □ Ampyrone—Fourier transform carbon-13 NMR spectra □ Aminopyrine—Fourier transform carbon-13 NMR spectra □ NMR spectroscopy—ampyrone and aminopyrine

The assignments of ^{13}C-NMR chemical shifts of synthetic and natural therapeutic agents (1–6) initiated the carbon-13 analysis of 4-amino-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one (I, ampyrone) and 4-dimethylamino-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one (II, aminopyrine), both of which possess antipyretic and analgesic properties. The natural abundance ^{13}C-NMR spectra, both proton noise decoupled and single frequency off-resonance decoupled (SFORD), of ampyrone and aminopyrine were recorded using the Fourier transform technique. The proton noise-decoupled spectra gave the chemical shift of various carbon resonances of I and II while the SFORD spectra differentiated the methyl, methine, and quaternary carbons.

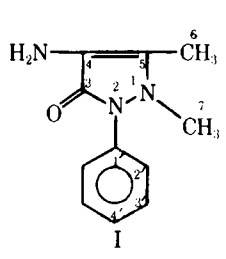
The assignments of carbon-13 signals are based on the chemical shift theory, multiplicity generated in SFORD spectra, percent intensity of signals, and carbon chemical shifts of the model compounds. These studies could possibly contribute toward the understanding of the biotransformation of I and II.

EXPERIMENTAL

The ^{13}C-NMR spectra of ampyrone, aminopyrine, and 1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one were obtained on a spectrometer[1] operating at 15.00 kHz. The samples were run in a 10-mm tube with deuterochloroform (30% w/v) as an internal lock and solvent and tetramethylsilane as a reference. The spectrometer settings during the experiment were: spectral width, 4 kHz; pulse width, 18 msec (90°); repetition rates, 5, 15, and 45 sec; and data points, 4 K.

The T_1 measurements of these two compounds were carried out in undegassed solution and were automatically calculated[2] by least-squares analysis (10) of the plot of ln $(I_\infty - I_T)$ *versus* T.

[1] Jeol FX 60 spectrometer.
[2] By FX-60 computer.



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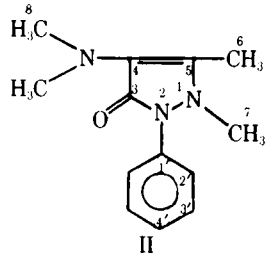

II

Ampyrone, aminopyrine, and antipyrine were obtained from commercial sources[3].

DISCUSSION

Ampyrone (I)—The carbon-13 chemical shifts of I are recorded in Table I, and its carbon resonances are illustrated in Fig. 1. The proton noise-decoupled spectrum of I gave seven signals in the lower field region and two signals in the higher field region. The quartets centered at δ 36.7 and 9.0 ppm could be easily assigned to C-7 and C-6, respectively, on the basis of chemical shift theory (7).

Feeney *et al.* (8) obtained the ^{13}C-NMR spectrum of 1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one (III, antipyrine) in dimethyl sulfoxide and reported the chemical shift of the ring carbon resonances relative to the upfield of carbon disulfide. To check the chemical shift of the carbons of two methyl groups present at positions 1 and 5 of the pyrazole ring in III, the proton noise-decoupled and SFORD spectra of III were recorded in deuterochloroform. The chemical shifts of the carbon signals of III obtained are represented on the structure of III while the figures in parenthesis indicate the chemical shifts reported earlier (8).

Table I—Carbon-13 Chemical Shifts of Ampyrone

Assignment[a]	Multiplicity[b]	Chemical Shift[c]	Relaxation Time[d], T_1, sec
C-3	s	160.7	27.4
C-5	s	136.3	18.9
C-1′	s	134.2	17.8
C-3′	d	127.8	1.08
C-4′	d	124.4	0.77
C-2′	d	121.4	1.11
C-4	s	118.0	13.8
C-7	q	36.7	1.55
C-6	q	9.0	2.90

[a] Numbering of carbons are shown in the structure. [b] Signal multiplicity obtained from SFORD; s = singlet, d = doublet, and q = quartet. [c] Chemical shifts are expressed in parts per million relative to tetramethylsilane. [d] The relaxation time was obtained in undegassed solution.

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

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