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Pharmacokinetic disposition of quinidine in the rhesus monkey

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PHARMACOKINETIC DISPOSITION OF QUINIDINE IN THE RHESUS MONKEY

by

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Pharm.D., University of California, San Francisco, 1967

[redacted]

To My Wife and My Parents

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To Dr. Malcolm Rowland, my mentor and friend who made my graduate education a very rewarding and meaningful experience. I am very grateful for his continued support and guidance and for the experiences which we shared together during the course of my graduate training.

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ABSTRACT

The pharmacokinetic disposition of quinidine and dihydroquinidine, a known impurity in commercial quinidine preparations, was studied in rhesus monkeys after intravenous bolus and infusion. It was observed that the fate of both drugs in the body can be described by a two-compartment open-system model. Quinidine is rapidly distributed in the body ($t_{.5}$ = 2 minutes). The half-life of elimination was 25 to 35 minutes. A similar distribution half-life was recorded for dihydroquinidine. However, the fractional rate of elimination of this compound was slower ($t_{.5}$ = 62 minutes).

Infusion of quinidine in doses of 1.44, 2.7, 5.4, and 10.7 mg/kg/hr for 200 minutes resulted in proportional steady state plasma levels of 1.75, 3.00, 5.66, and 11.8 mcg/ml, respectively. The plateau concentrations were predicted from plasma clearance estimated by the intravenous bolus data. At all steady state levels, the plasma clearance remained constant while at the 10.7 mg/kg/hr dosage only, upon cessation of the infusion, the apparent elimination half-life increased to 108 minutes. This last observation suggests an increase in the apparent volume of distribution at high infusion rates of quinidine.

Quinidine was also infused for a duration of 6 hours to plateau levels of 2.7, 6.3, and 8.6 mcg/ml. In each case, steady state plasma levels were rapidly attained and maintained. While plasma level decay from a steady state concentration of 2.7 mcg/ml was consistent with

the intravenous bolus data ($t_{.5}$ = 35 minutes), the decline from plasma levels of 6.3 and 8.6 mcg/ml was slow and curvilinear. These observations are consistent with a change in the apparent volume of distribution but not clearance with time and dose. This is an unusual example of dose-dependent kinetics.

Alterations in the protein-binding characteristics of quinidine in the plasma and a decrease in intracellular pH were investigated and were demonstrated not to be the mechanism(s) responsible for the observed changes in the elimination profile of quinidine. Several alternative mechanisms were presented which are consistent with known pharmacological properties of the drug.

A similar increase in the elimination half-life of dihydroquinidine ($t_{.5}$ = 160 minutes) was observed when plasma levels decayed from a peak concentration of 10.7 mcg/ml.

In the presence of dihydroquinidine, the fractional rate of quinidine elimination was decreased. Alterations in the plasma clearance and volume of distribution of quinidine were postulated as the mechanisms underlying this observation.

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INTRODUCTION

I. Quinidine - A Cinchona Alkaloid

Quinidine, a naturally occuring alkaloid, is used in the treatment of various cardiac arrhythmias. It is one of the principle pharmacologically active constituents found in the bark of trees and shrubs of the various species of two Rubiaceous genera, Cinchona and Remijia (1). Discovered in 1833 by Henry and Delondre, they concluded that it was quinine hydroxide because of its similarity in composition to quinine (2). However, it was not until 1853 that quinidine was prepared in pure form and given its present name by Pasteur.

Although the bark of the cinchona tree had been known to possess medicinal virtues as early as 1630, the ability of cinchona alkaloids to suppress atrial fibrillation was not recognized until 1749. A French physician, Jean-Baptiste de Sénac, successfully employed quinine for the treatment of what he called "rebellious palpitation" of the heart (3). This interesting and important observation was overlooked by the medical profession for nearly two centuries. It was not until the early 1900's that attention was renewed in the value of the cinchona alkaloids in disorders of cardiac rhythm.

In 1918, stimulated by an earlier report of the apparent ability of quinine to abolish auricular fibrillation (4), Frey (5) examined several members of the cinchona group of alkaloids. He observed quinidine to be the most effective in restoring normal cardiac rhythm when quinine, quinidine, and cinchonine were administered to

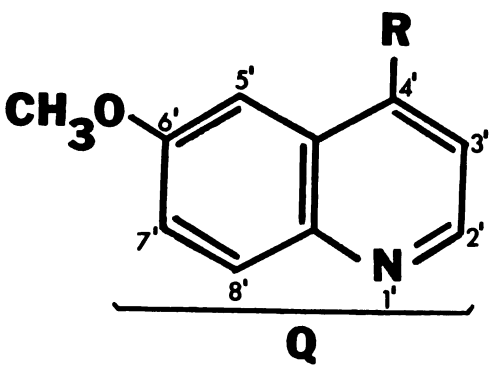
patients with atrial fibrillation. This observation initiated the modern day use of quinidine in cardiovascular chemotherapy.

It took many years after the discovery of quinidine before its value in the treatment of disorders of cardiac rhythm was recognized. Once established, however, the therapeutic usefulness of this drug has been appreciated. And although some of the earlier indications for quinidine have been replaced by modern therapeutic techniques, e.g., electroshock cardioversion to restore normal sinus rhythm, it still remains the treatment of choice in many arrhythmic conditions.

Quinidine is chemically 6'-methoxy-3-vinyl-9-rubanol. It is the d-isomer of quinine. The structural relationship between these two stereoisomers is shown in Figure 1. Quinidine differs from quinine only in the configuration about the C_8 and C_9 atoms. The absolute configuration at these two atoms in quinidine are rectus (R) and sinister (S), respectively. The fact that the C_9 hydroxyl and the C_3 vinyl groups in the quinidine molecule can react to form a cyclic ether while the corresponding groups in quinine cannot is evidence that these two functional groups (and thus C_3 and C_8) have a <u>cis</u> orientation in the case of quinidine and a <u>trans</u> relationship in the quinine molecule (1).

By virtue of the four centers of asymmetry within each molecule at positions C_3, C_4, C_8, and C_9, quinidine and quinine rotate the plane of polarized light in a dextrorotatory and levorotatory direction, respectively. The specific rotation for each compound varies markedly with solvent and pH.

Figure 1 The Configurational relationships of the three cinchona alkaloids, quinine, quinidine, and dihydroquinidine. Chemically, these compounds are expressed as derivatives of the parent heterocycle, ruban. They have one center of dissymmetry at C_8. The only structural difference between quinidine and dihydroquinidine is at the C_3 atom which are occupied by a vinyl and ethyl group, respectively.



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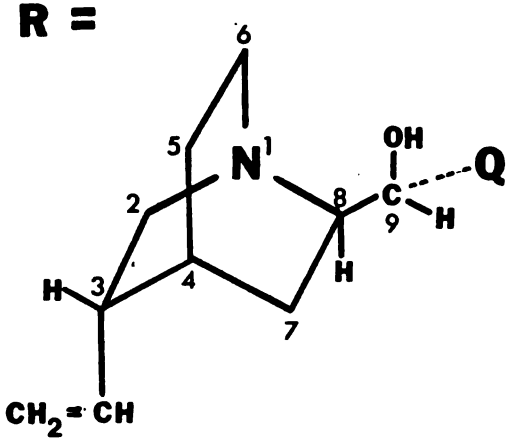

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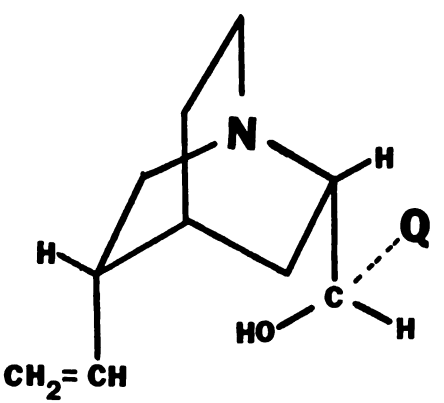
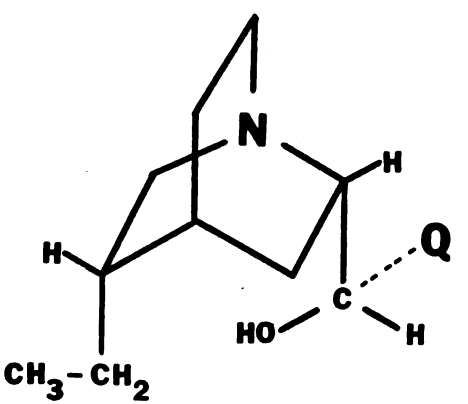


Quinidine



Dihydroquinidine

Quinidine and quinine account for approximately 0.25 to 3% and 50 to 67%, respectively, of the total alkaloidal yield of the cinchona bark (6,7,8). This material is used as a commercial source of quinidine. However, the majority of the quinidine that is utilized is prepared by isomerization of the levorotatory isomer, quinine, since it is available in much greater quantities (6,7,9). The isomerization procedure used is that described by Doering et al. (10).

In the event of a possible shortage of natural resourses, other means of obtaining quinidine and quinine were sought. A total synthetic procedure for the preparation of quinine was elucidated as early as 1944 (11,12). More recently several groups have reported on the total synthesis of quinidine and quinine (13,14,15). All of these methods can serve as potential sources of quinidine.

II. Dihydroquinidine - An impurity in Commercial Quinidine

Drug grade quinidine has long been known to contain dihydroquinidine as its chief impurity in quantities varying up to 30% (16,17,18, 19,20,21,22,23). It is also a member of the cinchona group of alkaloids and represents one of the twenty or more compounds which have been isolated from the cinchona bark. The existence of dihydroquinidine, which differs from quinidine only in the saturation of the C_3 vinyl side chain (Fig. 1), remained unsuspected until 1869. The structural and physiochemical properties of quinidine and dihydroquinidine are so similar that common methods of separation and isolation do not adequately remove one compound from the other (Table 1).

Table 1

PHYSICOCHEMICAL PROPERTIES OF QUINIDINE AND DIHYDROQUINIDINE

Property	Quinidine	Dihydroquinidine
Molecular Weight (7)	324.41	326.42
Melting Point (°C) (28)	170.5-171.5	169.5-170.5
$[\alpha]_D^{27}$ (95% ETOH) (28)	+ 269.6	+ 213
Solubility (7,25,121,122)		
Water	1gm/2000 ml	slightly
Benzene	very soluble	soluble
Chloroform	1 gm/1.6 ml	soluble
Ether	1 gm/56 ml	soluble
Ethyl Alcohol	1 gm/36 ml	soluble
pK_1 (24,26,27)	8.77, 8.57, 7.62	8.01
pK_2 (26,27)	4.20, 4.00	- - -

In 1922, Lewis et al. (16,23) obtained preparations of quinidine which contained less than 0.5% dihydroquinidine. They studied the effects of this "pure" quinidine sample and dihydroquinidine on the hearts of patients who were suffering from atrial fibrillation. The results of this study indicated that both compounds have very similar qualitative actions on the heart. On a weight basis, dihydroquinidine was found to be slightly more active than quinidine on decreasing atrial rate. The quantitative differences which apparently exist between these two compounds are discussed below.

III. Quinidine - A Cardiac Depressant

The heart is the principle organ of the cardiovascular system. Automaticity (rhythmicity), conductivity, and contractility are the primary physiologic properties of cardiac tissue. Conduction of electrical impulses throughout the heart is via a specialized network of conductive tissue. Depolarization of this conduction system initiates the contractile response. All tissues of this conduction system, namely, the sino-atrial and atrio-ventricular nodes, bundle of His, bundle branches, and the Purkinje system, undergo spontaneous diastolic depolarization to some degree and are therefore capable of initiating an impulse. In the normal heart, the sino-atrial node depolarizes more rapidly than adjacent conducting tissue and is referred to as the pacemaker of the heart. The contractile muscle of the atria and ventricles do not undergo spontaneous diastolic depolarization but respond to the propagated impulses of the pacemaking

and conducting tissue.

Quinidine is a cardiac depressant. It is used in the treatment of cardiac arrhythmias characterized by abnormal (ectopic) impulse generation. These include premature contractions (extrasystoles) originating in ectopic foci in the atria and ventricles, paroxysmal supraventricular tachycardia, atrial flutter and fibrillation, and ventricular tachycardia and fibrillation.

An arrhythmia may be the expression of (a) disturbances in cardiac automaticity, (b) alterations in the level of the threshold potential that is required for depolarization, or (c) changes in conduction (29). When the sino-atrial node is suppressed or when other conductive tissues depolarize more rapidly, an ectopic focus develops. If the rate of discharge from the abnormal focus is consistently greater than that of the sino-atrial node and the discharges exceed the threshold potential for propagation, the ectopic focus will continue as the predominant pacemaker and the arrhythmia will be sustained.

The exact nature of the etiology of ectopic impulse formation is not known. Although several alternative hypotheses have been invoked as possible mechanisms for their formation (30), the arrhythmias that are observed in man are best explained by the unitary theory of arrhythmia formation (30,31,32,33,34). It suggests that agents, such as quinidine, which are capable of suppressing atrial arrhythmias have the ability to decrease the rate of depolarization of an abnormal pacemaker. Since pacemaker activity in an extranodal site may give rise to an ectopic focus, the unitary theory suggests that the only dif-

ference between the arrhythmias is in the rate of depolarization of the ectopic focus responsible.

IV. Quinidine - Aspects of Pharmacology and Toxicology

Since the introduction of quinidine, clinical indications for administration of this drug have remained essentially unchanged. The therapeutic effects which are derived from quinidine when used in the treatment of cardiac arrhythmias are attributed to its action on the cardiovascular system of the body (30,34,35). Quinidine depresses excitability, conduction velocity, and contractility. The direct effects of quinidine on the intact heart are complicated to some extent by indirect effects resulting from an anticholinergic (vagolytic) action, antiadrenergic, and changes mediated by compensatory mechanisms. In addition, the drug is also active on the peripheral vasculature.

Quinidine decreases the excitability of conductive tissue. The rate of spontaneous pacemaker (normal and ectopic) discharge is depressed. Quantitatively, it appears that ectopic pacemaker tissue is selectively more sensitive to the effect of quinidine on conductive tissue than are the cells of the sino-atrial node. In therapeutic doses, quinidine is reported to have very little effect on normal sinus rhythm.

Two principle mechanisms are proposed to be operative in the ability of quinidine to depress or abolish abnormal cardiac rhythm. The threshold of the pacemaker tissue to electrical stimuli is eleva-

ted by the drug. The associated decrease in the slope (rate of rise) of slow diastolic depolarization results in a reduction of spontaneous discharge by ectopic pacemaking tissue. An alternate explanation for the effects observed on excitability with the administration of quinidine is the effect of the drug on the action potential of the myocardial cell. Quinidine prolongs the effective refractory period, which is defined as the period of time that must elapse following a response before a second propagated action potential can be initiated. Thus, the myocardial tissue remains refractory for an appreciable interval after full restoration of the resting membrane potential. This delay and interruption of the propagation of an impulse will result in a failure of the impulse to be propagated with resultant suppression of ectopic activity.

The atrioventricular and intraventricular conduction velocity is depressed by quinidine. This prolongation in conduction time is the result of a decrease in the rising phase of the action potential. When coupled with a reduction of excitability, the decreased rate of depolarization is probably the explanation for the depression of conduction throughout the cardiac tissue. Changes in conduction time in the heart are recorded with an electrocardiogram. The changes in the electrocardiogram induced by quinidine are prolongation of both the P - R interval and the duration of the QRS ventricular complex.

There are three pharmacological effects which are elicited by quinidine which are not particularly useful in the treatment of arrhythmias. In the laboratory animal, quinidine depresses contractility of

of the myocardium. This depression of contractility is regarded as a toxic response to the drug but there is no evidence that this action is significantly present during its clinical use. When myocardial contractility is markedly depressed, there may be an associated reduction in cardiac output.

The direct actions of quinidine in the intact heart are complicated by the vagal blocking actions of the drug. Quinidine prevents the slowing of cardiac rate produced by direct or reflex vagal stimulation. The vagolytic properties of the drug seen after the administration of larger doses tend to increase the heart rate and under certain circumstances, an antiadrenergic effect will tend to reduce it. Thus, despite the direct depressant action of quinidine on pacemaker cells, in unanesthetized animals and man in normal sinus rhythm, the net effect of these antagonistic actions is usually little change or even a slight increase in the heart rate (36).

Quinidine has a direct relaxing effect on vascular smooth muscle. The decreased arterial pressure or depressor effect which is observed after the administration of the drug in humans is due to peripheral vasodilatation. The hypotension observed after quinidine administration may occur as a result of this action and/or a reduction in the cardiac output. Reduction of arterial pressure of serious degree is very rarely seen with oral administration of quinidine and is more commonly observed after parenteral use of the drug (37). At toxic levels of the drug, concentrations in excess of 10 mcg/ml, the fall in blood pressure involves not only a peripheral vasodilatation action

but also central vasomotor effects and cardiac depression.

Extra-cardiovascular effects of quinidine of any significance is the observation of a weak curare-like ability to weaken skeletal muscle response.

The toxic effects which are observed after administration of quinidine are varied and unpredictable. Some clinicians, for example, Sokolow and Perloff (38), suggest that careful observation of patients with serial electrocardiograms and determination of serum quinidine levels permit early recognition of toxic manifestations and minimize the risks which are involved with the use of the drug.

The onset of quinidine toxicity is more pronounced after parenteral (intravenous and intramuscular) administration of the drug than it is after oral administration. The intravenous use of the drug is attended with such a high frequency of cardiovascular toxicity that its use is reserved for exceptional circumstances only. The toxicity of quinidine given intravenously is directly related to the rate of administration (39). Intravenous doses of the drug should be administered by slow infusion. Rapidly injected doses as small as 0.2 gm to man have resulted in cases of death (40).

The toxic effects observed in man are primarily those which are manifested as extensions of the pharmacologic activity of quinidine, namely, cardiac arrhythmias, myocardial depression, decreased cardiac output, hypotension, etc. (6,30,38,41). A rare idiosyncratic or hypersensitivity (allergic) reaction has been observed following the administration of the drug.

General toxic reactions affecting the gastrointestinal tract and the central nervous system are more frequently encountered in patients being treated with quinidine but they seldom require discontinuation of the drug. These reactions include symptoms of cinchonism, namely, nausea, diarrhea, vomiting, visual disturbances, and ringing of the ears, and have been related to dose and blood levels of quinidine.

Quinidine has a very narrow therapeutic index. Therapeutic responses to the drug are generally observed when the plasma concentration is in the range of 3 to 7 mcg/ml. Below this level, the effects are variable. However, when plasma concentrations exceed 8 to 10 mcg/ml, serious signs and symptoms of drug toxicity are readily apparent.

V. Dihydroquinidine - Aspects of Pharmacology and Toxicology

As previously indicated, on a qualitative basis the actions of dihydroquinidine on the cardiovascular system are similar to that observed with quinidine (18,28,42,43). Animal studies indicate that opinions vary concerning the quantitative differences between quinidine and dihydroquinidine in their ability to suppress disorders of cardiac rhythm. In attempting to extrapolate the observations made in animals to the clinical setting, one must be constantly aware of the fact that the arrhythmias which have been induced in these animals may not be representative of those observed in man.

The literature seems to clearly indicate that dihydroquinidine is more potent than quinidine in their ability to alter cardiovascular

functions. Scott et al. (28) reported that on the basis of intravenously administered doses (mg/kg) of both drugs, dihydroquinidine was three and one-half times as effective as quinidine in raising the threshold current that was required to produce permanent ventricular fibrillation in cats. On a weight basis, dihydroquinidine was observed to have greater activity than quinidine in suppressing chloroform-induced arrhythmias in the mouse (44). In addition, observations on isolated atria of the rat and guinea pig indicate that dihydroquinidine was 30% more effective in abolishing or inhibiting aconitine-induced arrhythmia when compared with an equal concentration of quinidine.

Although similar concentrations of the two drugs were not used in his study, Weisman (17) concluded that dihydroquinidine had very slight cardiovascular depressant activities in the dog and that the dihydroquinidine fraction of commercial quinidine may actually counteract to a great degree the cardiovascular depressant actions of pure quinidine.

Interestingly, in experimentally induced fibrillations in rabbits and cats, while dihydroquinidine gave a marked rise in resistance against electrical stimuli causing extrasystoles, tachycardia, and fibrillations of the atria and ventricles (lengthened the refractory period as well as the atrial and atrio-ventricular conduction times), pure quinidine was reported to have practically no effect on these disturbances of cardiac rhythm. The conclusion was made that the well known effects of quinidine are caused by the presence of

dihydroquinidine in the preparations (20).

In therapeutic doses, there were slight differences between the two drugs with respect to depressor effects. However, dihydroquinidine may have a shorter duration of action in cats (28).

Acute toxicity studies in mice proved dihydroquinidine to be somewhat more toxic than pure quinidine (28). A similar conclusion can be drawn from the studies of Weisman (17) in dogs.

There are very few studies with dihydroquinidine in human subjects. The effects of the drug were first observed by Lewis et al. (16,23). It was found to be slightly more potent than quinidine on a weight basis in their ability to suppress atrial and ventricular rate. Alexander et al. (18) concluded that dihydroquinidine was approximately two times as potent as quinidine in their effect on cardiac arrhythmias (primarily cases of atrial fibrillation). The results of 244 experiments conducted in 18 patients with atrial fibrillation demonstrated that the two drugs were of equal potency (45). While in 8 patients with chronic atrial fibrillation who were treated with dihydroquinidine, there were no cases in which there was restoration of normal sinus rhythm (17).

Although the effects of dihydroquinidine in the treatment of cardiac arrhythmias are clearly not known, the fact remains that it is present in quinidine preparations which are used to treat patients with disorders of cardiac rhythm. Thus, it remains to be ascertained unambigously what effects, if any, dihydroquinidine has on cardiac disease states themselves and on the disposition of quinidine in the

body.

VI. Quinidine - Aspects of Pharmacokinetics

Pharmacokinetics is the study of the kinetics of absorption, distribution, metabolism, and excretion of a drug and its metabolite(s) and the development of suitable models to describe the data. The time course profile of a drug in the body, as measured by the concentration or amount of drug in some biological tissue, primarily blood and/or urine because of the ease of sampling, is studied.

Modelling is a technique which is used to simulate and simplify real systems. The model should not be any more complex than is required to serve its function. Once a model is established, it serves to summarize the observed data; it increases the understanding of the process(es) involved; it provides a basis for predictions; and it suggests additional areas for investigation.

The mathematical notations which are used to describe the processes of absorption, distribution, metabolism, and excretion, as pointed out by Wagner (46), offer a precise mechanism by which the events may be quantitated and expressed. The brevity of the notation permits simultaneous consideration of a broad number of variables and their interrelationships. And the abstract nature of mathematics allows for a generality of expression and manipulation.

Aspects of Absorption

Quinidine free base is practically insoluble in water (1 gm in

2000 ml). The various salt forms of the drug are substantially more water soluble (47). In spite of the solubility characteristics of the drug, after oral administration of quinidine sulfate, essentially complete absorption is observed from the gastrointestinal tract. Only 1 to 3% of an administered dose is recoverable in the stool (48,49).

Although considerable individual variations in serum concentration are seen, within 15 minutes after a single dose of 0.2 to 1.0 gram of quinidine sulfate, the drug can be detected in the plasma. (50,51). This observation appears to indicate that the absorption process must begin in the stomach and/or stomach emptying is very rapid. The peak levels that are reached in the plasma appear within 1 to 3 hours after a single oral dose (32,38, 50,51). Plasma levels of the drug are on the average higher and appear earlier when the drug is administered on an empty stomach (51,52,53,54). Changes in gastric acidity did not appear to affect the absorption of quinidine (55).

Intramuscular administration is useful when there are contraindications to oral therapy, e.g., nausea and vomiting. Although some authors (37,56) have reported the intramuscular route to produce a more rapid onset of action, when compared with similar doses of orally administered quinidine, a delay in drug absorption was demonstrated from this route (52) and higher levels have been observed in the plasma at the end of 24 hours after a single dose of the drug (52,57).

A solution of quinidine sulfate or gluconate is acidic (pH approximately 4.5 to 7.0) owing to the high molar hydrogen ion concentration required to solubilize a therapeutic dose of the drug in a small

volume of injection fluid. The blood perfusion rate and buffer capacity of tissues at the injection site are sufficiently high that they will raise the pH of the injection solution. It has therefore been postulated that this change in pH causes the sparingly soluble quinidine base to precipitate at the site (58). Thus, drug absorption will be prolonged as the drug slowly dissolves into tissue fluids. This phenomenon would account for the higher levels of quinidine which persist after 24 hours.

When large doses of quinidine sulfate are administered rectally, only low levels can be demonstrated in the blood (38,52,57). Poor absorption from the rectum is the probable explanation for these observations due to the small surface area for absorption exposed to the drug and the relatively short contact time.

Aspects of Distribution

In vivo tissue distribution data in humans are virtually impossible to obtain. Reliance on studies determined in animals is therefore made out of necessity.

In the vascular compartment, quinidine is bound by plasma proteins, primarily the albumin fraction (60,61). The association constant for the binding to human serum albumin determined *in vitro* was 7.7×10^3 liters/mole. When the total plasma concentration of the drug is in the therapeutic range, 4 to 7 mcg/ml, approximately 80% of the drug is in the bound form (60,62,63). The binding to albumin is in a 1:1 stoichiometric relation under physiological conditions (60).

In 12 patients with chronic renal failure, Skuterud et al. (63) reported that in 9 patients the serum protein binding of quinidine was in the normal range. In 3 patients, however, the bound fraction of the drug averaged approximately 90%. They concluded that this higher degree of quinidine binding could be attributed to some extent to the presence of relatively higher amounts of α_1 and α_2 serum globulins.

Absorption of quinidine by human red blood cells has been demonstrated by Shaw (64). And binding to blood platelets has also been observed to occur (65).

Quinidine and some of its metabolic degradation products are also bound to tissue proteins. When plateau levels were achieved after constant infusion, quinidine concentrations which were 20 to 40 times greater than the corresponding plasma level have been observed in various tissues, e.g., kidney, lungs, liver, etc. of the dog (66). It should be noted that the method which was used to analyze the tissue samples may have lacked analytical specificity (see VII. Shortcomings of Previous Analytical Methods). Therefore the observed tissue/plasma ratios for quinidine may be considerably lower than those reported in the literature. Wegria and Boyle (67) reported that when myocardial tissue samples were obtained at various times after the administration of a single oral dose of quinidine in the dog, the tissue levels were 10 to 30 times higher than plasma samples collected at corresponding times. It was also demonstrated in these studies that the rise and decline of tissue and plasma levels

tend to be parallel indicating a ready equilibrium in the dog (66,67). Although no reference was cited, Conn (68) reports that the tissue uptake rate appears to approximate that of a blood-flow dependent system.

Tritium labelled quinidine was administered intravenously to rabbits (69). Radioautographs taken of myocardial tissue slices indicated that the major sites of quinidine concentration were located in the endoplasmic reticulum and the mitochondria.

Neff et al. (70) reported that the apparent volume of distribution in several species of domesticated animals varied from 1.25 to 6.32 liters/kg body weight for the swine and pony, respectively. In vitro protein binding determinations indicated that quinidine was bound to the extent of 98 and 82% in these two animals. When corrected for plasma protein binding, the apparent volume of distribution of unbound quinidine was calculated to be 49.5 and 34.0 liters/kg in the swine and pony, respectively. The importance of this observation is that for a given dose per body weight, the levels of the unbound, diffusible, and presumably pharmacologically active species of the drug in the body are virtually constant.

The apparent volume of distribution of quinidine in man has not been reported in the literature to the author's knowledge. However, an estimate of this parameter was obtained from the following relationships which assumes that all of the drug is absorbed following the administration of an oral dose. According to Wagner et al.(59), the average amount of drug in the body at steady state following a fixed

dose and time schedule is given by:

$$Vd\ C_{ave} = Dose/KdT \qquad \text{(Eq 1)}$$

where Vd is the apparent volume of distribution, C_{ave} is the concentration at steady state, Kd is the first-order elimination rate constant, and T is the dosing interval. Rearrangement of Equation 1 provides an estimate of Vd:

$$Vd = Dose/KdTC_{ave} \text{ or } 1.44\ Dose\ t_{1/2} \div TC_{ave} \qquad \text{(Eq 2)}$$

where $t_{1/2}$ is the half-life of drug elimination.

The observation that a 400 mg dose of quinidine sulfate administered orally every 6 hours produces plasma concentrations in the range of 4 to 7 mcg/ml (31) and assuming a plasma quinidine half-life of 7.5 hours (71), the computed volume of distribution is approximately 130 liters for man.

Aspects of Elimination

The decline of quinidine blood levels after a single oral dose to man is exponential (first-order) (72). Melmon and Morrelli (35) report the serum half-life of quinidine to be 4 hours. When the mean data of quinidine plasma levels obtained in 12 human subjects administered 400 mg of quinidine sulfate (73) was analyzed, Riegelman (71) found an elimination half-life of approximately 7.5 hours. Other observers have reported that 50% of the maximum blood levels remain after 8 hours (48,74).

It has been reported that quinidine levels disappear more slowly from the blood of patients who are suffering from congestive heart failure (72,75,76,77,78,79,80) and renal impairment (80). However, Kessler et al. (81), using a more specific assay method, found no such diminished rate of elimination of quinidine in these patients. They concluded that the slow excretion with subsequent accumulation of fluorescent metabolites rather than the decreased elimination of quinidine accounts for the erroneously high serum levels that are observed in these two disease states.

Neff et al. (70) studied the disappearance of quinidine from plasma after intravenous administration in several species of domesticated animals. Although there were wide variations in the disappearance profile between these species studied, all plasma levels of the drug declined in an exponential manner. Plasma half-life values varied from 0.85 to 5.59 hours in dogs and goats, respectively.

In man, the administration of intravenous quinidine is reserved for emergency situations only and as a result, there is very little data in the literature on the elimination profile of the drug from this route.

1. Metabolism

The fate of quinidine in the body has not been clearly established. The original report of Brodie et al. (82) and the recent work of Palmer et. al. (21) remain the most definitive studies concerning the metabolic profile of quinidine in man and their results are summarized

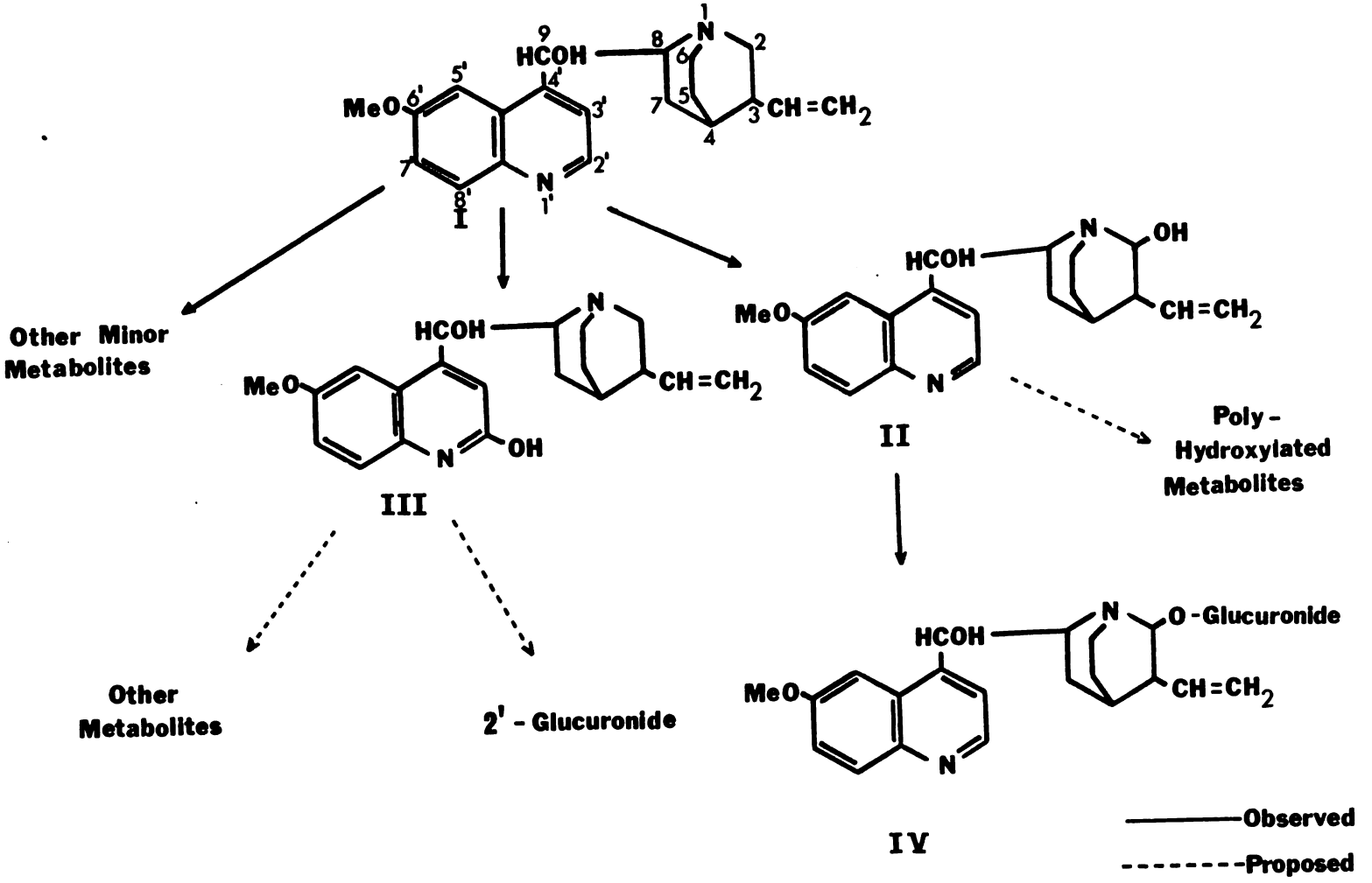
in Figure 2.

Since only 10 to 20% of an administered dose is recoverable in the urine as unaltered quinidine (32,48,53,78,83) (and possibly even less (81)), it appears that the fate of the drug in the body is primarily one of metabolic transformation. The major site of quinidine metabolism is believed to be in the liver. Enzymes are present in samples of rabbit liver homogenates which have been demonstrated to breakdown quinidine to a compound similar to that found the human urine (84). And it has been reported that the greater the extent of liver damage in dogs, the greater were the changes in the electrocardiogram, and the higher were the levels of quinidine following continuous intravenous administration (85). Other tissues of the body, including the heart, have not been demonstrated to have any significant role in the degradation of the drug in man (69).

Two major hydroxylated metabolites, II and III, exist for quinidine (Fig. 2). According to Brodie et al. (82), oxidation of the quinuclidine nucleus (II) is a major metabolic process. The formation of the 2'-hydroxy or carbostyril derivative (III) is a minor route of drug elimination in the body. In addition, poly-hydroxylated derivatives of quinidine have been observed and may represent a fraction of the 10% of the administered dose which is not accountable in the urine (21,82). A small but significant percent of glucuronide conjugates of the hydroxylated derivatives have been also demonstrated in the urine of humans (21).

The metabolites which were isolated from human urine by Brodie

Figure 2 A schematic representation of the metabolic profile of quinidine. The two hydroxylated species, II and III, comprise the major portion of the drug excreted in the urine. Glucuronide conjugation of the hydroxylated derivatives is reported to occur, however, other synthetic mechanisms, e.g., sulfate conjugation, have not been demonstrated.



Pathway of Quinidine Metabolism

et al. (82) were also isolated from the urine of dogs, rabbits, and humans and from dog and rabbit tissue following the administration of tritiated quinidine (86). In the urine of all three species the pattern of excretion was observed to be similar. Unchanged quinidine comprised approximately 20 to 40% of the total radioactivity. The 2-hydroxy metabolite (II) was usually the largest fraction found averaging 30 to 40% while the aromatic hydroxylated, carbostyril derivative (III) represented 20 to 30% of the radioactive material present. The remaining 10% of the radioactivity was found to be other minor metabolites.

The tissue levels of a drug and its metabolites vary as a function of time and dose. In the tissues (heart, kidney, skeletal muscle, and serum) of dogs and rabbits, quinidine represented 65 to 70% of the total radioactivity present, the 2-hydroxy compound, 15 to 20%, and the carbostyril derivative, 10 to 15%, respectively. The other metabolites were present in barely recognizable amounts. However, no information was given regarding the time that the tissue samples were collected relative to the administration of the drug.

The major products of quinidine metabolism are more water soluble, less bound to body proteins, and have much less antiarrhythmic activity when compared with the parent drug molecule (69,86). When isolated dog and rabbit hearts were perfused with the 2'-hydroxy derivative (III) of quinidine, it exhibited approximately 30 to 40% of the antifibrillatory activity of the parent drug. The 2-hydroxylated quinidine derivative (II) exhibited no effects in doses up to 10 times

that of quinidine. The unidentified metabolites were administered as a combined, non-purified fraction and were found to have no activity. Thus, it was concluded that the antiarrhythmic activity of quinidine is primarily the result of the actions of the parent drug molecule.

2. Excretion

Quinidine is presumed to be filtered at the glomerulus with partial tubular reabsorption (68). However, because of the structural and physicochemical similarities between quinidine and quinine, the demonstration of the presence of an active secretory process for quinine with passive pH dependent reabsorption would also be applicable to quinidine excretion (88).

Less than 20% of the total daily dose of quinidine is excreted unchanged in the urine (32,48,53,78,83). Quinidine and the end-products of metabolism (and their glucuronide conjugates) account for approximately 90% of the administered dose which can be isolated in the urine.

The renal clearance of quinidine diminishes with increasing urinary pH (27).

In patients with cirrhosis of the liver and azotemia, blood quinidine levels were not found to be abnormally high suggesting that reduction in the dosages are not necessary in cases of clinical hepatic or renal insufficiency (77,89).

Other minor excretory pathways exist for the elimination of quin-

idine. The drug has been reported to be present in the bile, however, biliary excretion does not appear to be an important route of drug elimination even in cases of extreme liver damage (66,85,90,91, 92). Fecal excretion accounts for less than 5% of an orally administered dose of the drug.

Conn et al. (19) were consistently able to isolate from the urine a total of 5 other compounds which represented the remaining 10% of the administered dose. However, it has not been definitely established which of these species represent administered impurities, e.g., dihydroquinidine (and its metabolites) and which represent true metabolites of quinidine.

VII. Shortcomings of Previous Analytical Methods

As indicated previously, quinidine has a very low margin of safety. In order to design rational dosing regimens, researchers have attempted to correlate serum concentration of the drug with observed pharmacologic or therapeutic effect. For example, after Sokolow and Ball (31), it appears that a serum concentration between 4 and 7 mg/liter will prevent the recurrence of atrial fibrillation and avoid serious drug toxicity. Other authors (32,37,50,52,67,93,94) have also observed gross parallel relationships between quinidine blood concentrations and cardiac effects.

A number of techniques for measuring the concentration of quinidine in biological samples have been described. Quinidine and its derivatives exhibit a natural fluorescence in an acidic media and are

therefore well suited for this method of analysis. In 1943, Brodie and Udenfriend (95) developed a fluorometric method of analysis which was capable of quantitating the levels of quinidine in biological specimen. Since that time this method (and many variations) has represented the basis of analysis for quinidine in the greater majority of quinidine studies to date. Prior to 1943, several less specific and sensitive methods were available for assessing the amount of quinidine present (96,97).

The method of Brodie and Udenfriend (95) consists of the dilution of a plasma sample with water and the precipitation of the plasma proteins with metaphosphoric acid. After centrifugation, the clear supernate is read fluorometrically. The amount of quinidine in this plasma is obtained by comparison with suitable known standard solutions.

The principle advantages of this method are the ease, simplicity, and rapidity of this procedure. The disadvantages are the inclusion of fluorescent metabolites and the presence of native substances, particularly chloride ions, in the plasma supernates which quench the fluorescence of quinidine (74,95,98).

The variations of the Brodie and Udenfriend method can be categorized into one of two groups: direct or protein precipitation method or extraction method. In both variants fluorometric techniques are used to determine the amount of fluorescing quinidine species in the sample.

When biological samples are analyzed by both methods, the direct

method yields values for quinidine which are consistently higher than the extraction method (99,100,101,102,103). This difference between the two methods is due to the inclusion of quinidine metabolites (99,100,101,102).

Quinidine fluoresces maximally in an acidic pH (about 1). In the concentrations used to cause precipitation of the plasma proteins, metaphosphoric and trichloroacetic acids produce supernatants which are sufficiently acidic. Quinidine was observed to exhibit less fluorescence in trichloroacetic acid than in metaphosphoric acid and the serum quenching effect was found to be greater with trichloroacetic acid((102).

Although the procedure is more time consuming, the extraction method has a higher degree of specificity. This method requires the alkalinization of the biological sample, usually with sodium hydroxide, and the subsequent extraction of quinidine with a suitable organic solvent. Fluorescence can be measured on an aliquot of this extract which was previously acidified with trichloroacetic acid (50,100,101, 104) (single extraction) or the organic solvent extract can be re-extracted with sulfuric acid which is then read fluorometrically (double extraction)(99,100,102,104).

Ethylene dichloride (50,100,101) and benzene (99,100,102,104,105) are the most commonly used extracting solvents. In addition, chloroform has been shown to be capable of removing quinidine and metabolites from various biological samples (21,106,107).

Pure quinidine was quantitatively extracted from biological

specimen by all of the following solvents: benzene,ethylene dichloride, chloroform, diethyl ether, and a 1:1 amyl alcohol-benzene mixture (105, 107). Excellent recovery (99.4%) of known amounts of quinidine added to plasma samples was demonstrated with benzene and ethylene dichloride using the double extraction technique. However, when actual plasma samples obtained from human subjects who were administered quinidine therapeutically, benzene and ethylene dichloride both appear to extract small amounts of metabolites (99,100,105,107). It was clearly demonstrated that benzene extracts a smaller amount of the fluorescing quinidine derivatives (99,107). For this reason, benzene has been reported to be the solvent of choice for the extraction of quinidine from biological samples (105) and specific for human plasma (104). Furthermore, the data indicate that the small fraction of metabolic products which do partition into the benzene layer may be removed by aqueous alkaline washes.

Up to this point the assumption has been made that studies were performed with pure quinidine. Commercial drug grade quinidine is reported to contain dihydroquinidine as an impurity as previously discussed. Because of the similarity in physicochemical properties, they would both be expected to have similar partitioning characteristics between aqueous alkaline solution and organic solvent, e.g., benzene. For this reason, the benzene extract and subsequent reextraction into sulfuric acid would be expected to consist of the two drugs. Preliminary investigations in this laboratory indicate that approximately 5 to 10% of the fluorescence which is exhibited by plasma

samples obtained from patients who were administered quinidine prophylactically is due to the presence of dihydroquinidine.

It is readily apparent that the present methods which are available for the determination of quinidine concentrations are inadequate if pharmacologic effects are to be correlated with "quinidine" levels. The need for an analytical method which was capable of separating the various fluorescing species in biological samples was obvious. A specific method for the analysis of quinidine and dihydroquinidine has been developed and is discussed in detail below (see pages 36 to 44 and 54 to 65).

VIII. The Rhesus Monkey as an Experimental Subject

Preliminary studies concerning the disposition and pharmacologic effect(s) of a drug in the body are usually performed in laboratory animals for obvious reasons, e.g., toxicity. The observations which are obtained from these investigations are then used as a basis upon which human experimental protocols are designed. Many important factors must be considered in the selection of the laboratory animal depending upon the nature of the investigation.

In the present study, the rhesus monkey was selected as the experimental subject. Many bio-medical investigations have been performed on this animal. For example, rhesus monkeys have been used in the study of gastrointestinal absorption of drugs (108,109), the enterohepatic circulation of bile (110), and the hemodynamic effects of various cardiovascular agents (111,112). Based on kidney function,

body fluid compartment, water, and electrolyte metabolism, the monkey is reported to resemble man more than any other experimental animal (113).

An animal preparation which can be used repetitively enables the animal to serve as its own control. This is a very desirable feature in view of the extreme inter- and intra-species variability that exists in nature. A chronically restrained monkey preparation ideally lends itself to long term, repetitive use. It allows the investigator the freedom to use an unanesthetized animal subject without the creation of acute adverse or abnormal environmental conditions. This is a particularly important consideration with agents which have their action on the cardiovascular system, since many physiological functions of the cardiovascular system are affected by environmental stimuli.

When pharmacokinetic studies are performed, biological samples must be appropriately collected at various times. In some animal preparations, these samples may be obtained with varying degrees of difficulty especially if the animal is unanesthetized. The rhesus monkey preparation which is described under Experimental Methods and Procedures is ideally suited for a pharmacokinetic study.

PURPOSES OF THE INVESTIGATION

Quinidine has been used clinically for many years but very little definitive data is available concerning the disposition kinetics of the drug in the body. Administration of the drug has been on an empirical rather than a rational basis. Knowledge of the changing levels of the drug with time in the body would be helpful in evaluating and understanding the pharmacologic (and toxicologic) responses observed and could lead to the establishment of a rational dosing regimen on an individualized basis.

The purpose of this investigation was two-fold. The principal objective was to study the pharmacokinetics of pure quinidine in normal rhesus monkeys and to develop suitable models which were consistent with the observed data. Since commercial drug grade quinidine is reported to contain dihydroquinidine in quantities varying up to 30% and because of the similarities in structure, physicochemical properties, and pharmacological activities between these two agents, the pharmacokinetics of dihydroquinidine and its influence on the pharmacokinetics of quinidine were also investigated.

EXPERIMENTAL METHODS AND PROCEDURES

I. Materials

United States Pharmacopeia (USP) grade quinidine sulfate was obtained from the University of California, School of Pharmacy Manufacturing Laboratory (New York Quinine and Chemical Works, New York, New York). Samples of pure quinidine gluconate USP reference standard (Lot Number X07108) and anhydrous dihydroquinidine free base (Lot Number 53950) were kindly supplied by the Eli Lilly and Company Laboratories, Indianapolis, Indiana and Fluka AG, Buchs SG, Switzerland, respectively.

All chemicals utilized were "Analytical Reagent" grade quality whenever possible.

Other materials, supplies, and apparatus which were used are listed in Appendix I and II.

II. Separation and Purification of Quinidine and Dihydroquinidine

Dihydroquinidine was separated from quinidine sulfate USP according to the method of Thron and Dirscherl (114). The reactivity of the vinyl group of the quinuclidine ring of quinidine and the relative unreactive nature of the corresponding saturated ethyl group of dihydroquinidine is the basis for the separation procedure. Quinidine and dihydroquinidine are weakly basic compounds (Table 1) and both are insoluble in alkaline solutions. Quinidine, but not dihydroquinidine, forms an addition compound with mercuric acetate

which is soluble in ammonium hydroxide. Dihydroquinidine can therefore be selectively extracted with a suitable organic solvent based on the solubility differences of these two compounds in aqueous ammonia.

A 10 gram sample of quinidine sulfate USP was dissolved in 60 grams of 10% sulfuric acid. To this solution was added 140 grams of a 10% mercuric acetate solution in 5% acetic acid and the mixture was heated for 5 hours at 40 to 50°C with occasional stirring. After cooling the mixture in ice, 100 grams of 28% ammonium hydroxide was added slowly. Dihydroquinidine precipitates out as a white precipitate. The dihydroquinidine was extracted with several 200 ml portions of ether. The combined ether extracts were washed with water. After drying with anhydrous sodium sulfate, the ether was evaporated and the dihydroquinidine sample was dried at 100°C.

The aqueous solution was acidified to litmus by the very careful addition of concentrated sulfuric acid. After the addition of 5 grams of phosphorous acid, the mixture was gently heated to boiling. Free metallic mercury which formed was separated by carefully decanting off the supernatant. After cooling in ice, the solution was made alkaline by the addition of 28% ammonium hydroxide. A dense white precipitate of quinidine was observed and extracted with ether as described above. The ether extracts were evaporated <u>in vacuo</u> and allowed to air dry.

The purity of the dihydroquinidine sample was examined by thin layer chromatography (TLC). A small sample of the drug was dissolved in chloroform and spotted on a pre-coated silica gel TLC plate. The

plate was developed with a solvent system consisting of a 4:1 mixture of methanol and acetone (107). The Rf values for dihydroquinidine and quinidine at room temperature are 0.08 and 0.19, respectively. The presence of trace amounts of quinidine was noted.

With slight modifications, the Heidelberger and Jacobs (115) method was used to purify the dihydroquinidine sample. Via this procedure, any quinidine which may be present is catalytically reduced to the dihydro derivative. The dihydroquinidine sample was dissolved in absolute ethanol and treated with 0.2 grams of 10% palladium on charcoal. Hydrogenation carried out under atmospheric pressure at room temperature was followed by TLC. Completion of the reaction was verified by the absence of the quinidine spot.

After filtration to remove the residual palladium on charcoal, the filtrate was diluted to three-times its volume with water and made alkaline to litmus with 28% ammonium hydroxide. The precipitated dihydroquinidine was extracted with ether. After drying with anhydrous sodium sulfate, the ether extracts were evaporated in vacuo. The solid drug was subjected to a three-fold recrystallization procedure with hot methanol and ether (1:1). The resultant needles of dihydroquinidine, which was chromatographically pure*, were dried to a constant weight at 75 to 80°C, melting point 169.6-170.6°C (Corr.); $[\alpha]_D^{22}$ = +214.9° (chloroform). The melting point, infrared and nuclear magnetic resonance (NMR) spectra, and TLC behavior compared

* The purity of this sample was also established by TLC analysis in a solvent system consisting of chloroform-methanol-diethylamine (80:20:1) according to Suszko-Purzycka and Trzebny (116).

favorably with that of pure dihydroquinidine base (Fluka AG) and the literature (7,23,28).

The quinidine obtained above was re-subjected to the separation procedure outlined above. The material obtained after the second purification procedure was found to be free of dihydroquinidine as determined by TLC. The white, crystalline quinidine was dried at 75 to 85°C to a constant weight, melting point 171.1-172.1°C (Corr); $[\alpha]_D^{22}$ = +244° (chloroform). The melting point, infrared and NMR spectra, and chromatographic behavior of this purified quinidine sample were the same as those for quinidine (as the gluconate salt) USP reference standard (Eli Lilly and Company).

III. Analysis of Quinidine and/or Dihydroquinidine in Biological Samples

An analytical method was developed which has specificity for quinidine and/or dihydroquinidine in various biological samples. A combination TLC-fluorometric method is used to separate and quantitate, respectively, the presence of quinidine, dihydroquinidine, or both. The procedure is outlined below and is directed towards the analysis of the two drugs in plasma and urine but it can be applied towards the analysis of these compounds in other biological samples. A detailed discussion of the method is presented under RESULTS AND DISCUSSION.

Preliminary observations have demonstrated that this method is applicable to various biological tissues, e.g., fat, heart, kidney, liver, muscle, etc. Known amounts of quinidine when added to these

tissue samples digested in 10 N sodium hydroxide were quantitatively recovered.

All blood samples were collected in 10 ml heparinized Vacutainers!™ The blood samples are immediately centrifuged at 3000 RPM for 5 minutes and a 0.5 ml aliquot of the plasma is removed and placed into a screw-cap (teflon lined) culture tube (16 x 150 mm). The plasma sample is diluted with an equal volume of water. This sample is analyzed immediately or it can be stored in the refrigerator (4°C) for future analysis. Under refrigeration conditions plasma quinidine was found to be stable for at least 10 days.

To the sample tube is added 50 µl of 10N sodium hydroxide and 100 µl of 95% ethyl alcohol. This tube is gently swirled to insure mixing. After the addition of 2 ml of benzene, the tube is tightly capped and placed on a shaker. Gentle agitation is required in order to prevent the formation of a dense, gelatinous emulsion which could not be separated by centrifugation.

After shaking for 4 hours, the tube is removed and centrifuged to separate the 2 layers. A 1.6 ml aliquot of the benzene extract is pipetted into a small culture tube (12 x 75 mm). After the addition of 100 µl of absolute alcohol which reduces the adsorption of the drug onto the glass, the sample is evaporated to dryness with a stream pf nitrogen or air.

After chilling the tube in ice, the evaporated benzene extract is reconstituted with 10 µl of chloroform. This chloroform extract in which quinidine (or dihydroquinidine) is very soluble is rapidly

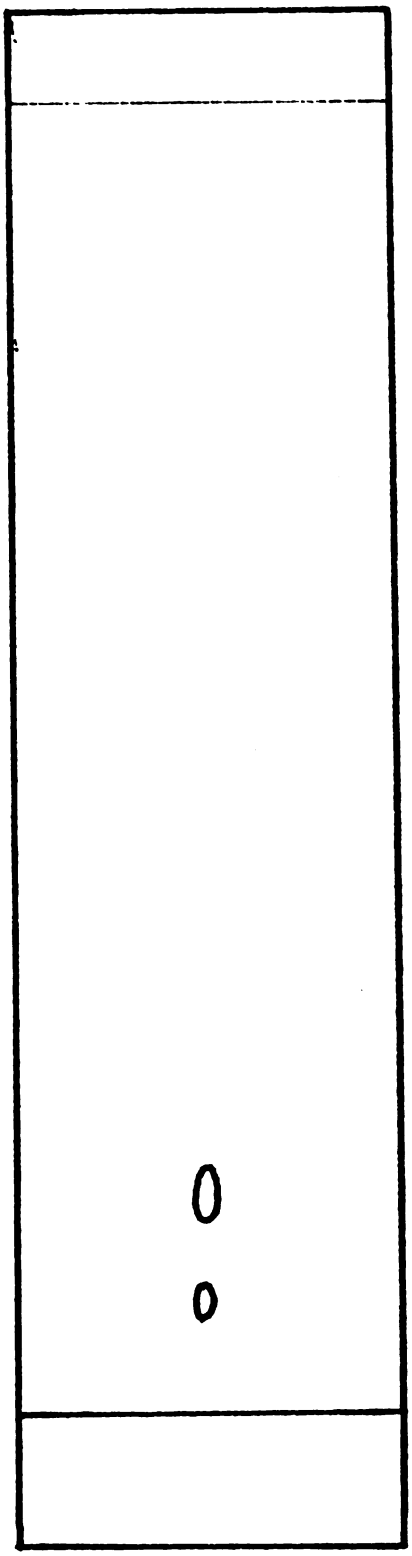
mixed by careful rotation of the tube. Using a Microcap^R pipette, a 5 µl aliquot of the chloroform solution is spotted on a pre-coated (0.25 mm) glass silica gel TLC plate. The plate is developed at room temperature via ascending TLC in a solvent system consisting of a 4:1 mixture of methanol and acetone.

After the development, the plate is air dried. The quinidine (and/or dihydroquinidine) spot(s) is located under an ultraviolet lamp. In the previously mentioned solvent system, quinidine and dihydroquinidine have Rf values of 0.19 and 0.08, respectively and are shown in Figure 3. In the course of the work-up of plasma or urine samples, other unidentified fluorescent species have been observed after the administration of either compound. The Rf values of these components are presented in Table 2.

The isolated spot(s) is scraped off of the plate and the powdered silica gel is placed into a culture tube (16 x 120 mm). The adsorbed drug is eluted with 3 ml of 0.1N sulfuric acid by shaking. After shaking, the tube is centrifuged to precipitate the suspended silica gel. An aliquot of the aqueous sulfuric acid is used to quantitate the amount of drug present in the sample using fluorometric analysis.

Quinidine and dihydroquinidine fluoresce maximally in an acidic solution. Care must be taken to avoid the presence of halogen ions since they are reported to quench the fluorescence of both drugs (98). Consequently all glassware was washed in concentrated nitric acid and rinsed with distilled water. Activation and fluorescence wavelength

Figure 3 A silica gel thin layer chromatogram after the development of a 5 µl sample of quinidine and dihydroquinidine in chloroform in the solvent system consisting of a mixture of absolute methanol and acetone (4:1). The developing time was approximately 75 minutes at room temperature. The Rf values for dihydroquinidine and quinidine under these conditions are 0.08 and 0.19, respectively.



TLC Chromatogram

Table 2

Rf VALUES OF OTHER UNIDENTIFIED FLUORESCENT COMPONENTS IN PLASMA OR URINE AFTER THE ADMINISTRATION OF QUINIDINE OR DIHYDROQUINIDINE

Quinidine

Component	Rf	Source
A	0.06	urine*
B	0.32	urine
C	0.40	urine and plasma
D	0.43	urine
E	0.51	urine and plasma

Dihydroquinidine

1	0.03	urine*
2	0.24	urine
3	0.33	urine
4	0.40	urine and plasma
5	0.50	urine and plasma

* only observed when chloroform is used as the extracting solvent.

maxima are 350 and 450 nm, respectively, for both compounds.

Plasma samples and aqueous solutions containing known amounts of quinidine were used to prepare standard calibration curves. The two curves were virtually identical. The standard solutions were processed concurrent with the plasma samples which were to be analyzed. In the concentration range used (0.3 to 9 mcg/ml), the relative fluorescence intensity was directly proportional to concentration (see Fig. 4 and Table 6, p. 62). A normalized least square regression analysis was performed on the standard curve in order to obtain the best fit equation of the line. The unknown concentration in the plasma sample could be readily obtained from the best fit equation of the standard curve after suitable correction for the plasma blank.

A standard calibration curve for dihydroquinidine was also prepared and processed with the plasma samples to be analyzed and is presented in Figure 5 (and Table 7, p. 63). In the concentration range of 0.3 to 9 mcg/ml, the relative fluorescence intensity is directly proportional to dihydroquinidine concentration.

Urinary concentrations of both drugs were determined in the same manner with one slight modification. A one ml sample of urine was directly subjected to the analytical procedure without the initial dilution step. All other procedural steps were idential as for plasma analysis.

Chloroform is an alternative extraction solvent that has been used with a fair degree of success. It possesses the added advantage that because of its more polar nature as compared to benzene, it will ex-

Figure 4 Quinidine standard calibration curve of relative fluorescence intensity (in arbitrary units) versus concentration (mcg/ml). The linear relationship in the concentration range of 0.3 to 9 mcg/ml is presented. The linear regression line equation is: $y = 0.758x - 0.109$.

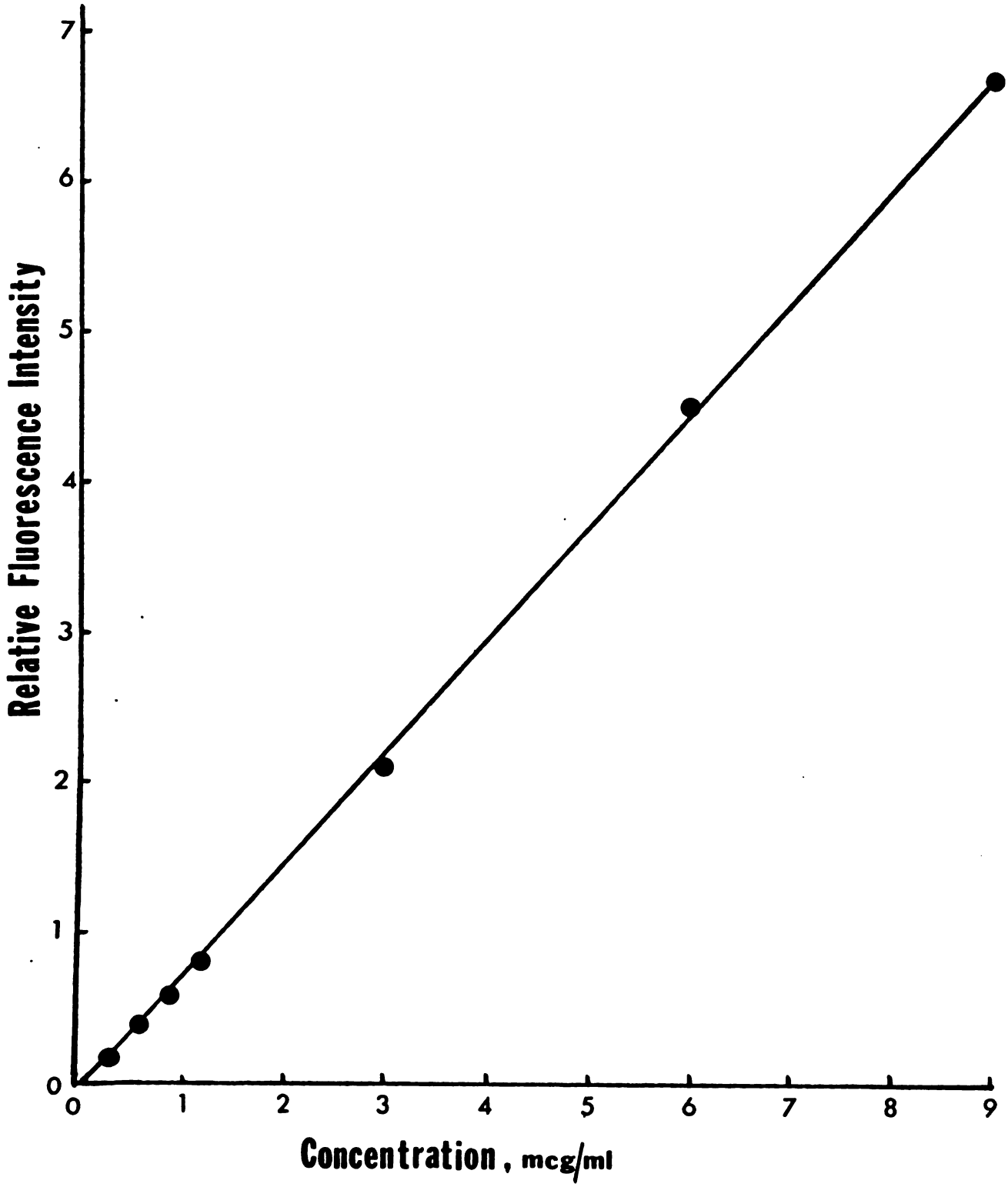
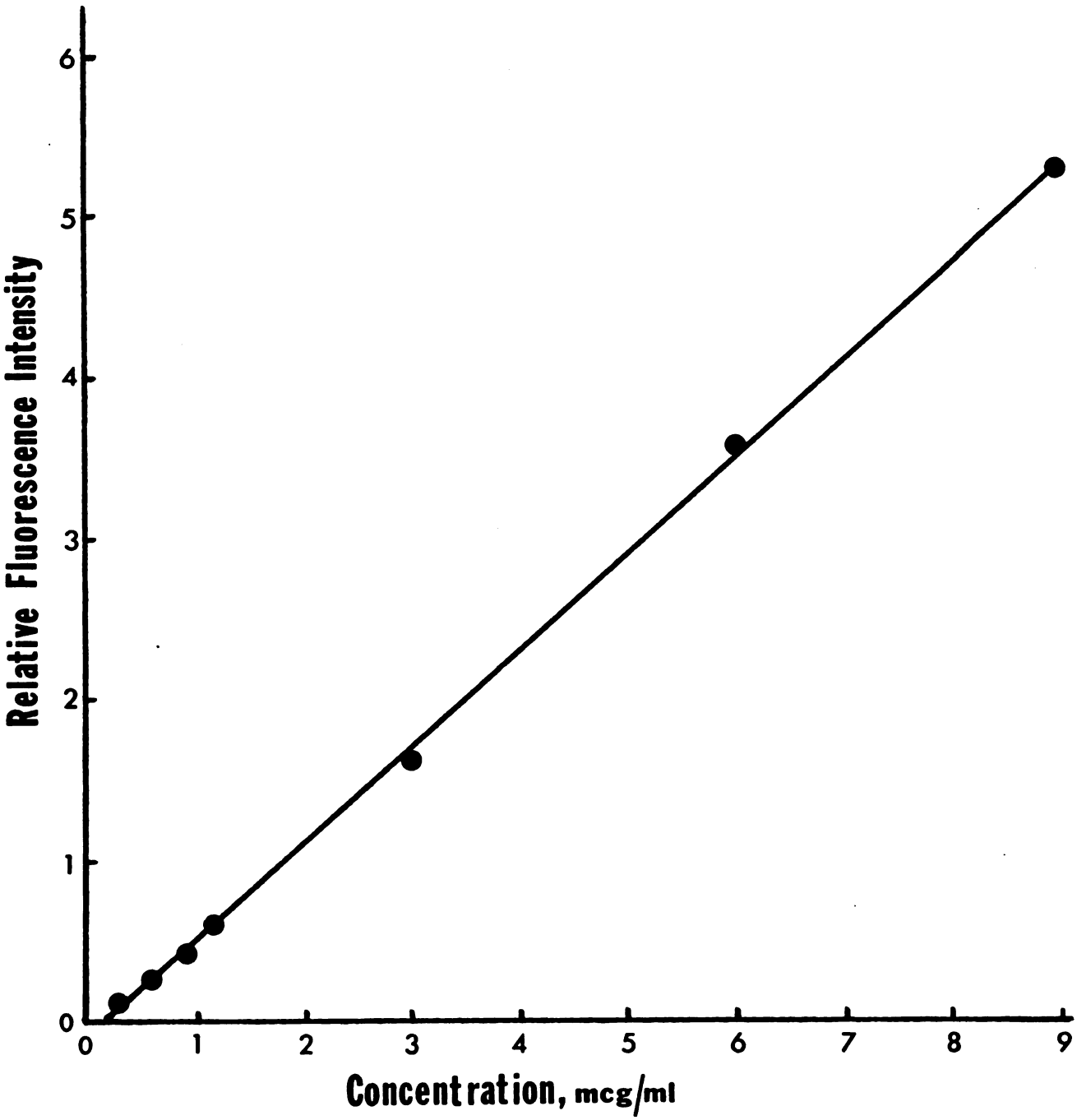

Relative Fluorescence Intensity
0
1
2
3
4
5
6
7
0
1
2
3
4
5
6
7
8
9
Concentration , mcg/ml

Figure 5 Dihydroquinidine standard calibration curve of relative fluorescence intensity (in arbitrary units) versus concentration (mcg/ml). The linear relationship in the concentration range of 0.3 to 9 mcg/ml is presented. The linear regression line equation is: $y = 0.599x - 0.118$.


Relative Fluorescence Intensity
0
1
2
3
4
5
6
0
1
2
3
4
5
6
7
8
9
Concentration, mcg/ml

tract in addition to the unchanged drug, the more polar metabolites of quinidine and dihydroquinidine. However, because of the lack of pure samples of these metabolic species with which to compare the relative extraction efficiency, the degree of extraction could not be ascertained. In the absence of pure metabolites, any attempts at quantitating the chloroform extractable metabolites based on the relative fluorescence of the parent drug molecule assumes that they have the same quantum yield as that of the parent molecule. This is probably an invalid assumption. Preliminary investigations indicated that based on the relative fluorescence intensity of quinidine, only 30 to 40% of the administered dose can be accounted for in the urine. This would seem to indicate differences in quantum yield for these metabolites, breakdown to non-fluorescent species, and/or elimination by other pathways.

IV. Preparation of Parenteral Solutions of Quinidine and Dihydroquinidine

Parenteral solutions of quinidine or dihydroquinidine sulfate containing 6 mg free base/ml of solution were prepared under aseptic conditions. To a 100 ml volumetric flask were added 600 mg (1.85 mmoles) of either base, 2.5 ml of 1N sulfuric acid (1.25 mmoles), and 80 ml of water for injection and shaken until all of the drug had dissolved.

The volume was brought up to 100 ml and bacteriologically filtered through a membrane filter (0.45 microns) into amber multiple dose vials. The vials were capped and stored in the dark. The final solution was clear and colorless and had a pH of 4.5.

V. Surgical Preparation of the Monkey

Permanent arterial and venous catheters were surgically implanted into two adult, male rhesus monkeys (Macaca mulatta) weighing 4.5 and 5.5 kg, respectively. The animals were free of disease and in apparent good health.

Prior to surgery, the animals are placed in suitably padded primate restraining chairs which were modified to allow for accommodation of the blood catheters (117). A 1 to 2 week period was allowed for the monkeys to acclimate themselves to their new environment. During this period, the monkeys were inspected for the development of pressure sores and observed for general adaptibility. Adjustments to the chair structure were also made at this time and any subsequent times when they were deemed necessary. Monkeys will adapt to residence in the chairs for many months (118). And although restrained, the animals have considerable freedom of movement and can feed and exercise themselves.

The monkeys were initially immobilized with Vetalar^{R} (20 mg/kg) intramuscularly. They were then anesthetized with sodium pentobarbital (Nembutal^{R}), 20 mg/kg intravenously. The surgical procedure used for the implantation of the blood catheters have been previously described by Forsyth and Rosenblum (117) and Werdegar et al. (118).

After surgery, the monkey is returned to the restraining chair. Panalog^{R} ointment is applied on and around the incision ad libitum and 1 ml of a suspension of Streptillin^{R} was administered intramuscularly daily for 5 to 7 days as a prophylactic measure to insure against

post-operative infections. Tissue reactions in the retroperitoneal operative site was reported to be negligible (118). The animals are able to tolerate the implanted catheters without difficulty and impairment of body functions are not noted.

Heparinized normal saline (5 units/ml) was continuously infused from a reservoir through the catheters at a rate of 1 ml/hr with a Harvard infusion pump. This procedure prevents the obstruction or occlusion (by clots) of the tip of the intact catheter. These relatively small volumes of saline that are administered would have a negligible effect on normal water balance in the monkey. The dose of heparin used in the infusate is insufficient to anticoagulate the animal.

VI. Methods of Drug Administration

All dosages of drug were administered through the venous catheter while blood samples taken at appropriate times were removed from the arterial line. Intravenous bolus doses were administered rapidly, usually within 10 seconds, and then immediately flushed with 2 ml of normal saline to clear the line of the drug solution. This procedure insures the total availability of the dose administered.

When intravenous infusion studies were performed, the following techniques were employed. A 50 ml disposible syringe was filled with the drug solution and carefully placed onto a pre-calibrated Harvard infusion pump. The intravenous catheter was removed from the continuous infusion reservoir and immediately connected to the syringe containing the drug via a three-way stopcock. The line from the

reservoir was capped with a sterile plug to prevent contamination of the system. Any entrapped air was removed with an auxillary 12 ml syringe.

In order to insure availability of the drug at the start of the infusion, the "void volume" defined as the volume of solution contained in the intravenous catheter from the tip of the syringe to the tip of the intact catheter located within the vascular system was estimated by rapidly removing the saline solution in the intravenous line with an auxillary 3 ml syringe until the presence of blood appeared at the three-way stopcock. This volume of drug solution (approximately 0.6 to 1 ml) was carefully infused manually with a fresh syringe. The system was opened to the drug solution, the infusion pump activated and an electric timer immediately started.

During the course of the infusion process, the rate of infusion could be changed at any desired time by simple re-adjustment of the gear speed lever. Termination of the infusion was performed in the following manner. At the desired time, the pump switch was deactivated and the system was closed to the animal. Removal of the residual drug which remains in the line was accomplished by withdrawing the solution with a syringe until venous blood appears in the syringe. One ml of blood is further withdrawn to insure complete removal of the drug solution. The venous catheter was re-attached to the infusion reservoir and the pump was removed.

In animal B, although infusion of solution into the venous system went without difficulties, after approximately one month, it

was not possible to withdraw samples from this line. In this case, the residual drug solution in the line was prevented from trickling in by creating sufficient negative pressure in the system. This was accomplished by attaching the terminal end of the catheter to a 12 ml syringe and withdrawing the syringe plunger, which was anchored firmly to the syringe barrel by passing a steel pin through the plastic shaft of the plunger. At the termination of the experiment, the solution in the line was infused into the animal (approximately 0.6 to 1 ml). The reliability of this procedure is recognized since comparable results are observed in animals in which withdrawal of solution is possible.

VII. Method of Blood Sampling

One ml of blood was removed from the arterial catheter per sample with a 3 ml disposible syringe. The stopcock was opened to the monkey and the blood was removed. The residual blood in the line was rapidly returned to the circulation by flushing 1.5 ml of normal saline through the catheter into the animal. The system was closed to the outside and the procedure repeated for later samples.

For experiments which required extended sampling, adherence to the removal of an arbitrary maximum of 10% of the total blood volume (approximately 40 ml) was strictly observed with a recovery period of 3 to 4 weeks between experiments. However, in the majority of the studies, approximately 5% or 20 ml of blood was removed per experiment. In this case, studies can be performed every 10 to 14

days on the same animal without running the risk of subjecting the animal to any traumatic episodes, e.g., anemia, shock, etc. The average control hematocrit was 33 to 34% and remained fairly constant throughout the entire course of this work. Supplementary injections of iron (Imferon^{R}) are administered every 10 to 14 days and a constant monitoring of the hematocrit is maintained.

VIII. Method of Urine Collection

In all experiments where urine was sampled, the total volume of urine voided in a period of 48 or 72 hours from the beginning of the experiment was collected. Control urine samples were obtained on the day preceeding each experiment. A stainless steel urinary collection pan (approximately 2 x 20 x 30 inches) equipped with a screen to separate the fecal material was placed immediately below the animal. The collection pan drained directly into sampling bottles packed in ice, The collected samples were pooled and frozen for future analysis.

IX. Method of Protein Binding Determination

The binding of quinidine to plasma proteins was studied *in vitro* using the Dianorm^{R} EQUILIBRIUM DIALYSIS System. This apparatus consists of 20 teflon cells each of which is divided into two 1 ml compartments (half-cells) by a section of Visking cellulose tubing with a thickness of 0.025 mm. The cells are mounted on a motor driven unit such that the contents of each cell is thoroughly mixed by continuous rotation of the cells. The entire apparatus is water tight so that it

may be immersed in a constant temperature water bath.

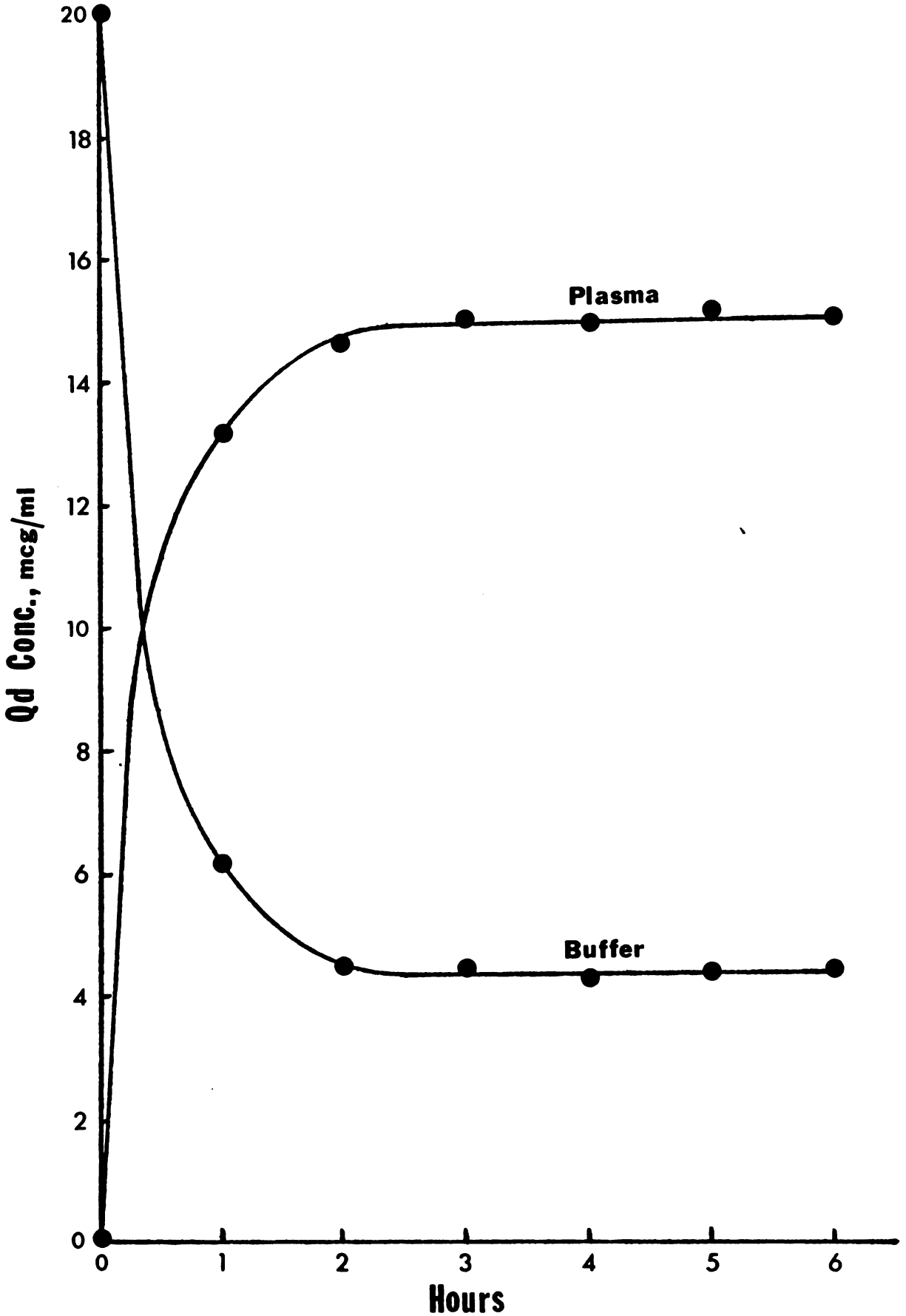
Solutions of quinidine sulfate were prepared in pH 7.4 Krebs-Ringer Bicarbonate buffer (119) in the concentration range of 2 to 30 mcg/ml. One ml of the quinidine solution was introduced into one half-cell with a disposible tuberculin syringe and 1 ml of fresh monkey plasma was simultaneously injected into the corresponding half-cell. The cells were then tightly sealed and placed into the constant temperature bath.

Duplicate and triplicate determinations were made for each quinidine concentration. Similar results were obtained when fresh frozen plasma was used. However, studies were made with fresh plasma which was collected just prior to use whenever possible.

Preliminary investigations were performed in order to establish the time required to reach equilibrium distribution of the drug within the dialysis cell. One ml of buffer containing 20 mcg/ml quinidine was dialyzed against 1 ml of monkey plasma. The contents of each half-cell were removed and analyzed at various times. As shown in Figure 6, equilibration was attained within 3 hours. The binding of quinidine to the dialysis membrane and/or cell surfaces was negligible (approximately 4 to 5%). Subsequently, all dialysis studies were carried out for 3.5 hours at 37 ± .5°C.

After allowing time for equilibration, the solutions in the buffer and plasma half-cells were removed and analyzed using a slight modification of the assay method (see p. 36). Since it was assumed that there are no interfering species, e.g., metabolites, these samples were

Figure 6 Quinidine concentrations in the plasma and buffer half-cells versus time following the dialysis of 20 mcg/ml of the drug at 37 ±.5°C. Note that equilibration is attained within 3 hours. The percent recovery was 95 to 98%.


20
18
16
14
12
10
8
6
4
2
0
Qd Conc., mcg/ml
Plasma
Buffer
0
1
2
3
4
5
6
Hours

analyzed by a direct double extraction method as described by Cramer and Isaksson (100). The 0.7 ml samples were alkalinized with 10N sodium hydroxide and extracted with 2 ml benzene. A 1.6 ml aliquot of the benzene layer was re-extracted with 3 ml of 0.1N sulfuric acid. This acid extract was read directly on the fluorometer. Suitable standard calibration curves were prepared and analyzed simultaneously with the samples. The unknown concentrations could be determined from the standard curve.

A similar procedure was utilized to determine the in vivo binding of quinidine to plasma proteins. One ml of the quinidine plasma samples was dialyzed against 1 ml of buffer solution. The 0.7 ml samples of plasma and buffer were analyzed as previously described (see p. 36). Standard plasma samples containing known amounts of quinidine were prepared and assayed concurrently with the unknown samples.

The total concentration of the bound plus free ligand was obtained from the analysis of the contents of the plasma half-cell after equilibration. The fraction of quinidine unbound can therefore be calculated by dividing the concentration of the drug in the buffer half-cell, which contains unbound drug only, by the concentration in the plasma half-cell.

X. Method of Protein Determination

A modification of the Lowry method (120) was utilized to determine the relative amount of protein which is present in a plasma sample. A 1:100 dilution of plasma in normal saline was prepared. This

reference solution ("100% protein") was suitably diluted in order to prepare standard samples containing 5 to 100% protein. Added to a Microfuge^{R} tube is 100 µl of a 1:100 dilution of the protein sample to be determined and the standard protein solutions. To each tube is placed 150 µl of 15% trichloroacetic acid. The solution is mixed well and allowed to stand for 15 minutes. The aqueous phase is carefully removed (as much as possible) after prior centrifugation. The tips of the Microfuge^{R} tubes are cut off and transferred to a 12 x 75 mm culture tube. One ml of a freshly prepared solution of 2% Na_2CO_3 in 0.1N NaOH (50 ml) and 0.5% $CuSO_4 \cdot 5\ H_2O$ in 1% sodium tartrate (1 ml) is added to the culture tube. The tube is gently mixed several times during a period of 20 minutes of standing. While mixing, 100 µl of 1N Folin-Ciocalteau Phenol Reagent is added. The colored solution which develops after one hour is read on a spectrophotometer at a wavelength of 750 nm. The amount of protein present in the unknown sample is determined from the standard calibration curve.

RESULTS AND DISCUSSION

I. Discussion of the Analytical Method

A detailed discussion and analysis of the previous methods utilized to quantitate quinidine in plasma and urine was presented in the section entitled Shortcomings of Previous Analytical Methods (p. 26). It was pointed out that the direct and extraction procedures represent the most commonly used methods today. Reports in the literature clearly indicate that when biological samples containing quinidine are analyzed by each method, the direct method yields values for quinidine which are consistently higher than the extraction procedure (81,99,100,101,102,103). These observed differences between the two methods were due to the inclusion of quinidine metabolites (81,99,100,101,102).

Benzene has been demonstrated to be an excellent extracting solvent for quinidine from biological samples (105,107). However, it was clearly shown that this solvent extracts small amounts of quinidine metabolites (99,100,107). Dihydroquinidine is also extractable with benzene and presumably some of its metabolites would be extracted too. The need for an analytical procedure which was capable of separating quinidine from its fluorescing metabolites and from dihydroquinidine and its metabolites in the plasma was a prerequisite before pharmacokinetic studies of quinidine and dihydroquinidine could be initiated. A specific and sensitive method based on TLC separation and fluorometric quantitation for the analysis of quinidine and dihy-

droquinidine in the plasma and urine was developed and has been described in detail (see Analysis of Quinidine and/or Dihydroquinidine in Biological Samples, p. 36). What follows is a discussion of the method which was developed.

When compared with the other methods of analysis, the TLC-fluorometric method has the principal advantage of specificity. Quinidine is selectively separated from its fluorescing metabolites in plasma and urine. In the presence of dihydroquinidine, the two agents are isolated as two distinct species in these biological fluids. When chloroform is used as the extracting solvent but not benzene, an unidentified fluorescent species observed in the urine only after administration of quinidine interferes with the isolation of dihydroquinidine (see Table 2, p. 40).

The principal disadvantages of the TLC-fluorometric method when compared with other existing procedures are (1) the length of time required for the analyses and (2) some loss in analytical sensitivity. The conventional methods require approximately 2 to 4 hours depending upon the number of samples to be analyzed. In contrast, this method requires 12 to 14 hours. However, up to 40 to 50 samples can be adequately accommodated in this period of time. In addition, while other analytical methods can detect quinidine concentrations as low as 1 to 10 ng/ml, the practical lower limit of sensitivity of this method is approximately 100 ng/ml of plasma or urine. However, this sacrifice in sensitivity for increased specificity might be considered an advantage when attempting to define the pharmacokinetics of quinidine.

Specificity

In order to test for the specificity of the TLC-fluorometric method which was developed, blank plasma, whole blood, and urine were obtained from various rhesus monkeys. These blank biological fluids were analyzed for quinidine and dihydroquinidine by this method. The specificity of the method is indicated by the data in Table 3. For quinidine and dihydroquinidine, there was no blank sample which gave a fluorescent reading greater than that equivalent to a mean value of 33 ng/ml quinidine and 30 ng/ml dihydroquinidine.

When post-infusion quinidine levels of 9.0 to 0.2 mcg/ml were achieved in the plasma (Fig. 7), visual inspection and an analysis of the TLC plate failed to demonstrated the presence of dihydroquinidine. The dihydroquinidine-equivalent concentrations were comparable to the plasma blank (Table 4). Following visual inspections of the TLC plate, a similar observation was noted for quinidine-equivalents in the presence of dihydroquinidine. Therefore, the presence of quinidine or dihydroquinidine metabolites will not interfere with the analysis of either drug when they are co-administered.

To test for the presence of interfering metabolites, a 72 hour urine sample was collected from a human subject who was being maintained on 1.6 grams of quinidine sulfate administered daily in divided doses. No other medications were consumed during this period. This urine sample insured the presence of quinidine metabolites in sufficiently high concentrations. The urine was concentrated and exhaustively extracted with 400 ml portions of benzene after prior alkali-

Table 3

VARIABILITY OF BIOLOGICAL FLUID BLANK SAMPLES

ANALYZED WITH THE TLC-FLUOROMETRIC METHOD

Compound	Sample	N*	Mean Drug Equivalents† (ng/ml)
Quinidine	Water	10	14.00 (8.3)
	Plasma	10	32.49 (7.3)
	Blood	5	25.08 (9.0)
	Urine	5	21.50 (6.4)
Dihydroquinidine	Water	6	18.52 (11.3)
	Plasma	6	29.41 (3.9)
	Blood	4	29.15 (11.1)
	Urine	5	16.58 (3.4)

* number of observations.
† numbers in parentheses refer to (± S.D.).

Table 4

PLASMA CONCENTRATION OF DIHYDROQUINIDINE EQUIVALENTS AT VARIOUS LEVELS OF QUINIDINE

Quinidine Concentration (mcg/ml)	Dihydroquinidine Equivalents (ng/ml)
0.040 (Control)	33.2
8.20	43.4
5.05	40.0
3.34	28.6
2.01	33.2
1.24	17.8
0.65	43.4
0.25	40.0
	Mean 34.9

nization to litmus with 28% NH_4OH. The benzene extracts were pooled and then dried over anhydrous sodium sulfate. The volume was reduced to approximately 20 ml in vacuo. Approximately 50 µl of this concentrate was streaked with a Hamilton^R syringe on a pre-coated preparative TLC silica gel plate and developed with the methanol and acetone (4:1) solvent system, i.e., the solvent system used in the final assay procedure. The bands corresponding to quinidine and dihydroquinidine were separated and eluted from the silica gel with 0.1N sulfuric acid. These eluates were made alkaline to litmus with NaOH and re-extracted with benzene. Five µl samples of these benzene extracts containing quinidine and dihydroquinidine were then spotted on silica gel TLC plates and developed with three solvent systems: methanol and amyl alcohol (10:1), methanol-acetone-chloroform (10:4:2), and benzene-methanol-chloroform-aqueous ammonia (20:10:10:1). In preliminary studies carried out in this laboratory, these systems were found to separate the unidentified fluorescent spots obtained from plasma and urine of patients receiving quinidine. Using these systems, only single spots were isolated corresponding to the Rf values for pure quinidine and dihydroquinidine (Table 5).

From these observations it was concluded that the TLC method developed was specific for quinidine and dihydroquinidine in plasma and urine.

Sensitivity

As previously indicated, this method is reasonably sensitive for

Table 5

COMPARISON OF THE Rf VALUES FOR THE EXTRACTED QUINIDINE AND DIHYDROQUINIDINE SPOTS ISOLATED WITH THE METHANOL-ACETONE (4:1) SOLVENT SYSTEM AND AUTHENTIC DRUG IN VARIOUS SYSTEMS

Sample	Rf System 1	Rf System 2	Rf System 3
Quinidine Spot	0.19	0.16	0.63
Authentic Quinidine	0.19	0.16	0.63
Dihydroquinidine Spot	0.13	0.11	0.60
Authentic Dihydroquinidine	0.13	0.11	0.60

System 1: Methanol-Amyl Alcohol (10:1)

System 2: Methanol-Acetone-Chloroform (10:4:2)

System 3: Benzene-Methanol-Chloroform-Aqueous Ammonia (20:10:10:1)

both quinidine and dihydroquinidine since concentrations as low as 100 ng/ml (approximately three times that of blank values) can be readily detected. However, the TLC-fluorometric method does not have the sensitivity exhibited by the more conventional methods.

Reproducibility

Quinidine and dihydroquinidine standard calibration curves were prepared for each experiment and the cummulative data were analyzed to demonstrate the variability of the method. A summary of these results for quinidine and dihydroquinidine in the concentration range of 0.3 to 9 mcg/ml is presented in Tables 6 and 7, respectively.

Recovery

Quinidine was added in known amounts to plasma and urine. In the concentration range of 0.2 to 10 mcg/ml, the mean percent recovery from these fluids was 13.2 (± 8.2) and 13.3% (±22.9) for plasma and urine, respectively (Table 8).

Comparison of the Methods

The improvement of the TLC-fluorometric method developed over conventional procedures was demonstrated in the following study. Quinidine was infused into animal B at a rate of 3.0 mg/kg/hr for a total of 135 minutes. The plasma samples obtained in this study were analyzed by the conventional double extraction method with benzene (100) and by the method developed (see p. 36). The quinidine concentration

Table 6

QUINIDINE STANDARD CALIBRATION CURVE

Concentration (mcg/ml)	Relative Fluorescence Intensity* (arbitrary units)	Percent Coefficient of Variation
0.3	0.145 (.01)	7.6
0.6	0.359 (.03)	8.1
0.9	0.564 (.04)	6.7
1.2	0.774 (.06)	7.1
3.0	2.124 (.10)	4.6
6.0	4.483 (.18)	4.0
9.0	6.691 (.19)	2.9

n = 63

Linear regression line: $y = 0.758 x - 0.109$

Standard deviation of slope: 0.004

Standard deviation of y-intercept: 0.020

Correlation Coefficient: 0.999

* numbers in parentheses refer to (± S.D.) for 9 determinations at each concentration.

Table 7

DIHYDROQUINIDINE STANDARD CALIBRATION CURVE

Concentration (mcg/ml)	Fluorescence Intensity* (arbitrary units)	Percent Coefficient of Variation
0.3	0.128 (.02)	14.1
0.6	0.276 (.03)	11.2
0.9	0.404 (.03)	6.2
1.2	0.562 (.06)	10.5
3.0	1.558 (.14)	8.7
6.0	3.572 (.13)	3.7
9.0	5.254 (.20)	3.8

n = 35

Linear regression line: $y = 0.599 x - 0.118$

Standard deviation of slope: 0.007

Standard deviation of y-intercept: 0.029

Correlation Coefficient: 0.998

* number in parentheses refer to (± S.D.) for 5 determinations at each concentration.

Table 8

RECOVERY OF QUINIDINE FOLLOWING THE ADDITION OF KNOWN AMOUNTS IN PLASMA AND URINE

Sample	Known Quinidine Concentration (mcg/ml)	Percent Recovery*
Plasma	0.2	14.4
	0.5	11.8
	0.8	13.7
	1.0	12.6
	2.0	13.8
	5.0	12.3
	8.0	13.4
	10.0	13.3
		Mean: 13.2 (2.2)
Urine	0.2	14.9
	0.5	11.9
	0.8	12.6
	1.0	13.8
	2.0	12.1
	5.0	13.2
	8.0	14.5
	10.0	13.0
		Mean: 13.3 (2.9)

* numbers in parentheses refer to (± S.D.)

was always higher and the decay curve obtained following analysis via the extraction method was observed to be curved (Fig. 7). When the samples were analyzed by the TLC-fluorometric procedure, the post-infusion curve was mono-exponential with an apparent elimination half-life of 40 minutes.

The differences in the observed quinidine levels when analyzed by the two different methods clearly illustrate the shortcomings of the extraction procedure. Presumably benzene extractable quinidine metabolite(s) account for the quinidine elimination profile observed in Figure 7. Thus, the method which has been developed for the analysis of quinidine is specific and sensitive and was utilized to determine the pharmacokinetic disposition of quinidine and dihydroquinidine in the rhesus monkey.

II. Quinidine Pharmacokinetics

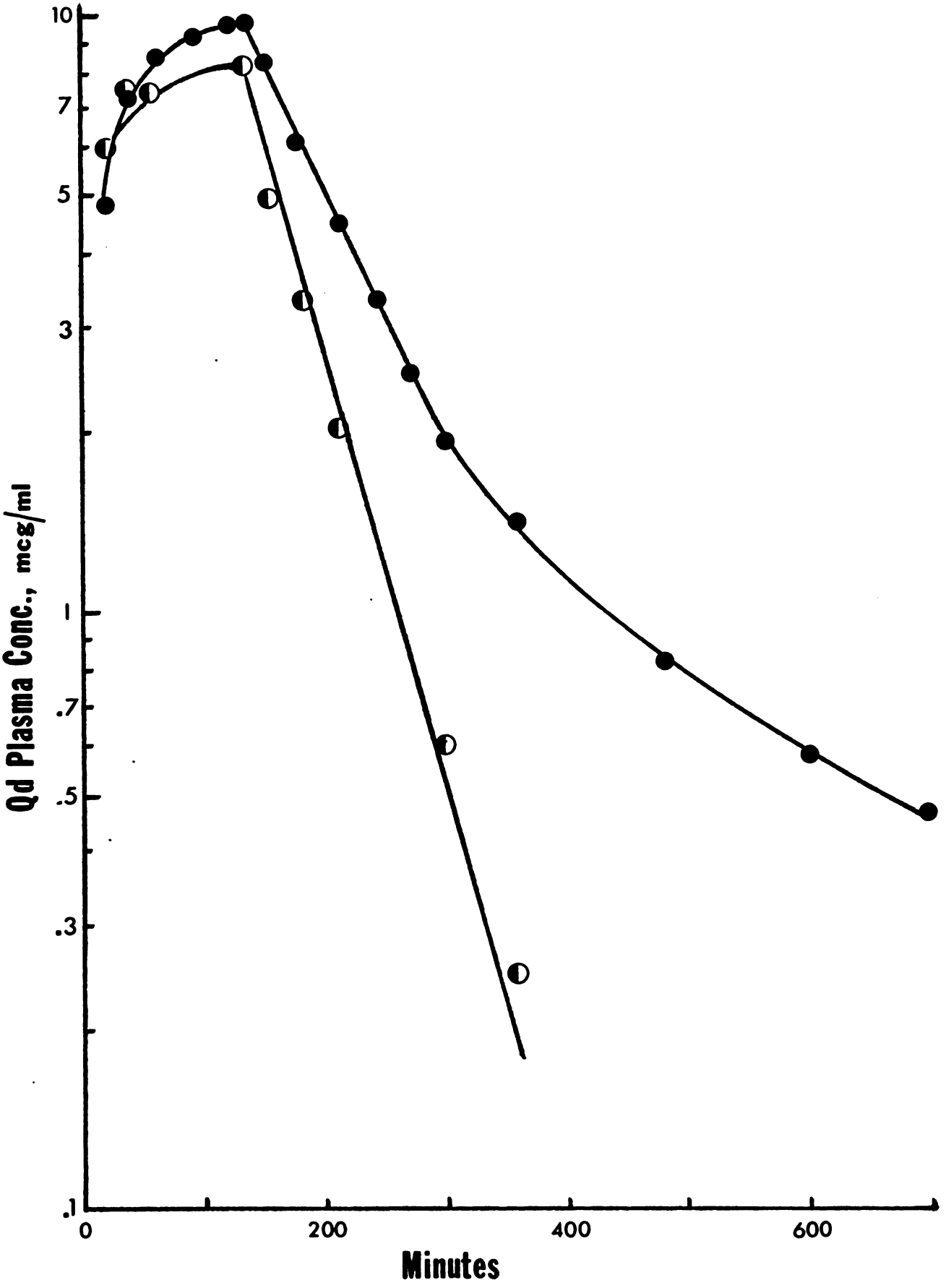
Single Intravenous Bolus Administration

After intravenous administration, the plasma level curves of quinidine exhibited a biexponential decay with time (see Fig. 9 and 10) according to Equation 1:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq 3)}$$

where Cp is the plasma concentration at any time (t) after the dose, α and β are the first-order hybrid rate constants of the fast and slow disposition processes, respectively, and A and B are the zero-time

Figure 7 Quinidine plasma level curves in animal B following infusion at a rate of 3 mg/kg/hr for 135 minutes analyzed by the extraction method with benzene (solid circles) and the TLC-fluorometric method (half-open circles). Note that the plasma levels analyzed by the extraction method are higher and the post-infusion decay time is curved. When the plasma samples were analyzed by the TLC-fluorometric method, the post-infusion elimination curve is exponential ($T_{.5}$ = 40 minutes).


10
7
5
3
1
.7
.5
.3
.1
Qd Plasma Conc., mcg/ml
0
200
400
600
Minutes

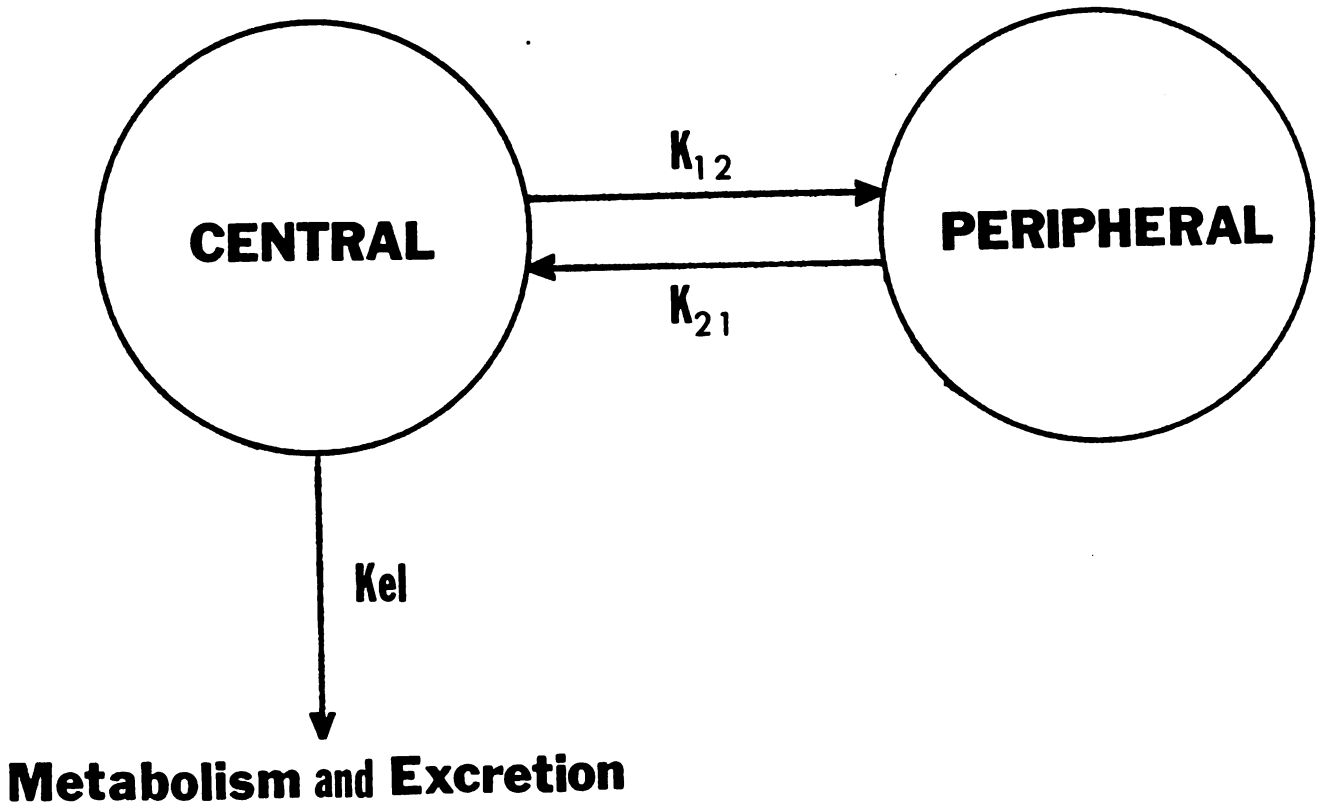
intercepts of the two components of the biexponential curve. This biexponential decay is consistent with a two-compartment open-system model as shown in Figure 8 and is proposed to describe the disposition of quinidine in the body.

In this model, quinidine is conceived to be distributed in the body within a central compartment and a peripheral or tissue compartment with which the drug interchanges. The transfer of drug between these two compartments is assumed to occur by first-order processes where k_{12} and k_{21} are the first-order rate constants for the movement of drug into and out of the peripheral compartment.

In general, most drugs are presumed to be taken up very rapidly by highly perfused (vascular rich) tissues of the body, such as the heart, lungs, liver, and kidneys. Because of this rapid uptake of drug, equilibration between the blood and these tissues is assumed to be quite rapid. It is therefore consistent to include these tissues physiologically and mathematically in the analysis of the central compartment. The inclusion of these and other tissues of the body as part of the central compartment has been discussed by Riegelman et al. (123). The tissue or peripheral compartment of a two-compartment open-system model is assumed to include those organs of the body which are comparatively less perfused (vascular lean tissues). They include the muscle masses, bone, skin, adipose tissue, etc. The size of each compartment will vary depending upon the physicochemical properties of the drug.

The liver and the kidneys are the principal organs which are

Figure 8 Two-Compartment Body Model. This pharmacokinetic model is proposed to describe the disposition of quinidine in the body. The arrows indicate first-order transfer processes with the appropriate rate constants. Metabolism and excretion of the drug occur from the central compartment. Cp is the concentration of intact drug in the central compartment with a volume, Vp.


CENTRAL
PERIPHERAL
K_{12}
K_{21}
Kel
Metabolism and Excretion

responsible for the elimination of quinidine from the body. In the present model, metabolism and excretion of quinidine are assumed to be first-order processes which occur from the central compartment. The rate constant, Kel, represents the sum of the simultaneous processes of metabolism and excretion of the drug.

After Riegelman et al. (124), the constants of the biexponential equation can be defined as follows:

$$Cp = \frac{Cp^{\circ}(k_{21} - \alpha)}{(\beta - \alpha)} e^{-\alpha t} + \frac{Cp^{\circ}(k_{21} - \beta)}{(\alpha - \beta)} e^{-\beta t} \quad \text{(Eq 4)}$$

where Cp° = concentration of drug in the body at zero time,

$= A + B$

$$\alpha = \frac{(k_{12} + k_{21} + Kel) + \sqrt{(k_{12} + k_{21} + Kel)^2 - 4(k_{21} + Kel)}}{2}$$

$$\beta = \frac{(k_{12} + k_{21} + Kel) - \sqrt{(k_{12} + k_{21} + Kel)^2 - 4(k_{21} + Kel)}}{2}$$

and Vp = volume of the central compartment,

$= Dose/Cp^{\circ}$

In the intact animal it would be virtually impossible to measure the actual or real volume of distribution of a drug. Most drugs distribute in the body in a complex and characteristic manner. The apparent volume of distribution, Vd, which is elaborated from observed data, is a parameter of the pharmacokinetic model. The computed Vd may or may not, therefore, bear any resemblance to the actual volume

occupied by the drug. Often Vd is a characteristic of the drug and may be correctly referred to as a volume constant (124,125,126,127).

Within the context of the model shown in Figure 8, the distribution volume at steady state, Vd_{ss}, is a measure of drug distribution uninfluenced by drug elimination. The parameter, Vd_{ss}, can be determined from the following relationship:

$$Vd_{ss} = (1 + \frac{k_{12}}{k_{21}}) Vp \qquad \text{(Eq 5)}$$

In view of the low margin of safety possessed by quinidine and the fact that the drug is not administered as an intravenous bolus in clinical medicine, preliminary studies were performed in an attempt to establish bolus doses which would be safe and provide post-distributive plasma levels within the therapeutic range. Quinidine was infused at a rate of 6.5 mg/kg/hr for a duration of 120 minutes. The post-infusion elimination half-life was 34 minutes. Although steady state plasma levels may have not been attained, a value of 10 mcg/ml was used as a reasonable estimate of the plateau concentration. From Equations 9 and 13 (pp. 75 and 79), a Vd of 0.6 L/kg of body weight was obtained and used to compute the requisite bolus dose, where Dose = Vd Cp°.

A single, rapid intravenous bolus dose of quinidine of 6.7 and 3.3 mg/kg was administered to animal A (4.5 kg) and animal B (5.5 kg), respectively. The plasma curves which were obtained following the administration of these doses are presented in Figures 9 and 10. In

Figure 9. The computer-simulated biexponential plasma level decay curve and experimental data points following the intravenous administration of 30 mg quinidine (6.67 mg/kg) to animal A. The distribution phase, obtained by feathering, is seen to be very rapid ($T_{.5}$ = 3.3 minutes). The slower elimination phase has a $T_{.5}$ = 35 minutes.

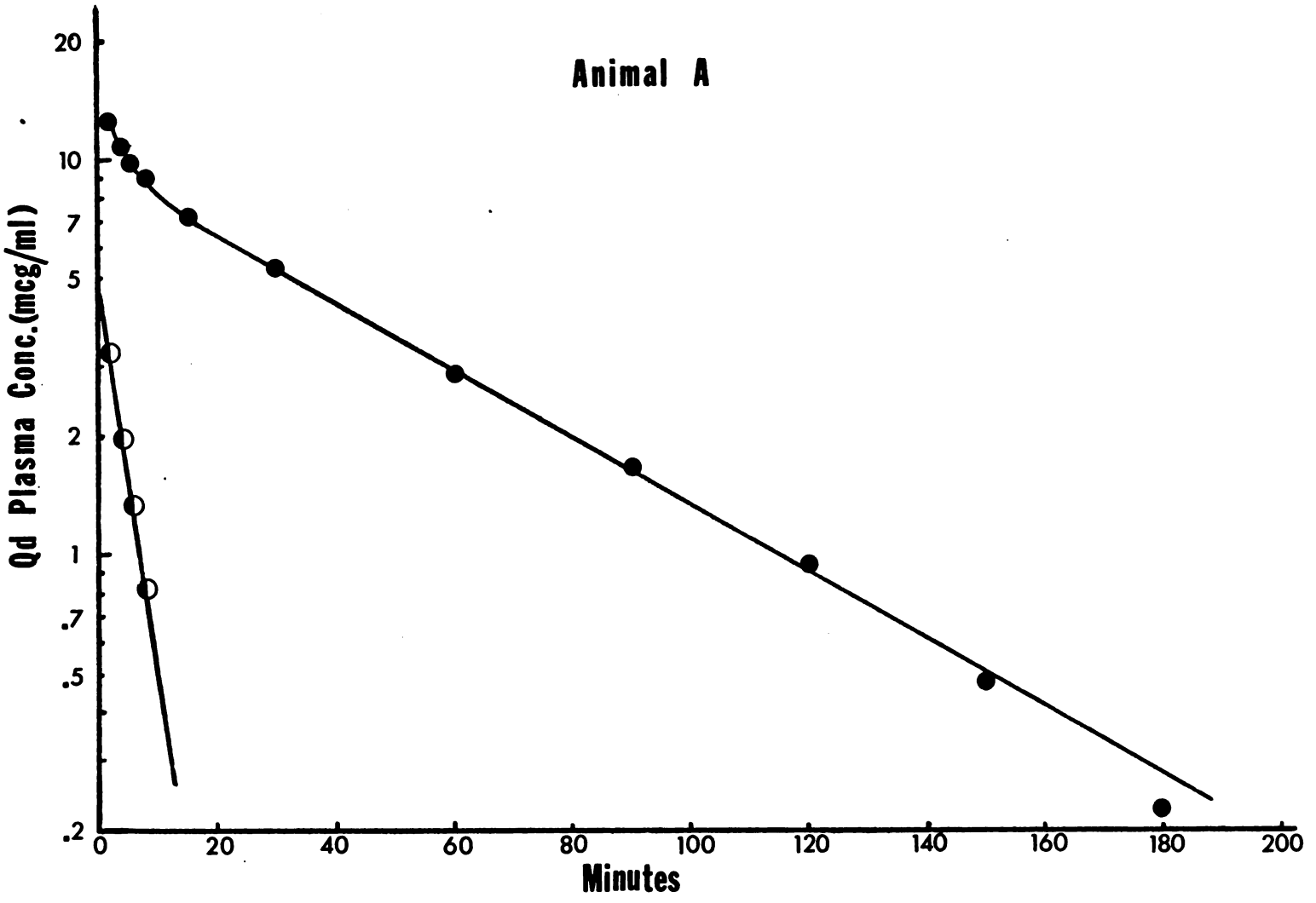
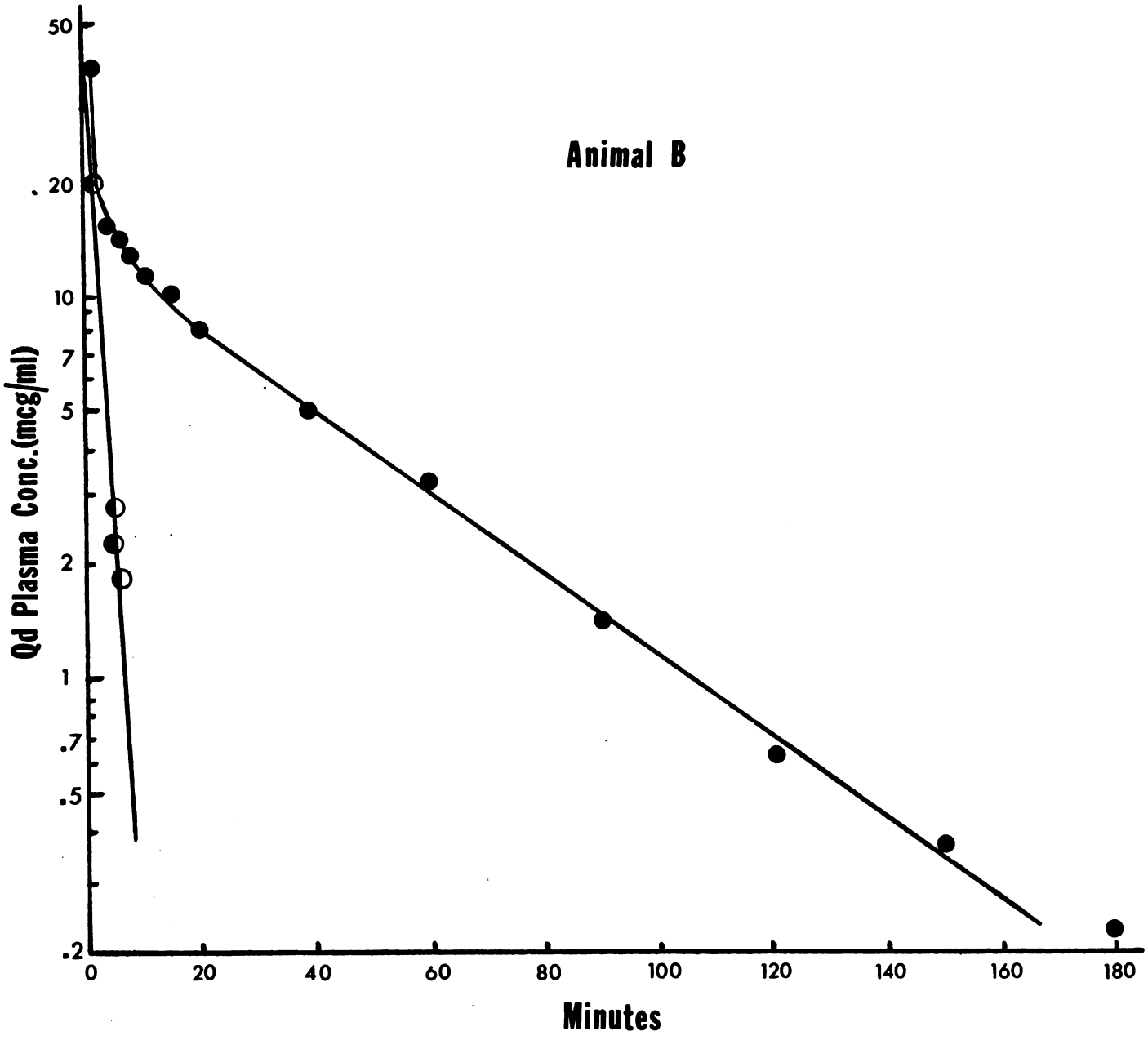

Animal A
Qd Plasma Conc.(mcg/ml)
20
10
7
5
2
1
.7
.5
.2
0
20
40
60
80
100
120
140
160
180
200
Minutes

Figure 10 The computer-simulated biexponential plasma level decay curve and experimental data points following the intravenous administration of 18 mg quinidine (3.3 mg/kg) to animal B. The distribution phase, obtained by feathering, is seen to be very rapid ($T_{.5}$ = 1.5 minutes). The slower elimination phase has a $T_{.5}$ = 28.8 minutes.


Animal B
Qd Plasma Conc.(mcg/ml)
50
20
10
7
5
2
1
.7
.5
.2
0
20
40
60
80
100
120
140
160
180
Minutes

each case, the curves decayed with a rapid distributive phase followed by the slower elimination phase.

The observed data were fitted to the biexponential equation (Eq 3) using the NONLIN program* and IBM 360/50 computer. This program uses an adaptation of Hartley's modification (128) of the Gauss-Newton method for the fitting of non-linear regression functions by least squares. Each of the observed data points, y_i, was weighted inversely with its variance. If it is assumed that the variance of y_i is proportional to y_i^2, each y_i observation may be weighted as $1/y_i^2$ (129,130). This weighting factor was used in all computer analyses reported herein.

Initial estimates of the parameters of the biexponential equation are required by the computer program. These parameters were determined from the semilogarithmic graph of the quinidine plasma level versus time curves. The best values of these constants were estimated by the NONLIN program. The pharmacokinetic constants of the two-compartment open-system model for these two animals are summarized in Table 9.

The very rapid and shallow distribution phase suggests significant distribution of quinidine into the highly perfused organs of the body, e.g., liver, kidneys, heart, lungs, etc. but insignificant distribution of the drug into vascular lean tissues, such as muscle and adipose tissue in the doses studied.

In certain circumstances the kinetics of a two-compartment system can be simplified to those of a one-compartment model. Upon integration of Equation 3, one arrives at the total area under the plasma level-

* This program was kindly supplied by Dr. Carl M. Metzler, The Upjohn Company, Kalamazoo, Michigan 49001.

Table 9

PHARMACOKINETIC CONSTANTS OF THE TWO-COMPARTMENT OPEN-SYSTEM MODEL FOR QUINIDINE

Subject	A (mcg/ml)	B (mcg/ml)	α (min^{-1})	$t_{.5\alpha}$ (min)	β (min^{-1})	$t_{.5\beta}$ (min)	k_{12} (min^{-1})	k_{21} (min^{-1})	Kel (min^{-1})	Vp (L/kg)	Vd_{ss} (L/kg)
Animal A	4.47	9.74	0.208	3.3	0.020	35.0	0.053	0.141	0.028	0.47	0.64
Animal B	37.47	13.65	0.460	1.5	0.024	28.8	0.260	0.140	0.085	0.06	0.18

time curve (A.U.C.):

$$A.U.C. = \int_0^\infty Cpdt = A/\alpha + B/\beta \qquad \text{(Eq 6)}$$

If the area, A/α, is small relative to the magnitude of B/β, i.e., A.U.C. $\propto B/\beta$ (which is the case for quinidine, c.f. Table 9), a negligible fraction of the dose is eliminated during the α phase. Then the disposition of the drug can be reasonably described by a single compartment model, in which case the apparent volume of distribution (Vd) is defined by the relationship:

$$Vd = Dose/B \qquad \text{(Eq 7)}$$

The plasma clearance of a drug is defined as the volume of plasma from which the drug is removed per unit time. This parameter is very useful for such matters as the assessment of physiological functions and for the prediction of drug concentration in the plasma following constant intravenous infusion. The plasma clearance after an intravenous bolus may be determined from the relationship:

$$\text{Clearance (Cl)} = Dose/A.U.C. \qquad \text{(Eq 8)}$$

which for quinidine, the Cl may be closely approximated by:

$$Cl = Vd\ \beta \qquad \text{(Eq 9)}$$

The total clearance in the sum of the drug's metabolic and renal plasma clearance, thus:

$$Vd\ \beta = kmVd + keVd \qquad \text{(Eq 10)}$$

where km and ke are the first-order rate constants for metabolism and excretion, respectively.

The apparent volume of distribution and plasma clearance of quinidine were calculated according to Equations 7 and 9 and are presented in Table 10.

The total volume of urine voided in a period of 48 hours commencing with the administration of the dose was collected and analyzed for quinidine. No attempt was made to control the pH of the urine. Approximately 1% of the administered dose was excreted in the urine as the unchanged drug molecule (Table 11). Since renal excretion of the drug plays an insignificant role in the elimination of quinidine, the total plasma clearance of the drug is probably associated with metabolism. Therefore, Equation 10 can be reduced to metabolic clearance according to:

$$Cl = Vd\,\beta \simeq kmVd \qquad \text{(Eq 11)}$$

Intravenous Infusion Administration

Probably the best means of examining the ability of an animal to eliminate drugs as a function of dose is through constant infusion experiments. The concentration-time course of a drug in the body following a constant zero-order infusion can be described by:

$$Cp = \frac{R^{\circ}}{Vd\,Kd}\,(1 - e^{-Kdt}) \qquad \text{(Eq 12)}$$

Table 10

CALCULATED VOLUME OF DISTRIBUTION AND PLASMA CLEARANCE

FOR QUINIDINE AFTER INTRAVENOUS BOLUS

Subject	Vd (L)	% Body Wt	Cl (ml/min/kg)
Animal A	3.08	68.5	13.5
Animal B	1.32	24.0	5.8

Table 11

48 HOUR URINARY EXCRETION OF QUINIDINE AFTER INTRAVENOUS BOLUS

Subject	Dose (mg/kg)	Volume (ml)	Concentration (mcg/ml)	Amount (mg)	% Dose
Animal A	6.67	538	0.46	0.25	0.83
Animal B	3.33	501	0.38	0.19	1.07

where R° is the rate of zero-order infusion and Kd is the first-order elimination rate constant. When plateau or steady state levels of the drug are reached, the rate of change in concentration is zero and has the following relationship:

$$R^\circ = Vd\ Kd\ Cp_{ss} = Cl\ Cp_{ss} \qquad \text{(Eq 13)}$$

where Cp_{ss} is the plasma concentration at steady state. Rearranging Equation 13, Cp_{ss} can be predicted from Equation 14:

$$Cp_{ss} = R^\circ/Cl \qquad \text{(Eq 14)}$$

When the clearance of the drug is constant, the Cp_{ss} levels attained should be directly proportional to the infusion rate. Any deviations from the direct proportionality between R° and Cp_{ss} would indicate that a dose-dependent system is present which is operating through changes in the clearance of the drug. In general, a dose-dependent system might be considered any system in which the pharmacokinetic parameters change with the amount of drug in the body. For example, the decline of drug levels in the body will not be exponential; the time required for elimination of 50% of a dose (half-life) will increase as the dose is increased; and the composition of the excretory products (unchanged drug and metabolites) will be affected by the dose of the drug.

The reasons for the existence of dose-dependent kinetics are numerous. Many physiological events which take place in the body are saturable processes, for example, active and facilitated trans-

port, metabolism, renal and biliary secretion, and protein binding of drugs. In most cases the concentration of drug is observed to be well below that approaching saturation and these events follow first-order kinetics. However dose-dependent kinetics are frequently seen after therapeutic doses of the drug. Salicylic acid (131), bishydroxycoumarin (132), phenylbutazone (133), and diphenylhydantoin (134) are a few compounds which have been demonstrated to exhibit dose-dependent kinetics in man.

The biotransformation of a drug is usually an enzymatic process and may be rate-limited by the capacity of the enzyme system(s) involved. Drugs which are primarily eliminated from the body by metabolic transformation might therefore be suspected to exhibit dose-dependent kinetics.

The reversible binding of drugs by plasma proteins, primarily albumin, and other substances which are normally present in the body is also regarded as a capacity-limited process. For most drugs in the dosage range used in man, however, the concentrations never approach the capacity of the binding system. Nevertheless, it is possible that a metabolite or another drug can exist in a sufficiently high enough concentration to alter binding and hence the pharmacokinetics of the drug in question.

Another important factor which can account for dose-dependent effects in pharmacokinetics is the action of a drug and/or its metabolites on the body. The effects may be mediated through changes in urine pH which will affect the urinary excretion of many weak acids and

bases. The drug and/or its metabolites may uncouple oxidative phosphorylation and so effect certain energy-dependent absorption, distribution, metabolism, and excretion processes; and/or they may act as stimulators or inhibitors of drug elimination processes (135).

In view of the above discussion, quinidine can be suspected to exhibit dose-dependent kinetics for several reasons. This drug is primarily eliminated from the body by metabolism. In man, as previously reported (see p. 22), only 10 to 20% of an administered dose is excreted in the urine as the unchanged drug molecule. Approximately 1% of intravenously administered quinidine was recoverable in the urine as the parent compound after administration to rhesus monkeys (see Table 11).

The binding of quinidine to plasma proteins has been reported to be approximately 80% in the therapeutic concentration in man (see p. 17). Plasma protein binding studies, which will be reported, indicate that the drug is bound in monkey plasma to the extent of 96 to 98% in the concentration range of 5 to 10 mcg/ml.

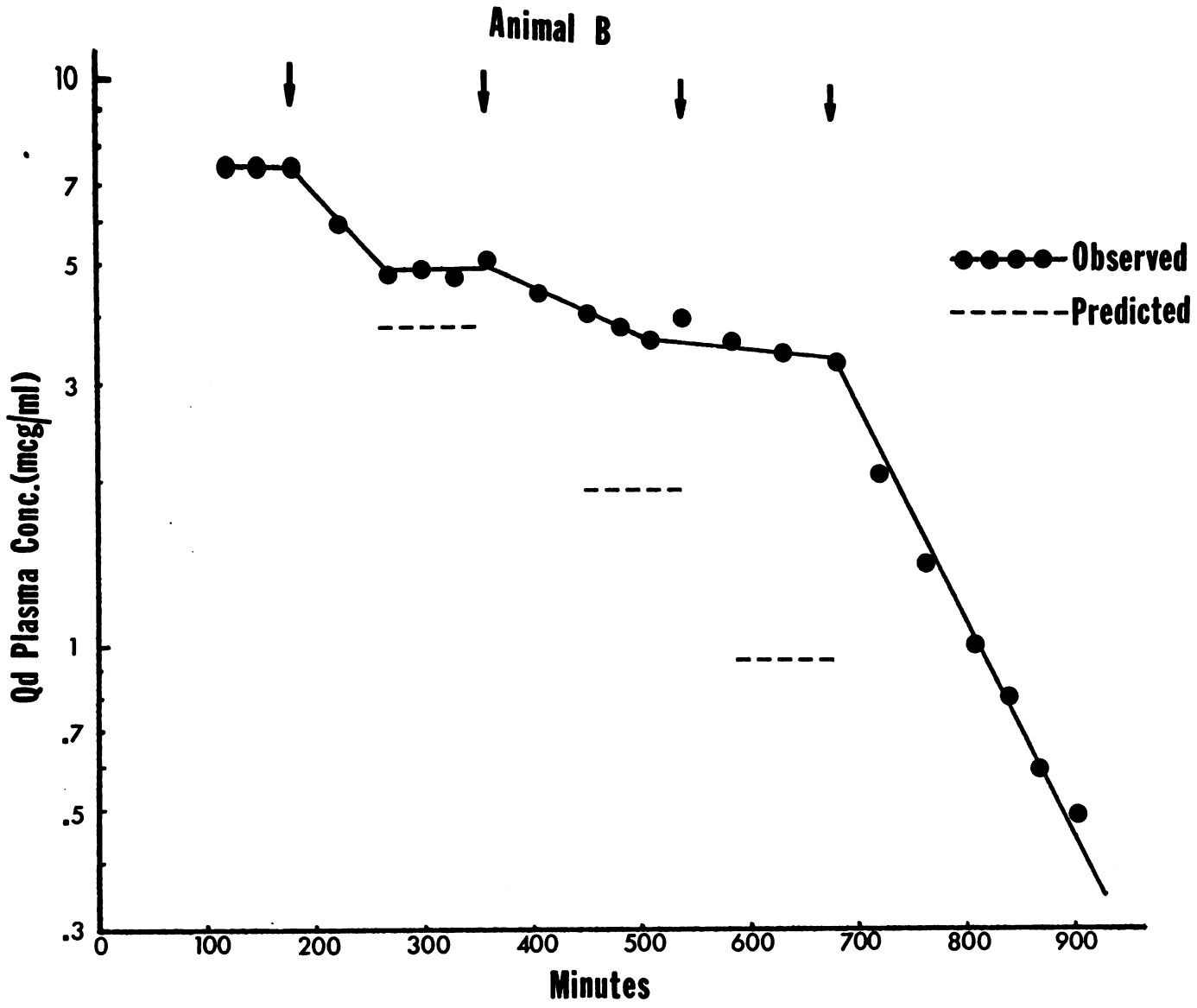
The cardiovascular network is the vehicle by which substances are distributed to tissues in the body. Agents which have their principal site of action on the cardiovascular system are unique because they can potentially affect their own fate in the body. Quinidine is such a drug. It can induce significant alterations in the cardiac output of the heart, heart rate, blood flow rates, and blood volume (see Aspects of Quinidine Pharmacology and Toxicology, p. 8). All of these changes can influence the disposition of the drug in the body.

Recognizing the possibility of dose-dependent quinidine kinetics but also realizing the unknown toxicity of quinidine metabolites which would accumulate with prolonged infusion of the drug, a preliminary constant infusion study was undertaken. Rather than the usual case in which the infusion rate is incrementally increased with time, quinidine was infused into animal B at decreasing rates. The initial rate was 8.77 mg/kg/hr. Each infusion rate thereafter was one-half of the previous input rate. The duration of each infusion was 180 minutes except for the lowest rate (140 minutes). The 180 minute infusion period, based on the short intravenous bolus half-life (29 minutes), should have insured the attainment of steady state concentrations. As shown in Figure 11, the initial level of quinidine reaches steady state concentration. However, the clearance estimated from the initial plateau level (107.2 ml/min) is not in agreement with the value previously observed in this animal (31.8 ml/min) and all subsequent levels were not those predicted for the drug. In addition, upon cessation of the infusion, the plasma levels decayed with an apparent half-life (99 minutes) which was significantly longer than that observed previously after the intravenous bolus (29 minutes).

From the results of this preliminary study, it was apparent that quinidine exhibits dose-dependent kinetics. Further experiments were designed in an attempt to elucidate the mechanism(s) responsible for these observations.

In view of the findings in the preceeding study, the infusion rate was increased with time during the course of two separate exper-

Figure 11 The quinidine plasma levels in animal B following the infusion of 8.77, 4.40, 2.22, and 1.16 mg/kg/hr. The arrows indicate the times at which the infusion rate was changed. The solid line is the observed plasma level. The dashed-lines are the plasma levels of quinidine which were predicted from proportional changes in the infusion rates. Upon cessation of the infusion at t = 680 minutes, the plasma level decayed with an apparent half-life of 99 minutes.


Animal B
10
7
5
3
1
.7
.5
.3
Qd Plasma Conc.(mcg/ml)
0
100
200
300
400
500
600
700
800
900
Minutes
Observed
Predicted

iments in the same animal. In these studies, the relationship between dose, quinidine plasma clearance, elimination half-life ($t_{.5}$), and volume of distribution were assessed.

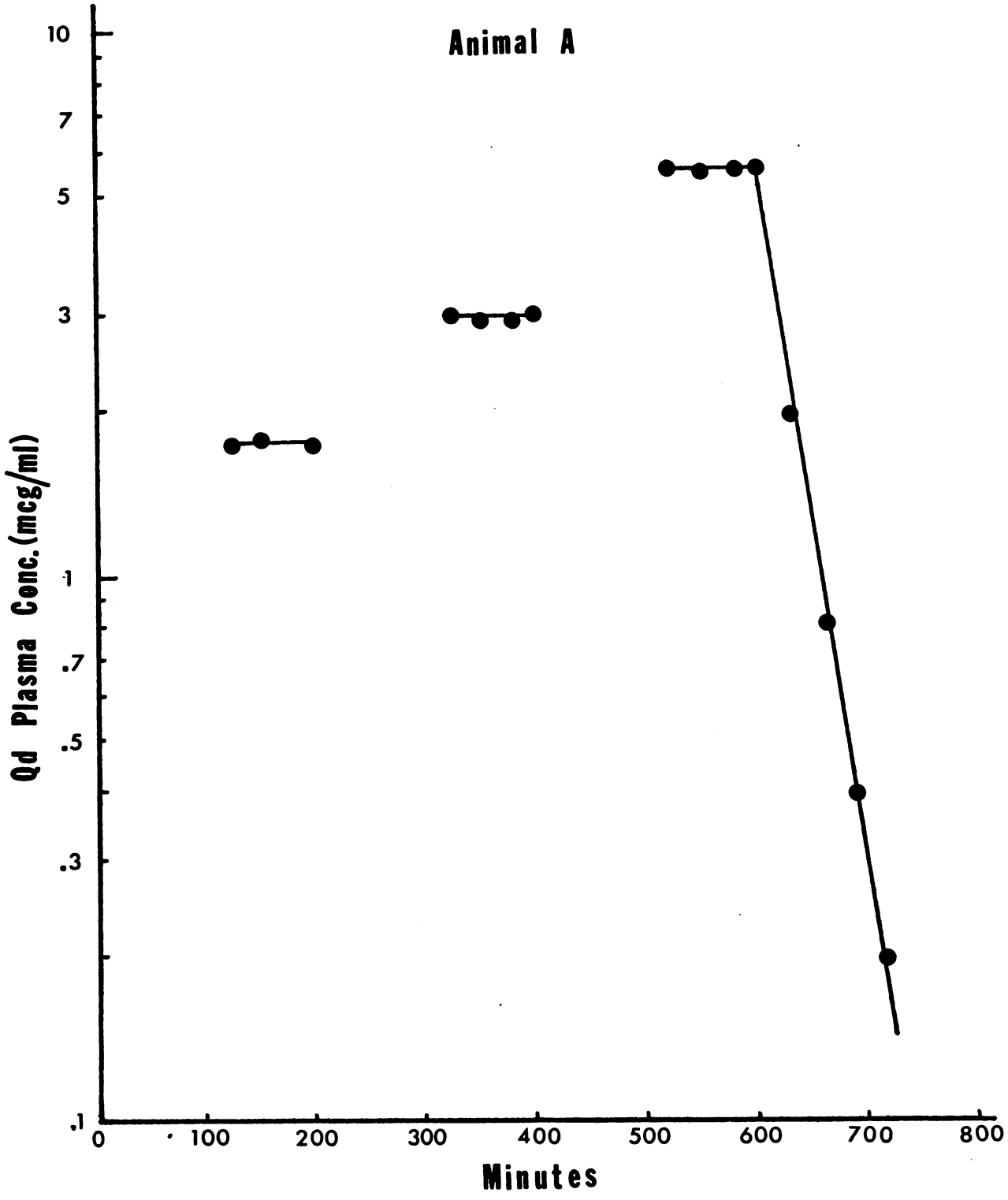
Because of the limited number of plasma samples that could be taken without subjecting the animal to any traumatic accidents, e.g., hemorrhagic shock, emphasis was given towards obtaining plasma samples at the steady state levels and the decay phase of the curves. Because of this limitation, additional information regarding the nature of the ascending portion of the plasma curve could not be ascertained.

Quinidine was infused into animal A in ascending doses of 1.44 2.7, and 5.4 mg/kg/hr for a duration of 200 minutes at each infusion rate. The infusion process was then terminated and the residual drug solution in the intravenous catheter was completely removed in order to insure against leakage of drug into the vascular system. Figure 12 presents the steady state and post-infusion levels of the drug which were achieved after the administration of these doses.

The apparent post-infusion elimination half-life is 25 minutes which is in agreement with that observed from the intravenous bolus experiment (p. 74). The absence of the distributive phase is consistent with the rapid, shallow distribution phase seen after an intravenous bolus. At the end of each infusion rate the plasma levels of quinidine are seen to reach steady state concentrations consistent with the drug's short elimination half-life. In addition, these plateau levels are proportional to the infusion rates.

In the next experiment of this series, after allowing 10 days for

Figure 12 Quinidine plasma levels in animal A following the infusion of 1.44, 2.7, and 5.4 mg/kg/hr, each for a duration of 200 minutes. Note that the plasma steady state levels are attained and maintained during the course of each infusion period. The post-infusion elimination half-life from the highest plateau level is 25 minutes and is consistent with that observed after an intravenous bolus.


Animal A
10
7
5
3
1
.7
.5
.3
.1
Qd Plasma Conc.(mcg/ml)
0
100
200
300
400
500
600
700
800
Minutes

the animal to recover, the drug was infused for 200 minutes at a rate of 1.44 mg/kg/hr. Immediately after the 200 minute sample was obtained, the infusion process was terminated, residual drug was removed from the intravenous catheter, and plasma samples were collected at appropriate times to determine the elimination half-life of the decay phase. Plasma level decay from this level occured with an apparent half-life of 30 minutes (Fig. 13). The plateau concentration attained in this experiment was identical to that observed in the previous study for this infusion rate (Fig. 12).

After allowing for sufficient data observations to be taken following the 1.44 mg/kg/hr dosage, infusion at a rate of 10.7 mg/kg/hr was initiated and allowed to proceed for 200 minutes. Apparent steady state levels were obtained. Upon termination of the infusion, post-infusion quinidine levels declined with an apparent half-life of 108 minutes.

As shown in Figures 12 and 13, at each input rate the time to reach steady state levels in consistent with the drug's short elimination half-life seen following the intravenous bolus. The plateau levels attained were proportional to the infusion rates (Fig. 14). Each plateau concentration could be predicted from the plasma clearance (60.9 ml/min) estimated from the intravenous bolus previously reported (Table 12).

When the drug was administered at rates (1.44 to 5.4 mg/kg/hr) which produce quinidine plateau concentrations in the therapeutic range, the plasma levels decline with an apparent half-life similar to

Figure 13 Quinidine plasma levels in animal A following the infusion of 1.44 and 10.7 mg/kg/hr, each for a duration of 200 minutes. Following the 1.44 mg/kg/hr infusion rate, the plateau level of the drug was identical to that observed in the previous study at this rate (1.75 mcg/ml). Post-infusion elimination half-life is 30 minutes. At t = 280 minutes, an infusion at the rate of 10.7 mg/kg/hr was initiated. Plateau concentration of the drug (11.8 mcg/ml) was maintained indicating no apparent change in quinidine plasma clearance. Post-infusion elimination half-life is 108 minutes.

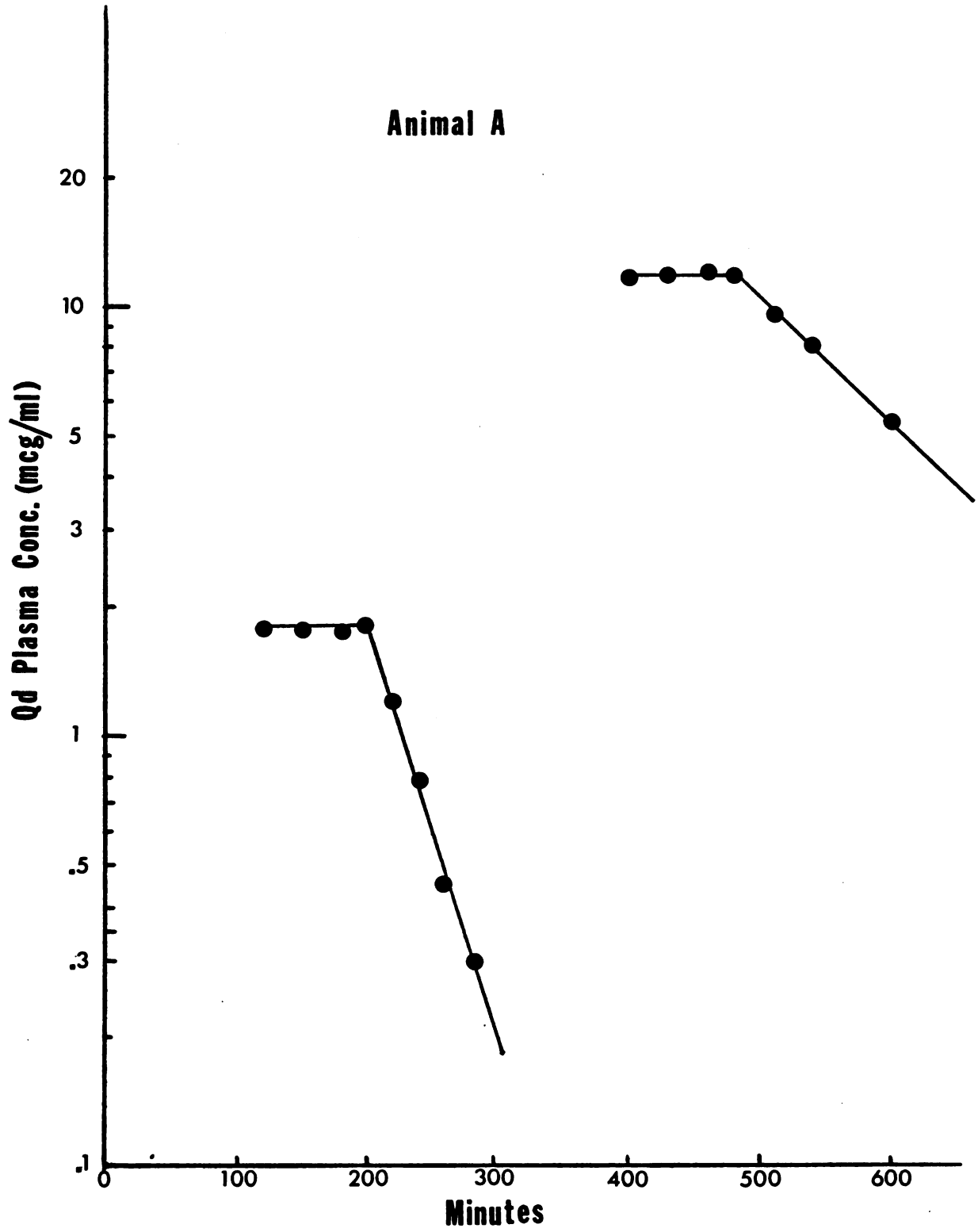

Animal A
Qd Plasma Conc. (mcg/ml)
20
10
5
3
1
.5
.3
.1
0
100
200
300
400
500
600
Minutes

Figure 14 Plot of the observed steady state quinidine plasma concentrations (Cp_{SS}) versus rate of infusion (R°). A linear regression line indicates the constancy of quinidine plasma clearance.

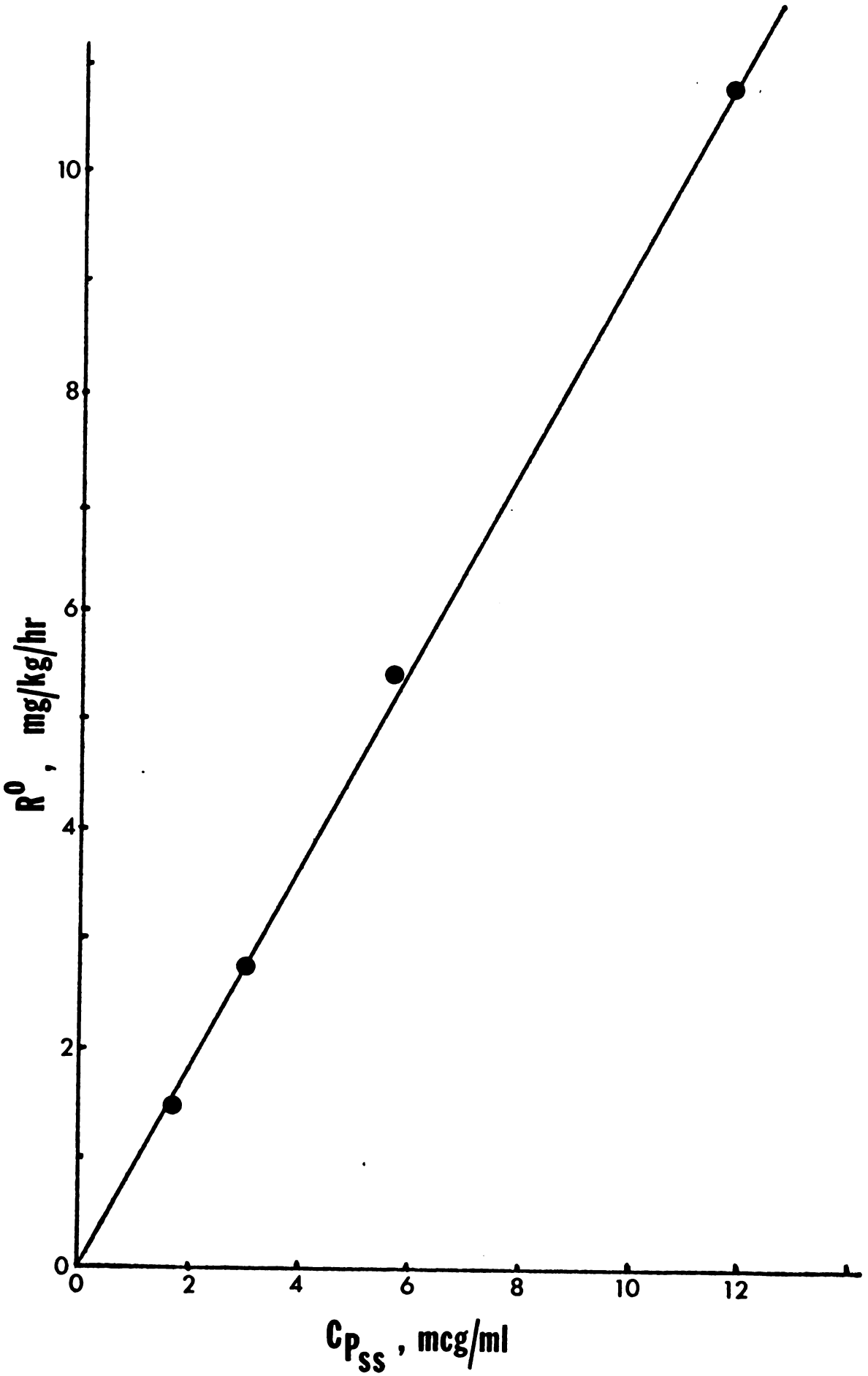

10
8
6
4
2
0
R^0, mg/kg/hr
0
2
4
6
8
10
12
$C_{P_{SS}}$, mcg/ml

Table 12

COMPARISON OF OBSERVED AND PREDICTED STEADY STATE LEVELS OF QUINIDINE IN ANIMAL A BASED ON CLEARANCE MEASUREMENTS

R° (mg/kg/hr)	Observed Cp_{ss} (mcg/ml)	Predicted Cp_{ss}† (mcg/ml)	Cl_{ss} (ml/min)	Cl_{ss}/Cl_{IV}
1.44	1.75	1.76	61.7	1.02
2.71	3.00	3.33	67.8	1.12
5.38	5.66	6.63	71.3	1.17
10.72	11.80	13.20	68.1	1.12

† Based on a plasma clearance of 60.9 ml/min calculated from the intravenous bolus data (Table 10).

that observed after a bolus dose ($t_{.5}$ = 25 to 30 minutes). However, as toxic concentrations of quinidine in the body are approached and maintained, as in the case after the 10.7 mg/kg/hr infusion rate, the apparent elimination half-life increased significantly ($t_{.5}$ = 108 minutes).

A similar observation was recorded at high concentrations in animal B. Quinidine was infused at a rate of 4.4 mg/kg/hr for 110 minutes. When plasma levels of quinidine were allowed to decay from an apparent plateau concentration of 13.2 mcg/ml, the elimination process appeared exponential with a half-life of 91 minutes (Fig. 15). This value for the half-life was significantly longer than that observed following a 3.3 mg/kg bolus ($t_{.5}$ = 29 minutes). The calculated plasma clearance was 30.6 ml/min and is in agreement with that following the intravenous bolus (Cl = 31.8 ml/min). Some possible mechanisms for this decrease in the fractional rate of quinidine elimination are discussed below.

Upon completion of these infusion studies, two experiments reported in the section entitled Influences of Dihydroquinidine on the Pharmacokinetics of Quinidine were performed in this animal. A chronological summary of the studies performed in animals A and B are presented in Appendix III and IV, respectively. After the infusion studies, an additional bolus of 2.7 mg/kg was administered to animal A to investigate whether quinidine kinetics had changed in this animal with time and previous treatments. The results of this experiment are shown in Figure 16. The pharmacokinetic constants of the two-

Figure 15 Quinidine plasma concentration in animal B following the infusion of 4.41 mg/kg/hr for 110 minutes. The observed elimination half-life is 91 minutes when the plasma concentration decayed from a plateau level of 13.2 mcg/ml.

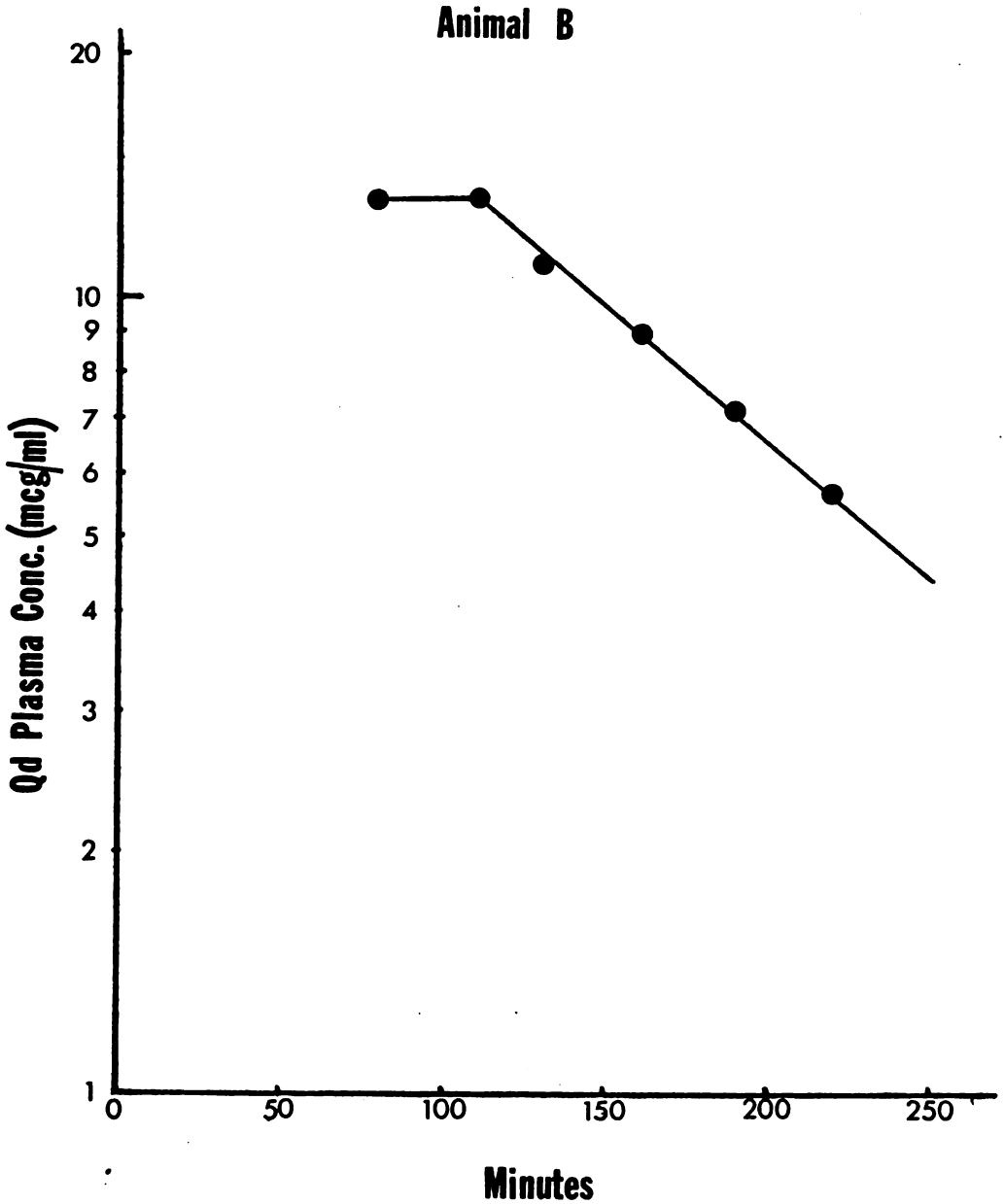
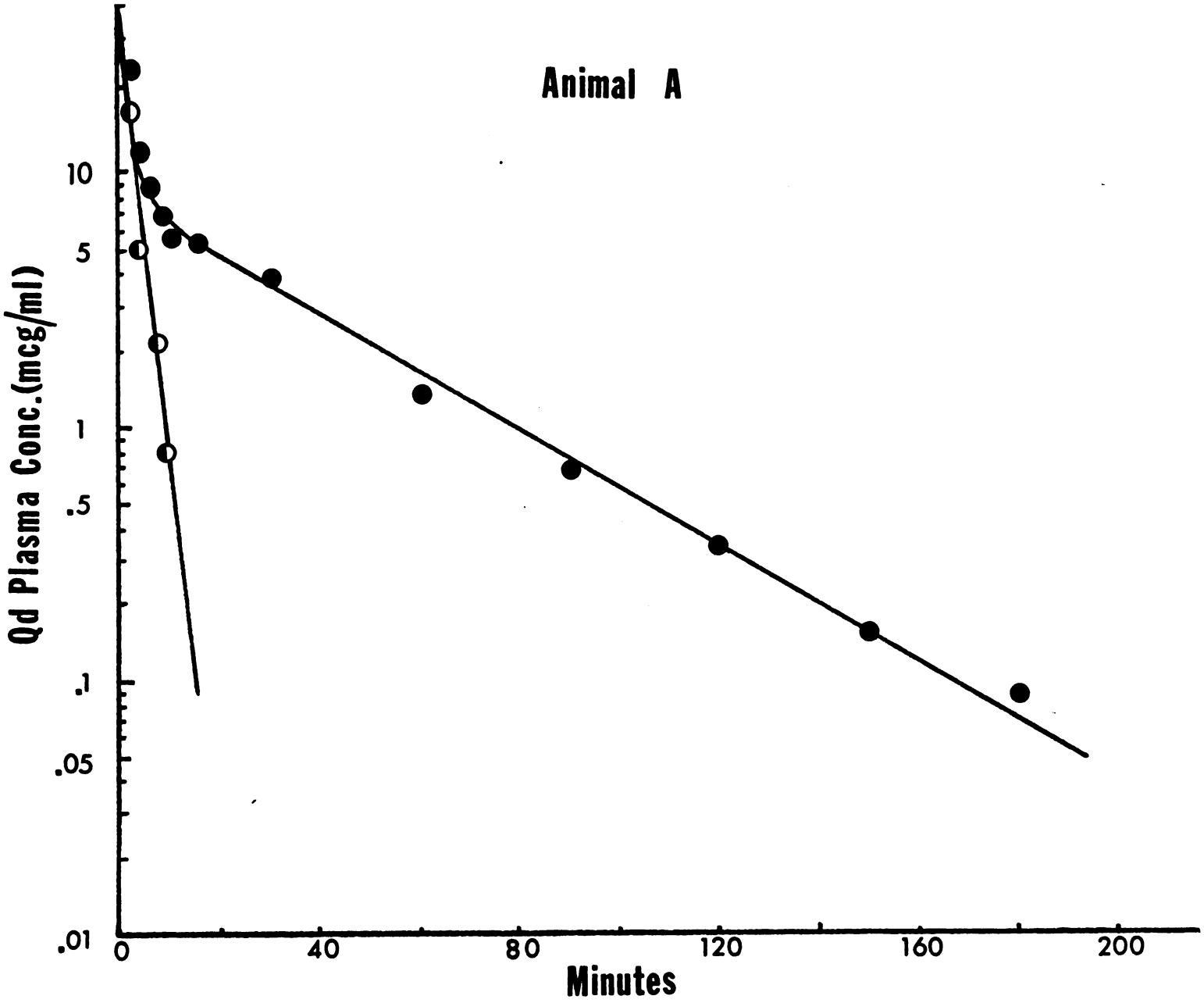

Animal B
20
10
9
8
7
6
5
4
3
2
1
Qd Plasma Conc. (mcg/ml)
0
50
100
150
200
250
Minutes

Figure 16 The computer-simulated biexponential plasma level decay curve and experimental data points following the intravenous administration of 12 mg quinidine (2.7 mg/kg) to animal A. The distribution phase, obtained by feathering, is seen to be very rapid ($T_{.5}$ = 1.6 minutes). The slower elimination phase has a $T_{.5}$ = 27.4 minutes.


Animal A
Qd Plasma Conc.(mcg/ml)
10
5
1
.5
.1
.05
.01
0
40
80
120
160
200
Minutes

compartment open-system model are summarized in Table 20, page 138. A rapid distribution phase was again noted and the apparent elimination half-life of 27.4 minutes was recorded. The computed values for plasma clearance and volume of distribution were 43 ml/min (9.56 ml/min/kg) and 1.7 liters (0.38 L/kg), respectively. When comparisons are made with the data in Table 10, both Cl and Vd appear to have decreased in this animal with respect to the disposition of quinidine. However, since equivalent doses were not utilized in the two studies, these observations cannot be definitively attributed to physiological changes in the animal with time. The change may reflect dose-dependent kinetics.

Long Term Intravenous Infusion Administration

The following series of experiments were performed in an attempt to ascertain whether the duration of quinidine administration affected the kinetics of drug disposition. In certain circumstances, a requisite period of time may have to elapse before changes are observed. For example, there is the time required for the concentration of metabolite(s) to reach levels in the body which might produce significant changes due to stimulation or inhibition of metabolic enzymes or to displacement of the parent compound from their binding sites on plasma or tissue proteins.

Quinidine was administered at rates of 2.7 and 2.2 mg/kg/hr for a duration of 6 hours to animals A and B, respectively. These infusion rates were chosen because previous observations in the animals

demonstrated that these rates would produce plateau plasma concentrations in the therapeutic range. The plateau plasma levels averaged 6.3 and 8.6 mcg/ml, respectively. The apparent steady state concentrations were rapidly attained and maintained for the duration of the infusion period. Upon cessation of the infusion, plasma level decay was observed to be slow and curvilinear as shown in Figures 17 and 18. While the log-linear portion of the post-infusion elimination phase in Animal B had an apparent half-life of 70 minutes, the rate of plasma decay from a plateau concentration of 6.3 mcg/ml in animal A approached that observed in previous experiments ($t_{.5}$ = 35 minutes). The steady state plasma clearance in animals A and B in these experiments were 32.3 ml/min (7.2 ml/min/kg) and 23.7 ml/min (4.3 ml/min/kg), respectively. These clearance values compare favorably with those observed in previous experiments (see Appendix III and IV).

In the preceeding experiments, it has been clearly demonstrated that infusion of quinidine at moderate rates for a duration of 6 hours produces significant changes in the elimination characteristics of the drug. The purpose of the following study was to investigate whether long term quinidine administration at a low infusion rate would result in any changes in kinetics from that observed following the intravenous bolus.

Steady state quinidine plasma levels of 2.7 mcg/ml were achieved and maintained for a period of 6 hours in animal A following infusion of the drug at a rate of 1.44 mg/kg/hr. At this concentration in the plasma, antiarrhythmic effects have been observed in humans (93).

Figure 17 Quinidine plasma levels in animal A following the infusion at a rate of 2.7 mg/kg/hr for a duration of 6 hours. Post-infusion decay from a plateau concentration of 6.3 mcg/ml is observed to be slow and curvilinear. The log-linear portion of the elimination phase approaches a $T_{.5}$ = 35 minutes.

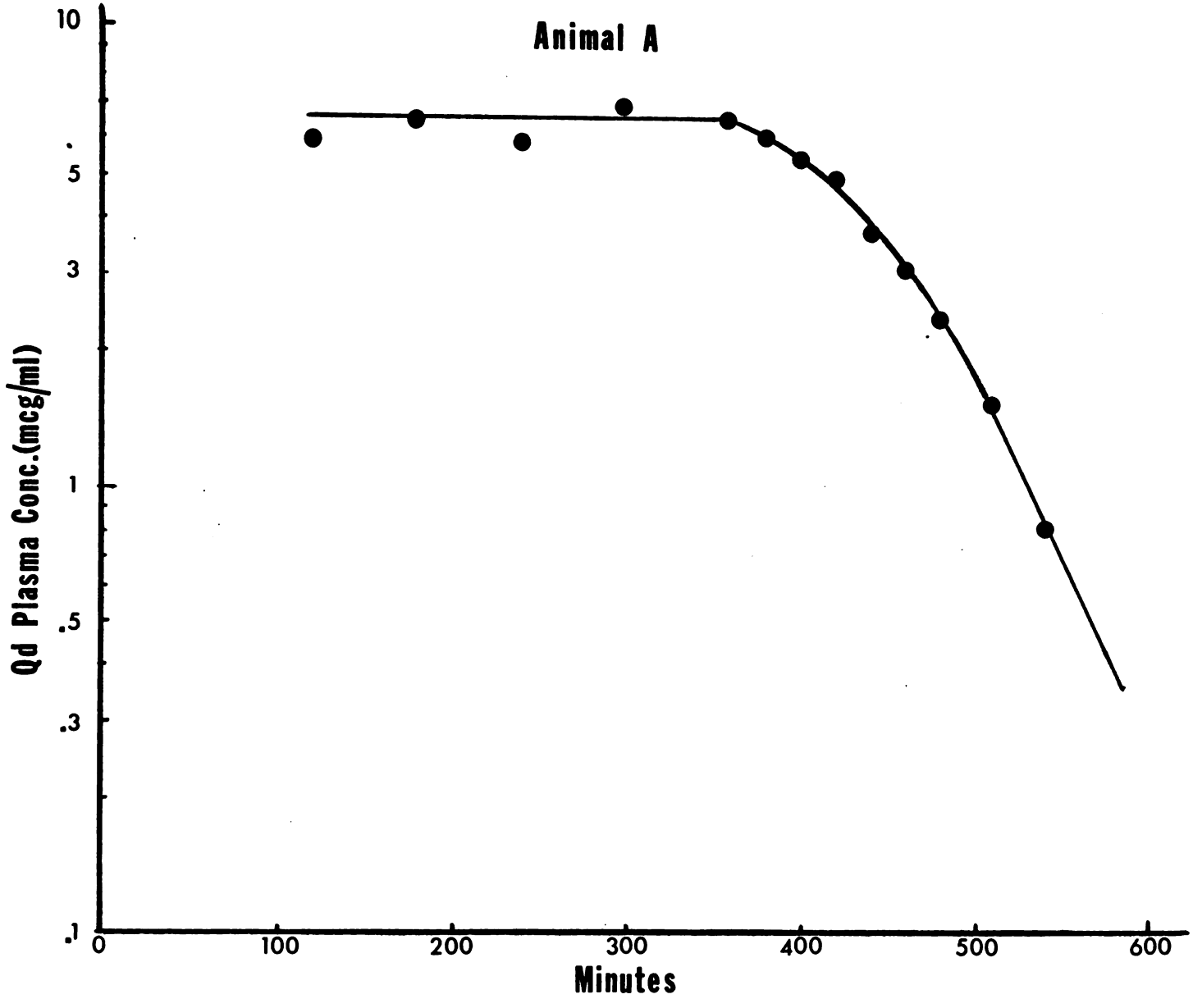
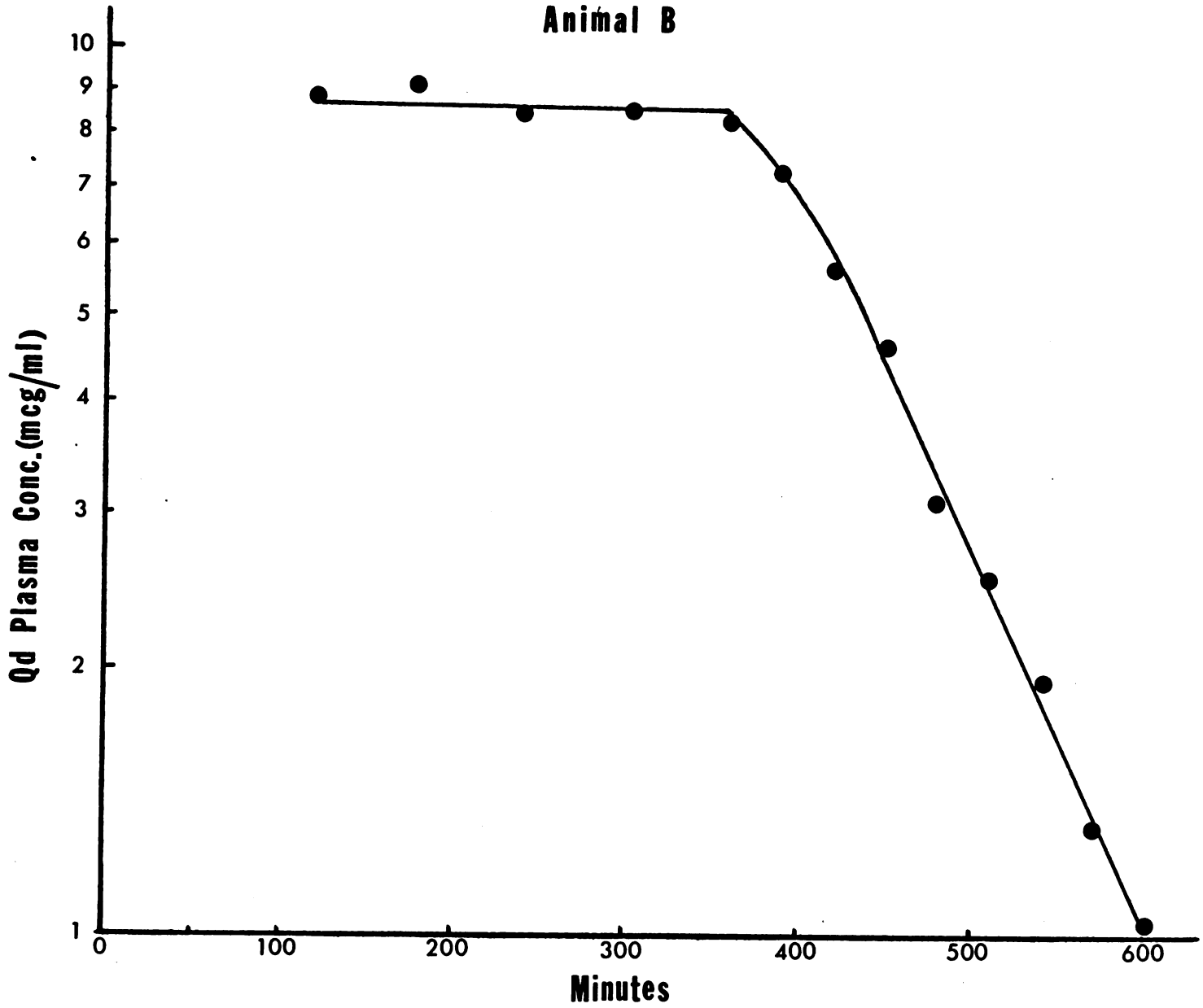

Animal A
Qd Plasma Conc.(mcg/ml)
10
5
3
1
.5
.3
.1
0
100
200
300
400
500
600
Minutes

Figure 18 Quinidine plasma levels in animal B following the infusion at a rate of 2.2 mg/kg/hr for a duration of 6 hours. Post-infusion decay from a plateau concentration of 8.6 mcg/ml is observed to be slow and curvilinear. The log-linear portion of the elimination phase has an apparent $T_{.5}$ = 70 minutes. And at steady state, the unbound fraction of quinidine is 1.6%.


Animal B
Qd Plasma Conc.(mcg/ml)
10
9
8
7
6
5
4
3
2
1
0
100
200
300
400
500
600
Minutes

After cessation of the infusion, plasma elimination of quinidine was exponential (Fig. 19). The half-life of drug elimination was 35 minutes and plasma clearance was 37.1 ml/min (8.24 ml/min/kg).

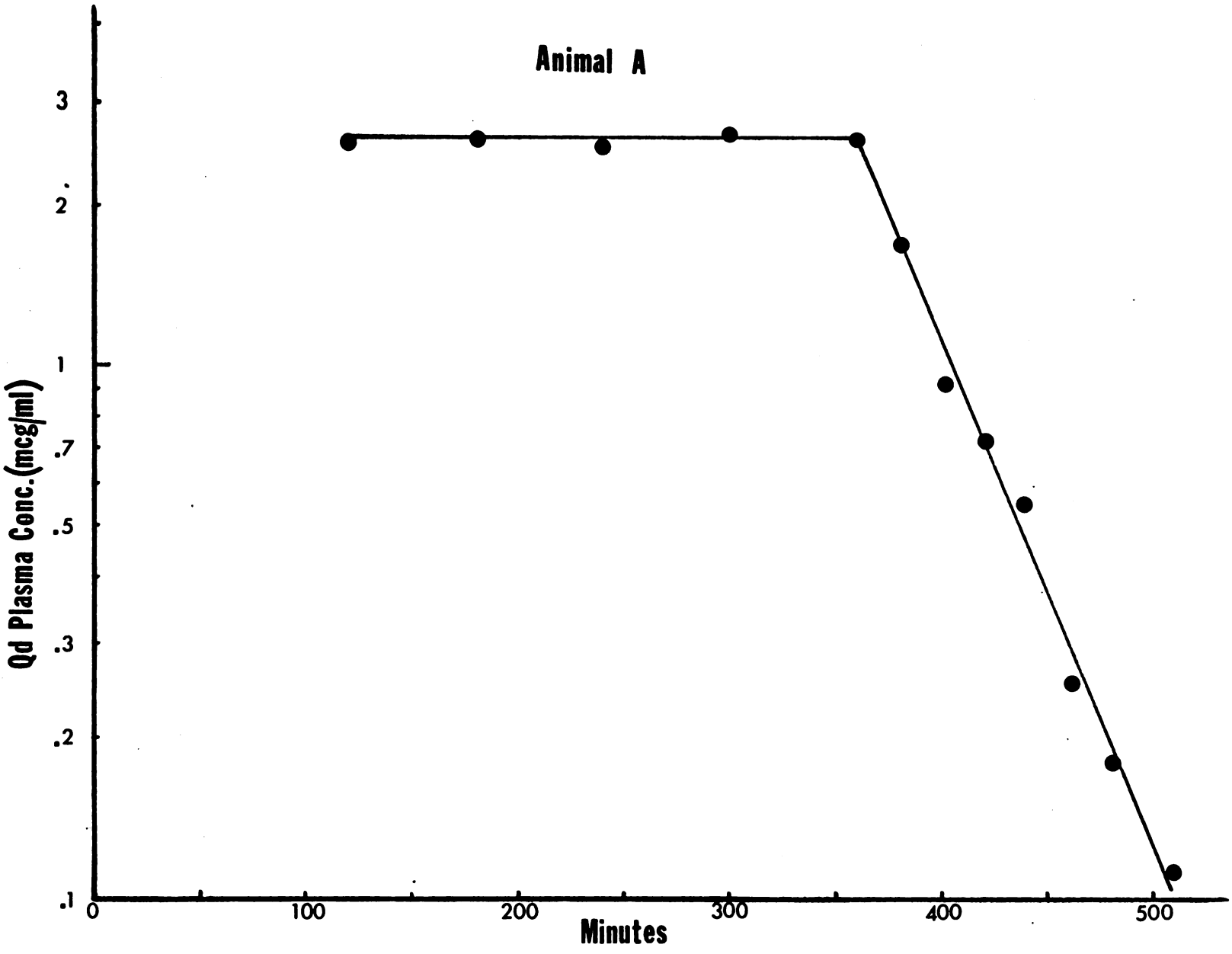
A chronological summary of the experiments performed in each animal is presented in Appendix III and IV. Several important observations were recorded during the course of these studies. In view of the fact that there were considerable differences in the computed Vd and Cl when individual quinidine bolus doses were administered 8 weeks apart, one distinct point of importance is the observation that the animal may slowly undergo physiological changes with time (see p. 116). However, for a period of approximately 4 weeks following the administration of an intravenous bolus, the animal appears to maintain physiological stability.

The fact that steady state plasma concentrations of quinidine are maintained throughout the entire period of drug infusion, it is apparent the plasma quinidine clearance does not change with concentration, length of infusion, or during the entire course of a single experiment.

Furthermore, it is evident that the half-life of quinidine elimination increases with plasma concentration and duration of drug administration.

Clearance is a measure of the ability of eliminating organs of the body to remove a drug from the plasma. The parameter relates the rate of drug elimination to the concentration of drug in the plasma according to:

Figure 19 Quinidine plasma levels in animal A following the infusion at a rate of 1.44 mg/kg/hr for a duration of 6 hours. Post-infusion decay from a plateau concentration of 2.7 mcg/ml was exponential with a $T_{.5}$ = 35 minutes.


Animal A
Qd Plasma Conc.(mcg/ml)
3
2
1
.7
.5
.3
.2
.1
0
100
200
300
400
500
Minutes

$$dA/dt = Cl\ Cp \qquad \text{(Eq 15)}$$

where dA/dt is the amount of drug eliminated per unit time and Cp is the corresponding concentration of drug in the plasma.

The rate at which the drug is eliminated (dA/dt) varies with the amount of drug and the extent of its distribution in the body. Vd describes how extensively the drug is distributed in the body. For a given clearance value, as Vd increases, β will decrease (hence $t_{.5}$ increases). Thus, it is evident that the rate constant of elimination (β) and therefore the half-life of drug elimination are dependent upon the plasma clearance of the drug and the manner in which the drug distributes in the body.

The present studies have demonstrated that the elimination half-life of quinidine changes with concentration and time, increasing as toxic concentrations in the plasma are approached and maintained, without changes in plasma quinidine clearance. This observation is consistent with an increase in the apparent volume of distribution. This is an unusual example of dose-dependent kinetics. Further studies were initiated in an attempt to elucidate the mechanism(s) of this phenomenon.

III. Possible Explanations for the Increase in Volume of Distribution

Diminished Plasma Binding

The interaction of a drug with tissue components influences the fate of the drug in the body. Some drugs form reversible complexes

with circulating and tissue proteins which effectively reduce the total amount of unbound or free drug in the body. The binding of a drug to plasma or tissue components, or both, influences the distribution of the drug, the rate at which it passes through membranes, and the drug's elimination rate (58,136,137).

The presence of plasma proteins in the vascular system act to retard the movement of drug out into tissues. Interactions of a drug with plasma proteins will therefore alter the drug's distribution pattern in the body since the protein-drug complex is unable to cross cell membranes and only free drug is capable of diffusing into tissues. As protein binding effectively decreases the concentration of free drug in the plasma, the gradient responsible for diffusion and passage of drug into tissues decreases and drug transfer diminishes.

If the concentration of drug in the plasma is high such that all available sites on the macromolecules are occupied, the net result would be an increase in the fraction of unbound drug. This increase in the diffusible form of the drug would allow more drug to distribute out of the vascular system into tissues. In this case, there will be a resultant increase in the apparent volume of distribution of the drug.

Protein binding studies in animals and man indicate that quinidine in plasma is bound to a large extent. In animals, the drug has been reported to be concentrated in highly perfused tissues of the body. An increase in Vd can be due to diminished plasma protein binding or an increase in binding in the tissues. In view of expense and

the fact that it would be most difficult to study changes in in vivo tissue distribution with dose and time in the monkey without sacrificing the animal, studies were initiated in an attempt to ascertain whether alterations in plasma binding could be responsible for the large apparent increase in the volume of distribution.

1. Saturation of Plasma Binding Sites

In the first part, in vitro binding of quinidine to monkey plasma was studied with the Dianorm^R equilibrium dialysis system as previously described (see Method of Protein Binding Determination, p.49) to ascertain the degree of binding with concentration. In addition to animals A and B, plasma samples were obtained from various other M. mulatta subjects and were used in these studies. A total of 4 binding experiments were performed in vitro and the data are presented in Table 13. The percent of unbound quinidine as a function of concentration is shown in Figure 20. In the concentration range of 2 to 10 mcg/ml the binding of the drug is relatively constant. Only 3 to 4% of quinidine is in its free or diffusible form.

When the nature and concentration of the macromolecule responsible for binding is not known, Rosenthal (138) has advocated the use of a Scatchard-type plot to allow calculation of binding constants according to the relationship:

$$\frac{D_b}{D_f} = nkP_t - kD_b \qquad \text{(Eq 16)}$$

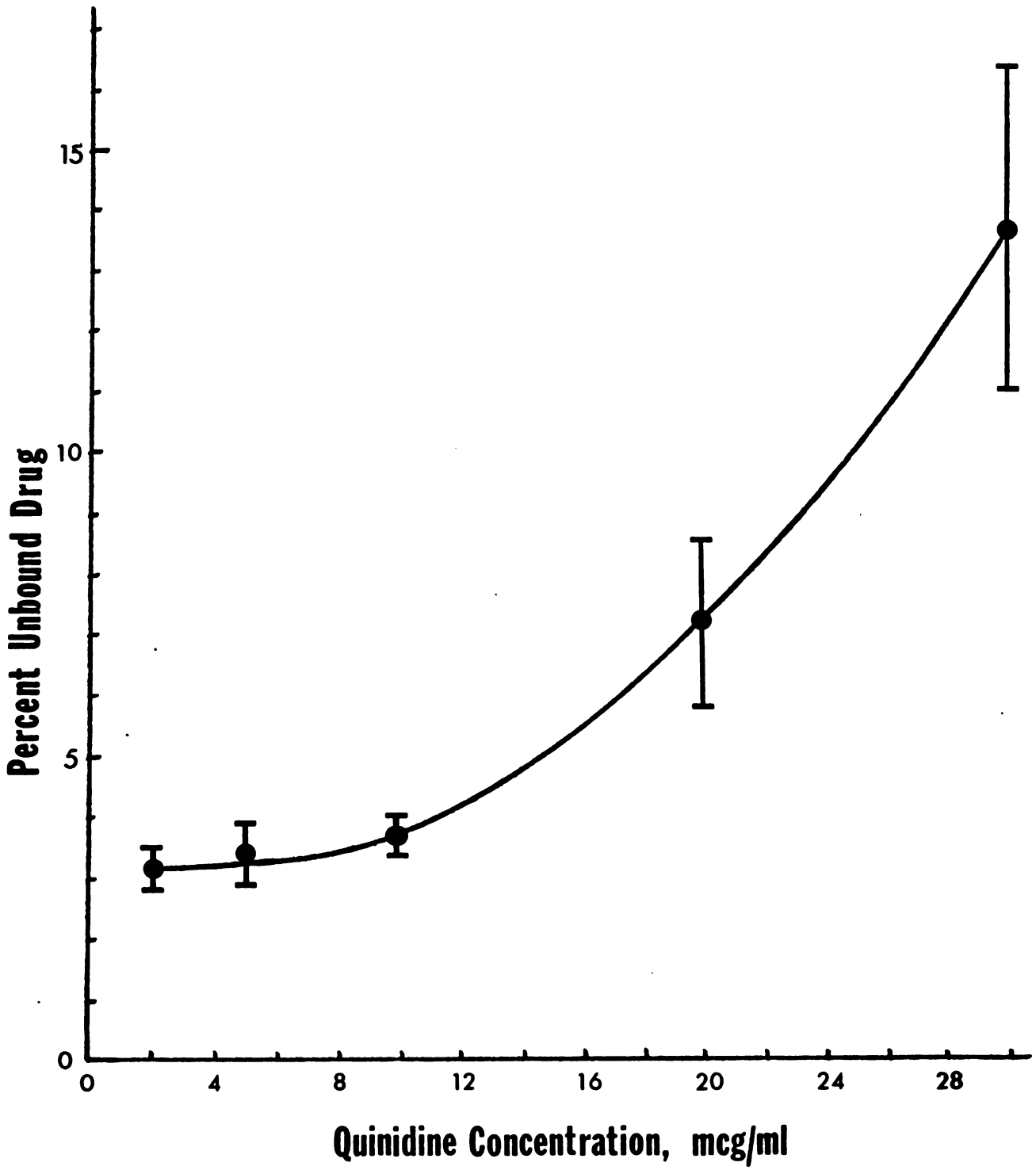
Table 13

IN VITRO BINDING OF QUINIDINE TO PLASMA PROTEINS AT VARYING CONCENTRATIONS

Quinidine Concentration (mcg/ml)	Percent Unbound Quinidine*
2.0	3.14 (0.3)
5.0	3.40 (0.5)
10.0	3.76 (0.3)
20.0	7.14 (1.4)
30.0	13.61 (2.7)

* numbers in parentheses refer to (± S.D.) for 4 determinations at each concentration.

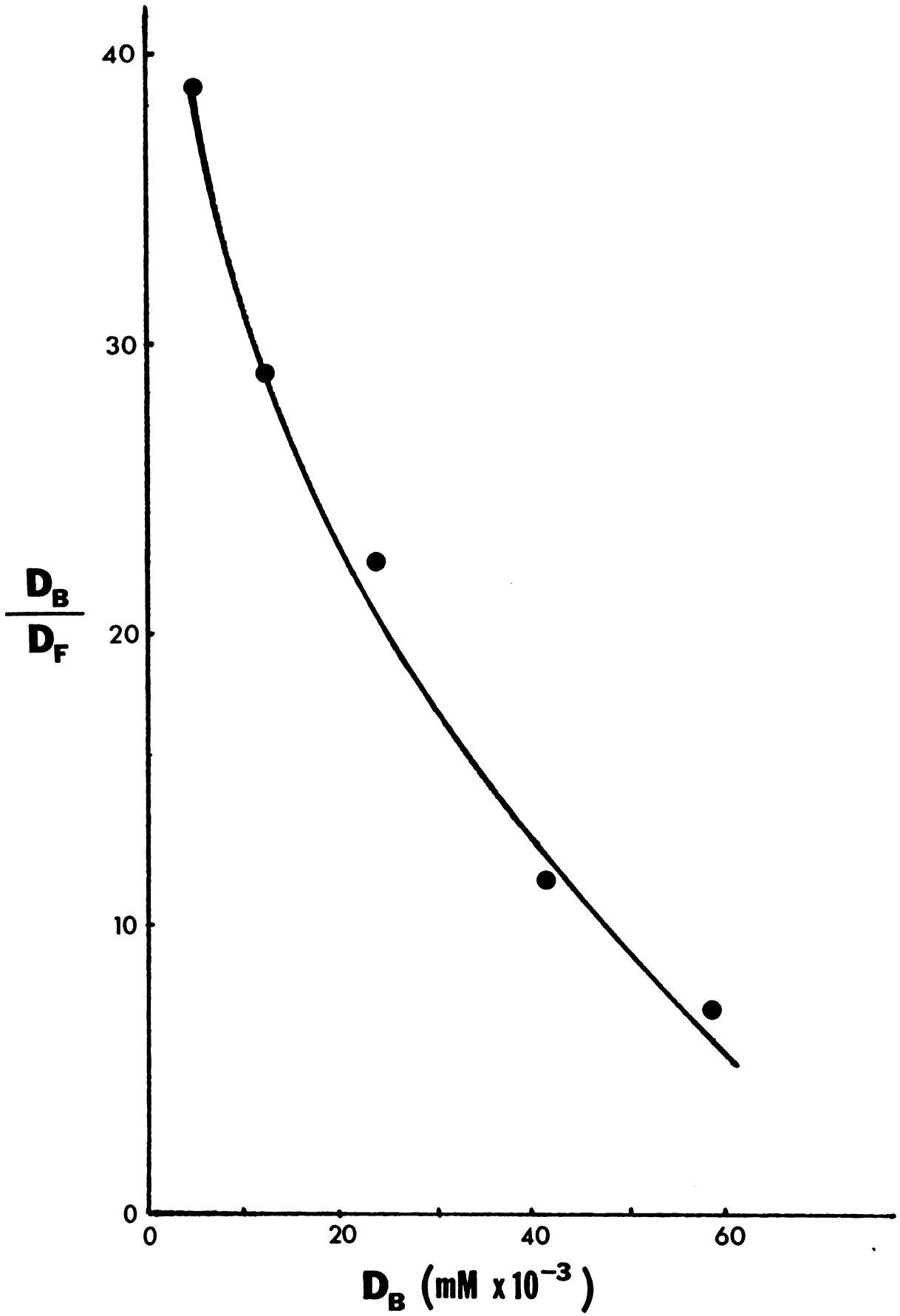
Figure 20 A plot of the percent unbound quinidine versus total plasma quinidine concentration. The mean values and bars representing (± S.D.) for n = 4 determinations is presented. Note the rapid rise in the percent unbound quinidine as the concentration in the plasma increases beyond 12 mcg/ml.


Percent Unbound Drug
0
5
10
15
0
4
8
12
16
20
24
28
Quinidine Concentration, mcg/ml

where D_b and D_f are the molar concentration of bound and unbound drug in the plasma, respectively, P_t is the total molar concentration of macromolecules, k is the affinity constant in M^{-1}, and n is the number of binding sites. A plot of D_b/D_f as a function of D_b permits an estimation of the parameters nP_t and k from the intercept and slope, respectively. The binding characteristics of quinidine in the concentration range of 5 to 30 mcg/ml is presented in Figure 21. This plot shows a trend towards convex-decreasing curvature which is indicative of more than one type of macromolecule or more than one class of binding sites on a single molecule being involved in the binding of the drug.

Because of the curved nature of the graph, realistic estimates of the above binding parameters could not be properly assessed from the data. Nonetheless, these <u>in vitro</u> data do demonstrate that the primary binding site in monkey plasma has a low binding capacity for quinidine at low concentrations. As shown in Figure 20, the percent unbound quinidine increases from 3.1 to 13.6% over the concentration range of 2 to 30 mcg/ml (0.62 to 9.25 x 10^{-2} mM). This observation suggests that the binding constituent in the plasma must be in low concentration and is therefore unlikely to be albumin. Albumin represents approximately 50% (139) of the 5.8 mg/100 ml of total plasma protein fraction in rhesus monkeys (140). The total plasma protein concentration in a control monkey in this study was 8.5 mg/100 ml. Albumin represented 35% (3 mg/100 ml) of this total; the remaining fraction being globulins. In view of the minor differences in the

Figure 21 A plot of the ratio of bound/unbound quinidine concentration (D_b/D_f) versus bound quinidine concentration (D_b) in plasma. Note the convex-decreasing curvature of the graph.


40
30
20
10
0
$\frac{D_B}{D_F}$
0
20
40
60
D_B (mM x 10^{-3})

average molecular weight of albumin (70,000) among mammalian species (141), an albumin concentration of 0.4 mM would be anticipated in normal rhesus monkeys. Tucker et al. (142) observed a similar absence of significant binding of bupivacaine, lidocaine, and mepivacaine, all weak base local anesthetics, to albumin.

These changes in quinidine binding to plasma constituents cannot explain the large (2 to 3 fold) increase in Vd observed in vivo when the drug concentration is maintained at approximately 8 to 13 mcg/ml for 2 to 3 hours or longer. From Figure 20, it can be readily seen that increasing the quinidine plasma concentration from 2 to 13 mcg/ml only increases the unbound drug fraction from 3.1 to 4%. This corresponds to a maximum possible increase in the volume of distribution for quinidine of only 20 to 25% (and this assumes that virtually all drug in the body resides outside of the plasma compartment).

2. Displacement from Plasma Binding Sites

In vivo, two events in plasma could account for the large observed increase in quinidine volume of distribution at high plasma concentrations and following the prolonged administration of moderate doses. The first, and most like explanation, could be the liberation of a significant concentration of an endogenous displacer substance or the accumulation of a significant concentration of quinidine metabolites which are capable of competing for the plasma quinidine binding sites. The second, and less likely explanation, is a decrease in the concentration of the plasma binding constituents. This would also serve as

a mechanism by which the volume of distribution of quinidine increases.

To test these hypotheses, the binding of quinidine to plasma proteins was studied in vivo under conditions of drug administration which had produced changes in the post-infusion elimination profile of the drug. Quinidine was infused into animal A at a rate of 10.7 mg/kg/hr. A control plasma sample was taken immediately before and samples were collected during and after the infusion. An average plateau concentration of 10.4 mcg/ml was attained in the plasma within 135 minutes and was maintained for a total of 178 minutes (Fig. 22).

As previously noted in this animal (p. 86), in this study the post-infusion plasma level decay of the drug was significantly slower ($t_{.5}$ = 252 minutes) than that observed following the administration of a small intravenous bolus (see Appendix III). Using the Dianorm^{R} apparatus, the percent of quinidine unbound at the plateau concentration averaged 14% as shown in Table 14. While these values for the percent unbound quinidine differed significantly from that previously recorded at this same concentration (see Fig. 20), they did not vary significantly from the 15% value observed when the quinidine plateau plasma concentration (approximate) was added to the control plasma collected immediately before the infusion.

A similar observation was recorded after prolonged administration of quinidine in animal B. Following infusion at a rate of 2.2 mg/kg/hr for a duration of 6 hours (Fig. 18), in vivo plasma samples obtained during the infusion showed that a steady state concentration of 8.6 mcg/ml was readily attained and maintained in the plasma. The unbound

Figure 22 Quinidine plasma levels in animal A following the infusion at a rate of 10.7 mg/kg/hr for a duration of 178 minutes. Post-infusion decay from a plateau concentration of 10.4 mcg/ml was exponential with a $T_{.5}$ = 252 minutes. The unbound fraction of quinidine at steady state is 14%.

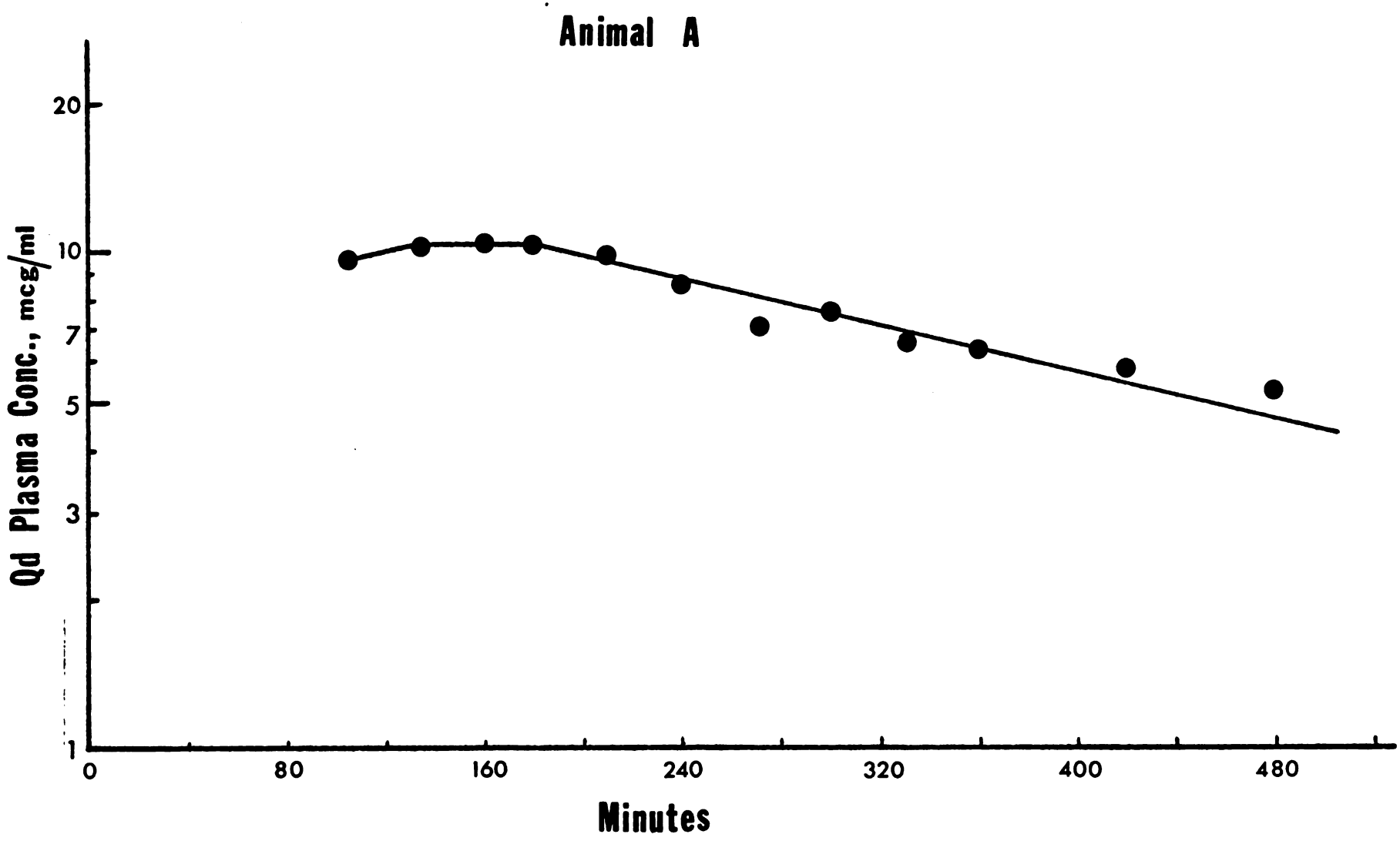

Animal A
Qd Plasma Conc., mcg/ml
20
10
7
5
3
1
0
80
160
240
320
400
480
Minutes

Table 14

IN VIVO BINDING OF QUINIDINE TO PLASMA PROTEINS AT STEADY STATE IN ANIMAL A

Time (min)	Plasma Concentration (mcg/ml)	Quinidine Fraction Unbound
Control	11.4	.151
	11.4	.148
	11.4	.152
		Mean: .150
112	9.71	.114†
137	10.27	.133
160	10.54	.152
178	10.38	.132
		Mean: .139

† This value was not used in the computation of the mean.

quinidine fraction at this level averaged 1.9% (Table 15) and was demonstrated to be virtually identical to the value of 1.6% observed when a quinidine concentration of 7.38 mcg/ml was produced in the control plasma sample.

From these two studies in different animals, it is concluded that alteration in quinidine-plasma binding due to either displacement or dilution of plasma binding sites is not the mechanism responsible for the observed large increase in the volume of distribution.

3. Increase in Plasma Volume

While dilution of plasma binding sites cannot be the mechanism for the observed changes in the volume of distribution of quinidine, it is worthwhile noting that quinidine causes peripheral vasodilatation (143,144). Rose and Fries (145) observed that hexamethonium, a ganglionic blocking agent which produces peripheral vasodilatation, increased the blood volume an average of 15% in dogs. Other authors (146,147,148) have reported similar changes in the blood volume after the introduction of various agents which cause dilatation of the peripheral vessels. Presumably the mechanism involves an inward movement of extracellular water into plasma with a commensurate dilution of high molecular weight plasma constituents.

In order to examine whether quinidine produces an increase in the plasma volume, the plasma protein concentration was measured at various times following the administration of the drug at a rate of 10.7 mg/kg/hr in animal A (Fig. 22). The results indicate a gradual fall

Table 15

IN VIVO BINDING OF QUINIDINE TO PLASMA PROTEINS

AT STEADY STATE IN ANIMAL B

Time (min)	Plasma Concentration (mcg/ml)	Quinidine Fraction Unbound
Control	7.38	.016
	7.38	.016
		Mean: .016
180	9.14	.021
240	8.53	.018
300	8.53	.017
360	8.23	.019
		Mean: .019

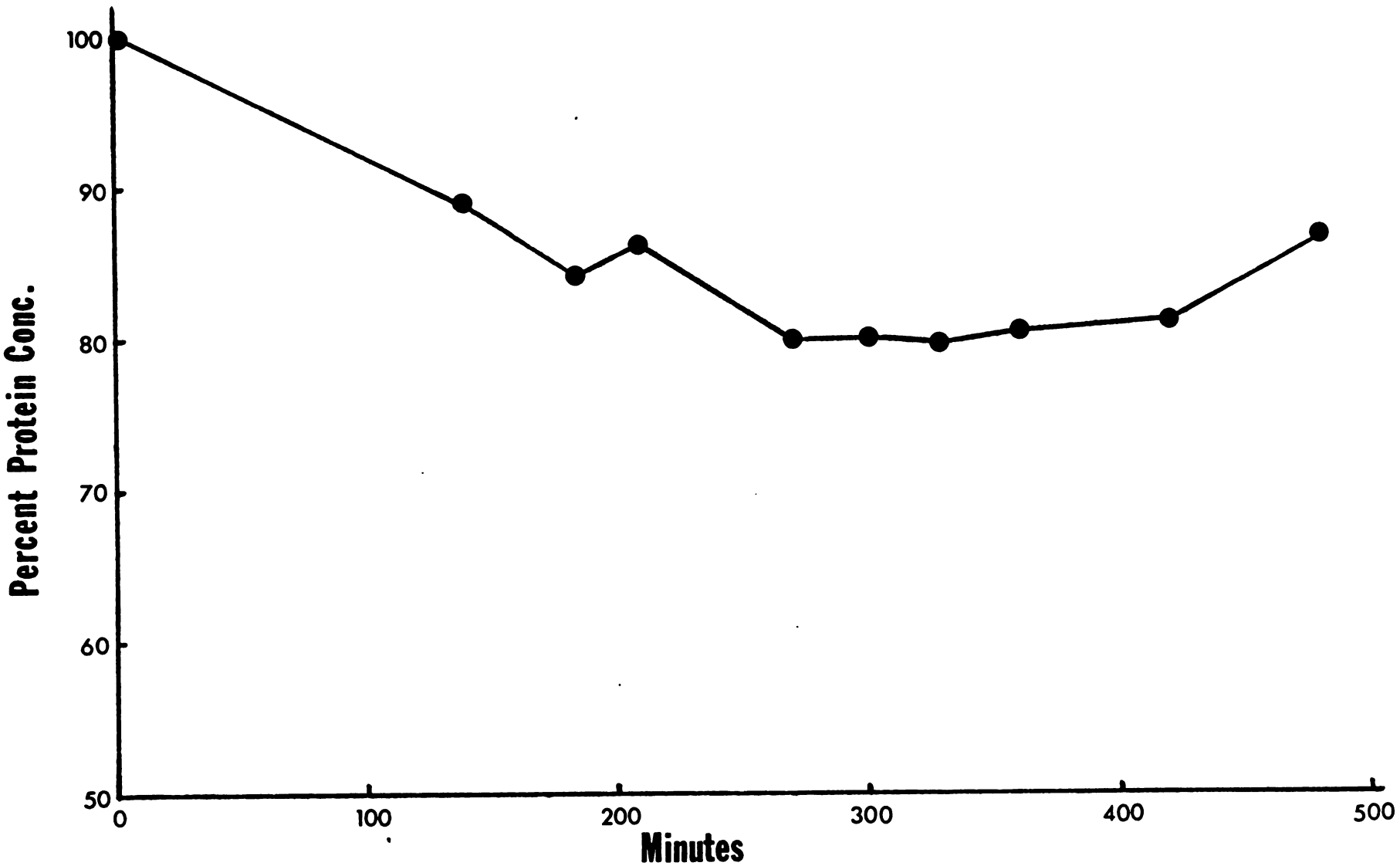
in plasma protein concentration throughout quinidine infusion (Fig. 23). However, even at maximum fall in protein concentration, it only corresponded to a 20% change in plasma volume. Although this observation clearly indicates that quinidine is capable of diminishing the concentration of plasma proteins and presumably quinidine binding sites, this change is too small to explain the observed volume of distribution changes for quinidine.

From the *in vivo* quinidine binding and protein concentration measurements obtained during a high rate of drug infusion, one must conclude that alterations in tissue distribution of quinidine is the apparent mechanism underlying the changes in Vd in the body. The direct measurement of *in vivo* tissue distribution changes is very difficult to study. Out of necessity, one must therefore rely on indirect observations.

Modifications in the Tissue Distribution of Quinidine

Based on known pharmacological properties of quinidine, it is possible that induction of modifications in the internal environment of tissue cells would favor enhanced intracellular movement of the drug from extracellular fluids. And as a consequence of the physicochemical properties of quinidine, a change in cellular pH would alter the distribution of quinidine in the body. It is well known that sodium and potassium are the principal extra and intracellular cations in the body, respectively and that the pH of intracellular fluids is slightly more acidic (149,150).

Figure 23 Plot of the percent plasma protein concentration versus time following the administration of quinidine in animal A at a rate of 10.7 mg/kg/hr for a duration of 178 minutes. Note that the average fall in protein concentration is approximately 20%.


Percent Protein Conc.
100
90
80
70
60
50
0
100
200
300
400
500
Minutes

Cardiac muscle excitability, conduction, and rhythm are markedly affected by changes in the potassium concentration in the extracellular fluid. Both an increase and a decrease of extracellular potassium diminishes excitability and conduction rate (151). Clinically, potassium deficiency is seen in cases of liver cirrhosis and congestive heart failure and cardiac arrhythmias are often produced (151).

If the extracellular potassium is depleted there is usually a concommitant decrease in intracellular potassium concentration (152, 153). The available evidence suggests that a reduction in intracellular potassium results in an intracellular increase of hydrogen ion concentration (153,154,155), thus lowering the pH of the tissues. Under these conditions the extracellular pH is unchanged or actually increased depending upon the progress of the disease state. In view of the fact that quinidine is a weak base (pKa_1 = 8.57), these cellular pH changes create a gradient which favors the enhanced entry of quinidine into tissue cells.

Quinidine is known to produce arrhythmias, a condition for its purported use, and other cardiovascular changes when toxic concentrations are approached in the plasma. The observations that quinidine may abolish various cardiac arrhythmias by interfering with the mechanisms of sodium and potassium exchange of the cardiac cell (156) and that intracellular potassium concentration is decreased in the presence of the drug (157) would lend support to an hypothesis that changes in intracellular or tissue pH towards as acidotic state might explain the apparent increase in the volume of distribution discussed

above.

To test this hypothesis, a state of intracellular acidosis was induced in the following manner. Animal A was maintained on a low potassium diet for 72 hours prior to the induction of potassium depletion with a thiazide diuretic (sodium chlorothiazide, Diuril^{R}). Before initiation of the study, chlorothiazide was administered at a rate of 1 mg/kg/hr for 24 hours followed by an infusion of the diuretic at a rate of 2 mg/kg/hr for an additional 24 hours immediately before and during the course of quinidine infusion. The normal range of serum potassium in M. mulatta is 4.5 to 5.8 mEq/L (158). Control level in this animal was 4.6 mEq/L. At the start of the infusion, the serum potassium concentration was 2.7 mEq/L. In man, the normal serum potassium levels (3.5 to 5.0 mEq/L) are similar to that observed in the rhesus monkey (159). The presence or absence of clinical symptoms of hypokalemia is closely related to serum potassium concentration. In most patients, serious signs and symptoms of hypokalemia, including metabolic alkalosis, are observed when serum potassium levels fall below 2.5 mEq/L (160). In view of the similarities between the rhesus monkey and man with respect to serum potassium levels, it was assumed that a tissue acidotic state was induced in the animal when the serum potassium level reached 2.7 mEq/L.

Quinidine was infused into this animal at a rate of 1.44 mg/kg/hr for 201 minutes. Following this input rate, prior observations of the drug elimination half-life were consistent with that obtained following an intravenous bolus in this animal (see Appendix III). If an

alteration in tissue pH is the mechanism responsible for the changes in the volume of distribution and elimination half-life observed at elevated quinidine concentrations, then similar observations would be expected under the conditions of this study. The results of this experiment are shown in Figure 24. Plasma level decay from a plateau concentration of 3.4 mcg/ml was exponential with an apparent elimination half-life of 47 minutes.

This value for the elimination half-life was somewhat longer than that previously observed and is suggestive of possible changes in the physiological state of the animal with time. In view of this possibility, an additional bolus experiment was performed. A 4 mg/kg intravenous bolus was administered to animal A. The biexponential plasma level decay curve with time and pharmacokinetic constants are presented in Figure 25 and Table 16, respectively. The distribution phase was very rapid ($t_{.5}$ = 1.4 minutes) as noted previously. The apparent elimination half-life was 46.9 minutes. Plasma clearance and Vd were 38.4 ml/min (7.68 ml/min/kg) and 2.59 liters (0.52 L/kg), respectively.

The computed plasma clearance and volume of distribution at steady state were 31.4 ml/min (6.9 ml/min/kg) and 2.13 liters (0.47 L/kg), respectively. The observation that the volume of distribution did not change when quinidine was infused in a potassium deficient state supports the conclusion that a change in tissue pH (acidosis) is not the mechanism responsible for the large increase in the volume of distribution of quinidine following prolonged and high levels of drug administration in the rhesus monkey.

Figure 24 Quinidine plasma level curve obtained following infusion at a rate of 1.44 mg/kg/hr in animal A for a duration of 201 minutes in a potassium depleted state. Potassium depletion was accomplished by (1) low potassium diet for 72 hours prior to the induction of diuresis, (2) infusion of sodium chlorothiazide at a rate of 1 mg/kg/hr for 24 hours followed by (3) an infusion of the diuretic at a rate of 2 mg/kg/hr for an additional 24 hours immediately before and during the course of quinidine administration. At the start of the infusion, the serum potassium level was 2.7 mEq/L. Post-infusion decay from a plateau level of 3.4 mcg/ml is observed to be virtually linear with an elimination half-life of 47 minutes.

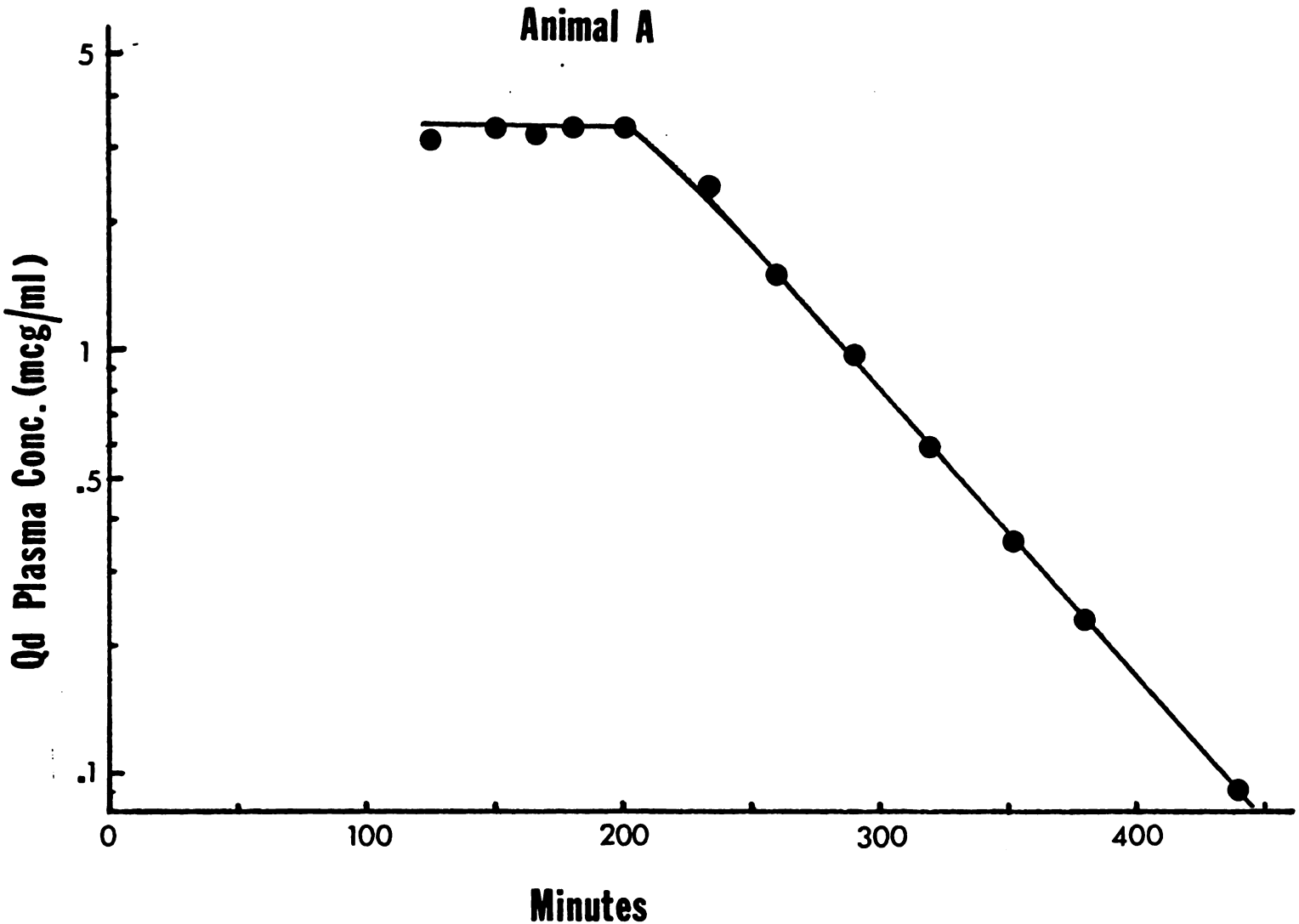

Animal A
Qd Plasma Conc. (mcg/ml)
5
1
.5
.1
0
100
200
300
400
Minutes

Figure 25 The computer-simulated biexponential plasma level decay curve and experimental data points following the intravenous administration of 18 mg quinidine (4.0 mg/kg) to animal A. The distribution phase, obtained by feathering, is seen to be very rapid ($T_{.5}$ = 1.4 minutes). The slower elimination phase has a $T_{.5}$ = 46.9 minutes.

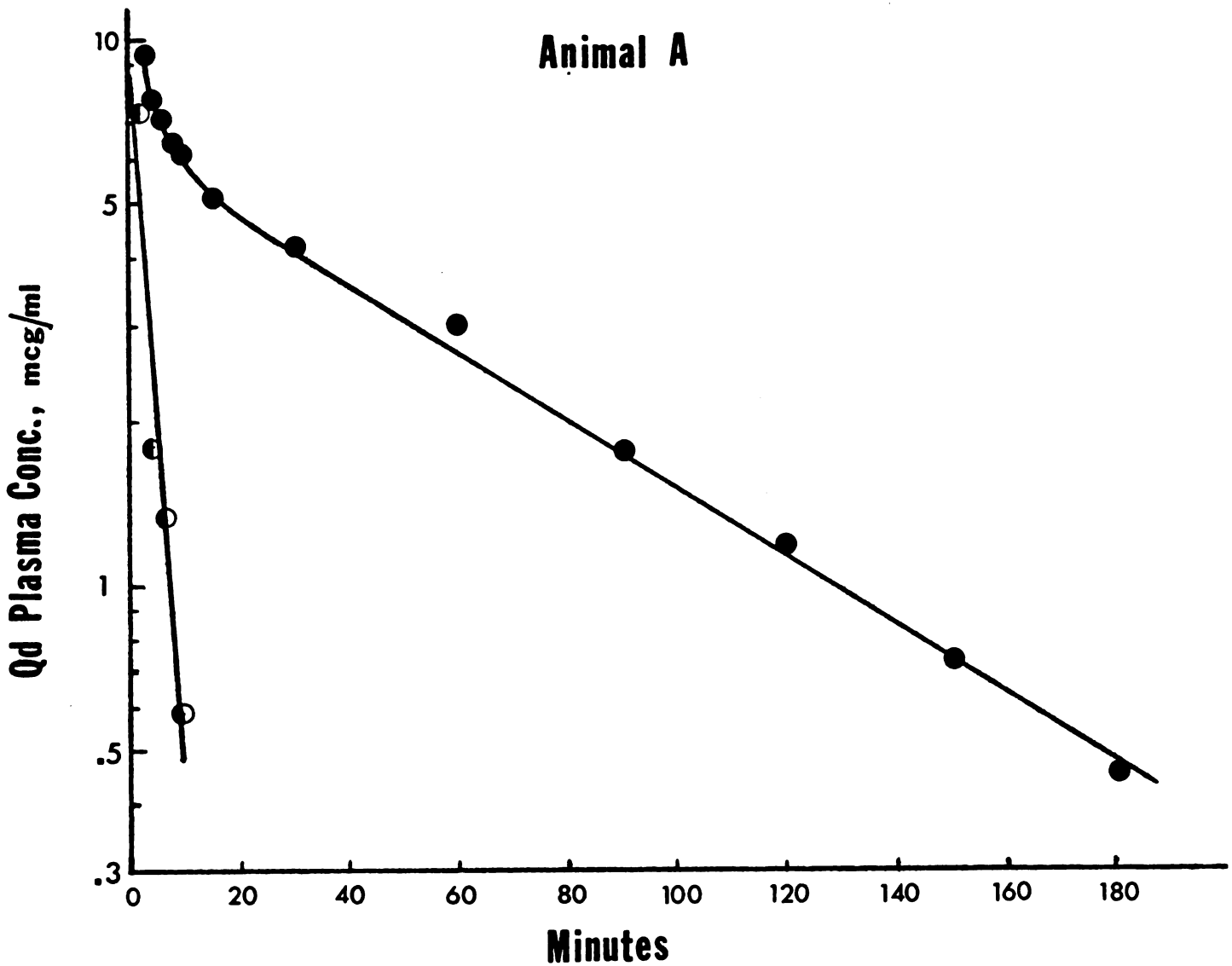

Animal A
Qd Plasma Conc., mcg/ml
10
5
1
.5
.3
0
20
40
60
80
100
120
140
160
180
Minutes

Table 16

PHARMACOKINETIC CONSTANTS OF THE TWO-COMPARTMENT OPEN-SYSTEM MODEL FOR QUINIDINE IN ANIMAL A

A (mcg/ml)	B (mcg/ml)	α (min^{-1})	$t_{.5\alpha}$ (min)	β (min^{-1})	$t_{.5\beta}$ (min)	k_{12} (min^{-1})	k_{21} (min^{-1})	Kel (min^{-1})	Vp (L/kg)	Vd_{ss} (L/kg)
8.32	6.94	0.49	1.41	0.015	46.9	0.243	0.231	0.031	0.24	0.48

IV. Quinidine Pharmacokinetics - Discussion

The data presented indicate that after prolonged administration of quinidine at levels approaching drug toxicity and when therapeutic drug concentrations are surpassed in the plasma, the distribution volume of quinidine increases. Under these circumstances, a constant clearance is maintained throughout the course of drug administration and accordingly, the half-life of elimination is significantly increased.

Alterations in the plasma binding and modification of the pH in intracellular or tissue environment were examined as possible explanations for the observed distributional changes of quinidine in the body with time and dose. It was demonstrated that quinidine-protein interactions in the plasma remain virtually unaltered under conditions where the volume of distribution changes. In a study which produced conditions favoring the enhanced entry of the drug into tissues, the computed Vd was not observed to increase. Alternative explanations must be sought.

In view of the large increase in the volume of distribution of quinidine, it would appear that the likely explanation for this observation would be an increase in the extent of quinidine binding in the tissues and/or an increase in the distribution of the drug in the body, for example, into skeletal muscle with time and dose. As briefly eluded to earlier, the rapid and shallow distribution phase observed following the administration of a quinidine bolus was suggestive of distribution of the drug into highly perfused organs of the body,

e.g., liver, kidneys, lungs, etc. and that distribution of quinidine into muscle, bone, and adipose tissue was insignificant in the doses studied. However, in addition to a possible increase in the extent of quinidine binding to various tissue components in the body with time and dose, distribution of the drug into skeletal muscle following prolonged drug administration at moderate and high doses could be an alternative explanation for the large increase in Vd. An increase in the perfusion rate to voluntary muscles due to the peripheral vasodilatation activity of quinidine might explain the observed phenomenon. Quinidine has been reported to possess curare-like activity in skeletal muscle (34,161). Although the author has seen no reports in the literature, at low doses of quinidine, the curare-like action is apparently negligible and is presumably observed only after high doses have been administered. The postulate that there might be a large increase in the distribution of quinidine into skeletal muscle with time and dose could account for the increase in the volume of distribution of quinidine in these studies.

Wagner (127) presented evidence for biscoumacetate, probenecid, and diphenylhydantoin in which similar increases in distribution volumes and elimination half-lives occured with an increase in dose. He has pointed out that if the volume of the peripheral compartment in a two-compartment open-system model increases with increasing dose, then the slope of the log plasma (or blood) level versus time line will decrease. The possibility that the distribution of a drug increases as the dose is raised such that the drug reaches sites following high

doses which are not reached following low doses was discussed. However, the location(s) and mechanism(s) by which these changes occur in the body were not examined.

The observation that quinidine modifies cardiac function and is active on the peripheral vasculature is a possible alternative explanation (which still must be examined) for the changes seen in these studies. These cardiovascular effects could alter blood flow to peripheral tissues and thereby modify the distribution of quinidine. The precise mechanism(s) responsible for the changes in the elimination profile of quinidine observed in these studies remains to be elucidated.

The observation that the fractional elimination rate of quinidine is decreased at elevated plasma levels of the drug is clinically significant and would explain the slow recovery of patients once quinidine toxicity has been induced (162). In two of the present studies, apparent drug toxicity was observed. The signs included intense spells of vomiting, malaise, and flushing followed by loss of coloration of fascial skin. These signs were initially observed at quinidine plasma concentrations corresponding to 9 mcg/ml or greater and continued for several hours despite drug plasma levels which dropped below this concentration.

V. Dihydroquinidine Pharmacokinetics

Single Intravenous Bolus Administration

In view of the similarities in physicochemical properties of quinidine and dihydroquinidine, it would be expected that both compounds might have similar distribution volumes in the body. In addition, in attempting to select an appropriate size bolus, consideration was given to reports that dihydroquinidine was slightly more toxic in animals (17,28). Thus, two-thirds of the quinidine bolus or 2.2 mg/kg of dihydroquinidine was administered to animal B as a single, rapid intravenous bolus. The disposition of the drug in the body can also be described by a two-compartment open-system model as previously presented in Figure 8. The observed data were fitted to the biexponential equation (Eq. 3) using the NONLIN computer program. The biexponential disposition curve and pharmacokinetic constants are presented in Figure 26 and Table 17, respectively. The initial distributive phase is very rapid ($t_{.5}$ = 2 minutes) and is very similar to that observed after a bolus of quinidine. The slower β phase which is associated with the elimination of dihydroquinidine from the central compartment has an apparent half-life of 62 minutes. When Vd was computed according to Equation 7, it was observed to be 1.2 liters (0.22 L/kg). This observation that the volume of distribution is similar for the two compounds indicates that the plasma clearance of dihydroquinidine is considerably less than that shown previously for quinidine (2.46 versus 5.80 ml/min/kg). A comparison of the pharmacokinetic parameters in the same animal for these two agents is presented in Table 17.

Figure 26 The computer-simulated biexponential plasma level decay curve and experimental data points following the intravenous administration of 12 mg dihydroquinidine (2.2 mg/kg) to animal B. The distribution phase, obtained by feathering, is seen to be very rapid ($T_{.5}$ = 2 minutes). The slower elimination phase has a $T_{.5}$ = 62 minutes.

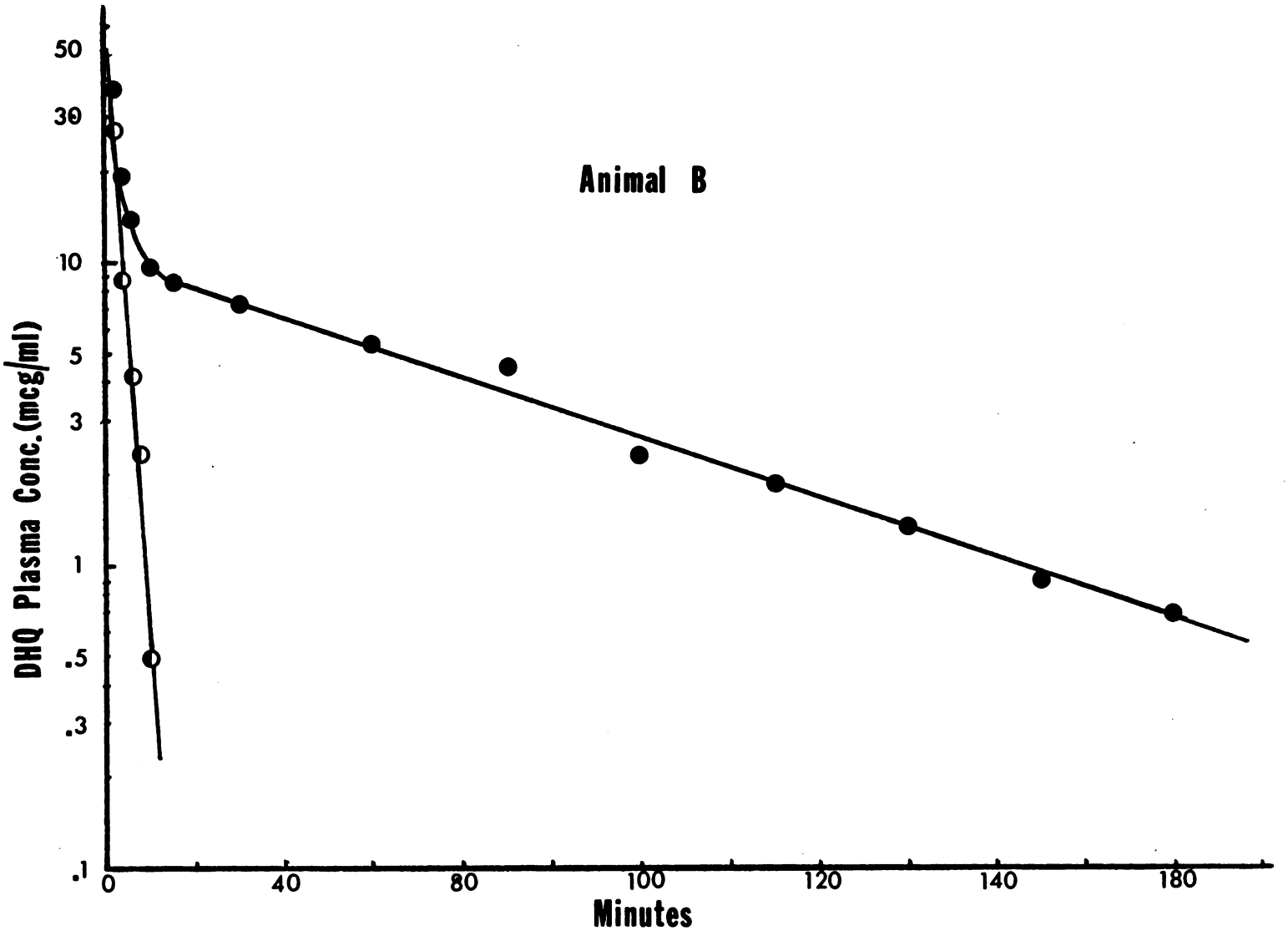

Animal B
DHQ Plasma Conc.(mcg/ml)
50
30
10
5
3
1
.5
.3
.1
0
40
80
100
120
140
180
Minutes

Table 17

COMPARISON OF THE PHARMACOKINETIC CONSTANTS OF THE TWO-COMPARTMENT BODY MODEL FOR QUINIDINE AND DIHYDROQUINIDINE IN ANIMAL B FOLLOWING EQUAL DOSES

Parameter	Quinidine	Dihydroquinidine
A, mcg/ml	37.47	13.65
B, mcg/ml	56.94	10.11
α, min^{-1}	0.46	0.43
$t_{.5\alpha}$, min	1.50	2.00
β, min^{-1}	0.024	0.011
$t_{.5\beta}$, min	28.80	62.00
k_{12}, min^{-1}	0.26	0.30
k_{21}, min^{-1}	0.14	0.07
Kel, min^{-1}	0.085	0.065
Vp, L/kg	0.064	0.033
Vd_{ss}, L/kg	0.183	0.164
Vd, L/kg	0.240	0.220
Cl, ml/min/kg	5.800	2.460

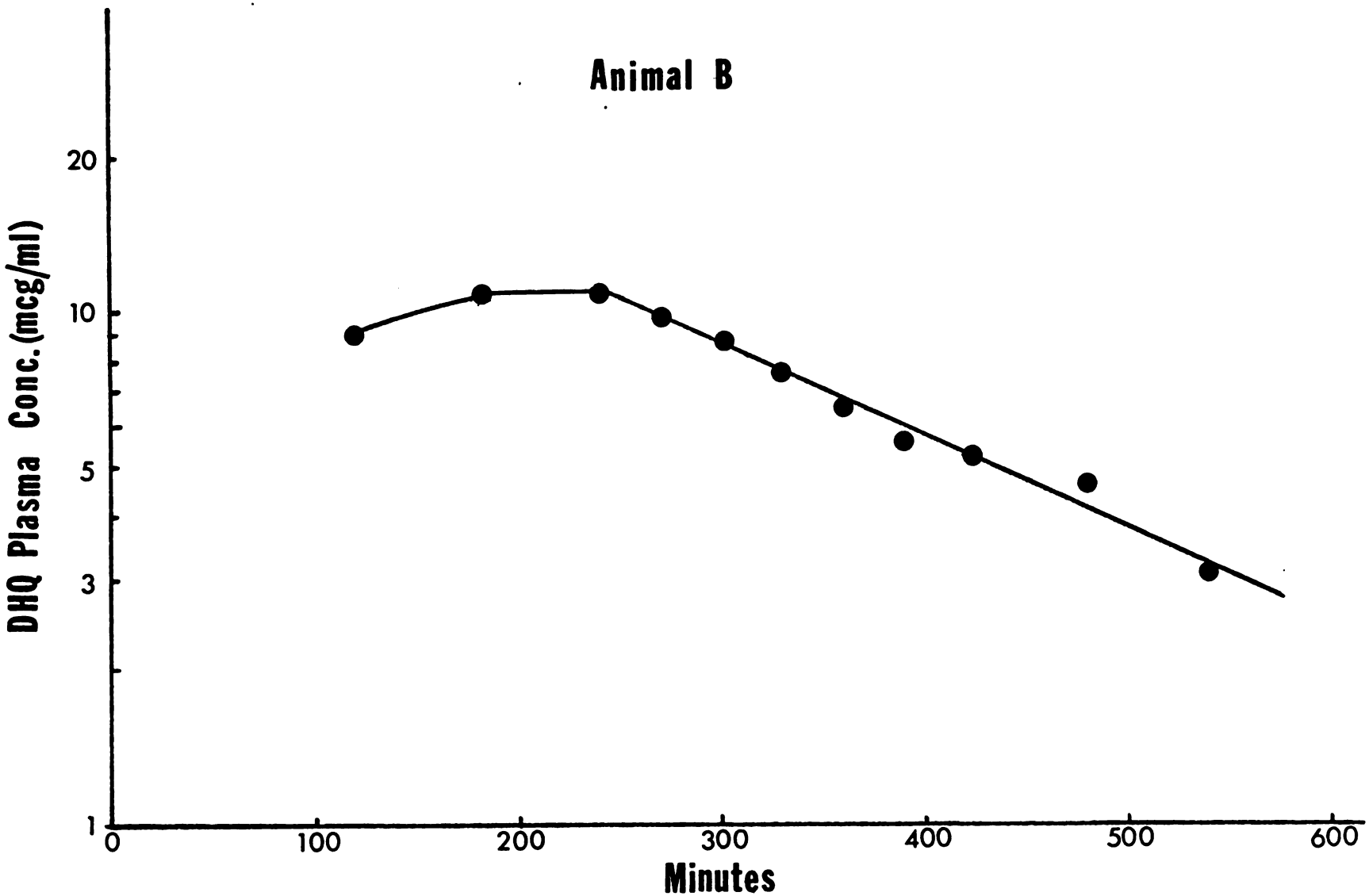
Intravenous Infusion Administration

On the basis of the information obtained from the dihydroquinidine bolus, an infusion at a rate of 1.76 mg/kg/hr in animal B was initiated in this study. The predicted steady state was 12 mcg/ml and Figure 27 shows the plasma level versus time curve of dihydroquinidine following infusion at this rate for a duration of 240 minutes. When the levels of the drug were allowed to decline from a peak concentration of 10.7 mcg/ml, plasma level decay was exponential with an apparent half-life of 160 minutes. The absence of a distributive phase is consistent with a rapid and shallow distribution phase seen after the intravenous bolus. The increase in the half-life with prolonged elevated plasma concentrations is similar in magnitude to that previously observed for quinidine. In this study, the monkey appeared to lose skin coloration and to be suffering from malaise. Vomiting episodes were also observed.

Because of the similarities in drug elimination characteristics between quinidine and dihydroquinidine with respect to dose, it was assumed that at lower dosages there would be no alteration in the elimination kinetics of dihydroquinidine with respect to the intravenous bolus. No further studies were conducted with this drug at lower dose levels.

The total volume of urine voided in a period of 72 hours following administration of the bolus and infusion was collected and analyzed for dihydroquinidine. No attempt was made to control the pH of the urine. Approximately 3% of the dose administered (bolus and infusion)

Figure 27 Dihydroquinidine plasma levels in animal B following the infusion at a rate of 1.76 mg/kg/hr for a duration of 240 minutes. Post-infusion decay from a plateau concentration of 10.7 mcg/ml is exponential with a $T_{.5}$ = 160 minutes.


Animal B
DHQ Plasma Conc.(mcg/ml)
20
10
5
3
1
0
100
200
300
400
500
600
Minutes

is excreted in the urine as the parent drug molecule (Table 18). As seen with quinidine, it is apparent that the primary mode of dihydroquinidine elimination in the rhesus monkey is that of metabolic transformation.

VI. Influence of Dihydroquinidine on the Pharmacokinetics of Quinidine

The presence of dihydroquinidine in commercial quinidine preparations has been firmly established. In 8 quinidine products (both as the sulfate and gluconate salts) selected at random from pharmacy shelves, dihydroquinidine was present in all samples in amounts varying up to 17% (Table 19). Both compounds have been reported in the literature to possess antiarrhythmic and cardiovascular activities (see pp. 8 to 15). The structural and physicochemical similarities between these two agents were discussed previously. However, despite all of the information that is currently known about the two compounds, no information is available concerning the effects of dihydroquinidine on the disposition kinetics of quinidine in the body. Studies were therefore initiated in an attempt to elucidate the influence of dihydroquinidine on the pharmacokinetics of quinidine.

There are many potential mechanisms through which dihydroquinidine may influence the fate of quinidine in the body. The following examples are consistent with observations and known facts concerning both compounds. Quinidine and dihydroquinidine are both eliminated from the body by metabolism. The properties of these two agents suggest that they might compete for the same metabolizing enzyme system(s)

Table 18

72 HOUR URINARY EXCRETION OF DIHYDROQUINIDINE FOLLOWING ADMINISTRATION OF A BOLUS AND INFUSION IN ANIMAL B

Dose	Urine Volume (ml)	Concentration (mcg/ml)	Amount (mg)	% Dose
2.20 mg/kg	1004	0.35	0.35	2.95
1.76 mg/kg/hr	852	1.45	1.23	3.18

Table 19

EXTENT OF DIHYDROQUINIDINE IN VARIOUS COMMERCIAL QUINIDINE PREPARATIONS

Preparation	Amount Dihydroquinidine* (mg)	Amount Quinidine* (mg)	Percent Dihydroquinidine
A	21.91	189.28	10.4
B	21.61	207.43	9.4
C	17.11	194.46	8.1
D	13.89	186.68	6.9
E	39.02	184.09	17.5
F	12.80	184.09	6.5
G	16.51	194.46	7.8
H	27.97	303.22	8.5

* as the salt form.

Quinidine Sulfate 0.2 Gm Tablets

A = Parke Davis Co., Lot # EJ577

B = Person and Covey Inc., Lot # 324930

C = Stayner Labs., Lot # 4FR140A

D = Eli Lilly Co., Lot # XA4562

E = Davies, Rose and Hoyt, Lot # 602036

F = Strong Cobb Arner, Inc., Lot # 5202-3

G = University of California Mfg. Lab., Lot # AAK287

Quinidine Gluconate 5 Gr Tablet

H = Cooper Labs., Lot #P1135

and thus, alter the elimination characteristics of both compounds.

It has been previously demonstrated that the protein fraction responsible for the binding of quinidine in the plasma has a low capacity for the drug (see p. 104). If dihydroquinidine competes with quinidine for the binding sites on the macromolecule, the fraction of quinidine bound to plasma constituents will be altered. The resultant temporary increase in the unbound levels of quinidine in the presence of dihydroquinidine would tend to lower the total plasma concentration of quinidine due to redistribution into tissues.

The ability of quinine and quinidine (163) and therefore probably dihydroquinidine to inhibit drug metabolizing enzymes in the liver has been reported in the literature. The inhibition of quinidine metabolism would obviously decrease the rate of its removal from the body.

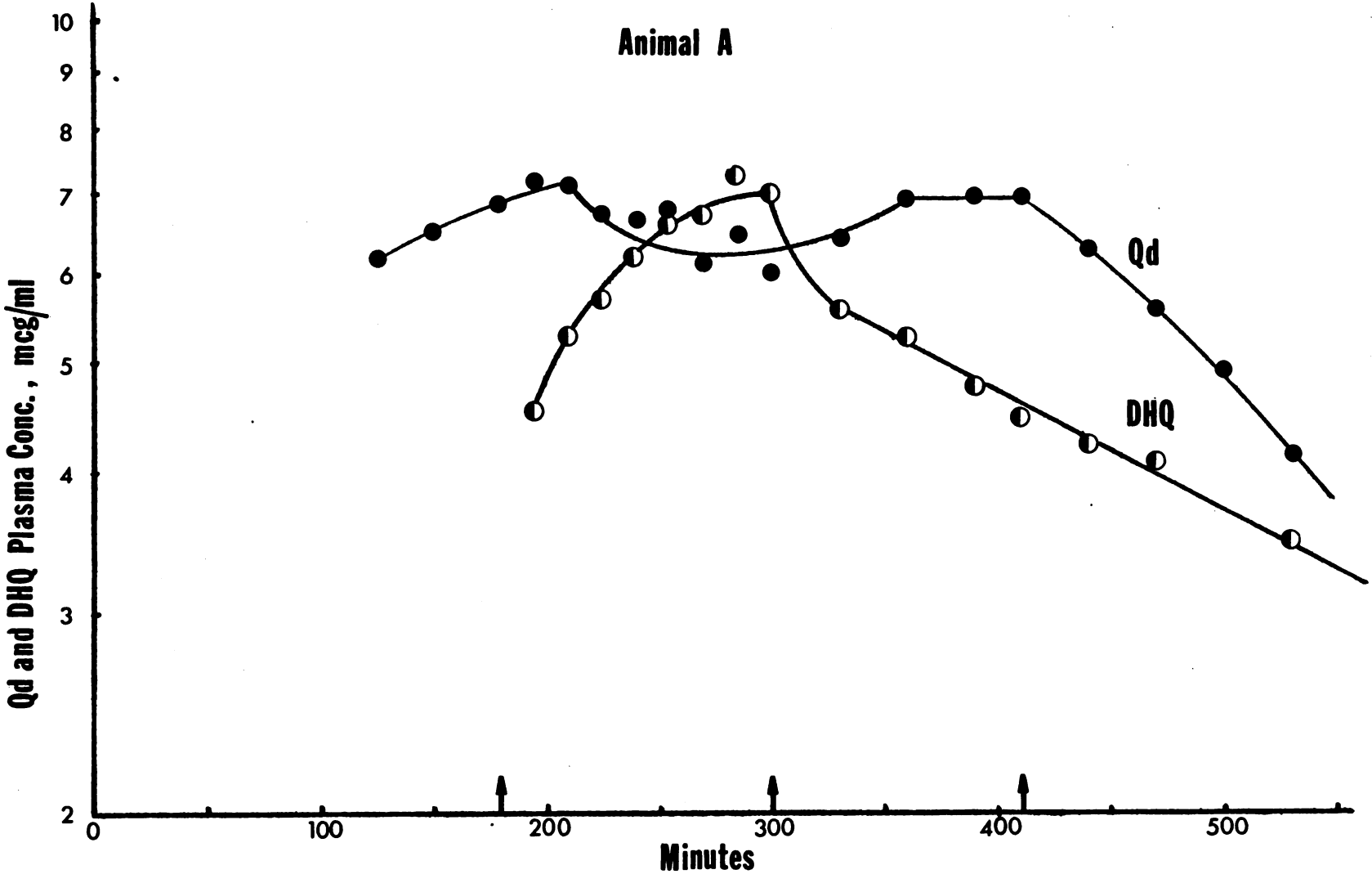
To examine the influence of dihydroquinidine on the pharmacokinetics of quinidine, a study was performed in which quinidine was administered by constant zero-order infusion at a rate of 2.7 mg/kg/hr in animal A for 180 minutes until a plateau concentration of 7.2 mcg/ml was attained in the plasma. Without changing the rate of quinidine infusion, at t = 180 minutes an infusion of dihydroquinidine at the same rate was initiated. The administration of dihydroquinidine was continued for a duration of 120 minutes. At the end of this time, dihydroquinidine infusion was terminated while the administration of quinidine was permitted to proceed for an additional 120 minutes. The plasma levels of these two compounds obtained in this study are shown

in Figure 28. Several important observations were recorded. After the administration of dihydroquinidine, the plasma levels of quinidine were observed to slowly decline. The levels of quinidine proceeded to fall until the infusion of dihydroquinidine was terminated. As the concentration of dihydroquinidine dropped off in the plasma, the concentration of quinidine rose to approximately pre-dihydroquinidine steady state concentrations. The post-infusion elimination profile of quinidine was very slow and curvilinear. And the decay of dihydroquinidine plasma levels was apparently biexponential with a β phase elimination half-life of 280 minutes. The plasma clearance of quinidine at steady state was 34 ml/min (7156 ml/min/kg) and is similar to that which was observed following a small intravenous bolus (see Appendix III).

This fall in quinidine concentration in the presence of dihydroquinidine suggests either an increase in plasma quinidine clearance or an increase in the volume of distribution of quinidine. Alterations in the plasma volume would explain the apparent decrease in the quinidine concentration in the plasma. The influx of extracellular water into the vascular system with a resultant dilution of the plasma binding constituents would decrease the quinidine fraction bound to plasma proteins. This phenomenon produces a temporary increase in the volume of distribution of quinidine and a decrease in the total plasma concentration of this highly bound drug.

The vasodilatation activity of dihydroquinidine could produce such changes. As previously discussed, several authors have observed in-

Figure 28 Plasma level curves following the co-administration of quinidine and dihydroquinidine in animal A. Quinidine was infused at a rate of 2.7 mg/kg/hr. A plateau concentration of 7.2 mcg/ml was attained and maintained in the plasma. At t = 180 minutes, dihydroquinidine infusion was initiated at a rate of 2.7 mg/kg/hr for 120 minutes. The infusion of quinidine was continuous for a duration of 420 minutes. The solid circles and the half-open circles represent quinidine and dihydroquinidine, respectively. The arrows indicate the initiation and termination of the two drugs at their respective times. Post-infusion quinidine elimination was slow and curvilinear. The β phase elimination half-life for dihydroquinidine is 280 minutes. Note the fall in quinidine plasma levels upon the infusion of dihydroquinidine.


Animal A
Qd and DHQ Plasma Conc., mcg/ml
10
9
8
7
6
5
4
3
2
0
100
200
300
400
500
Minutes
Qd
DHQ

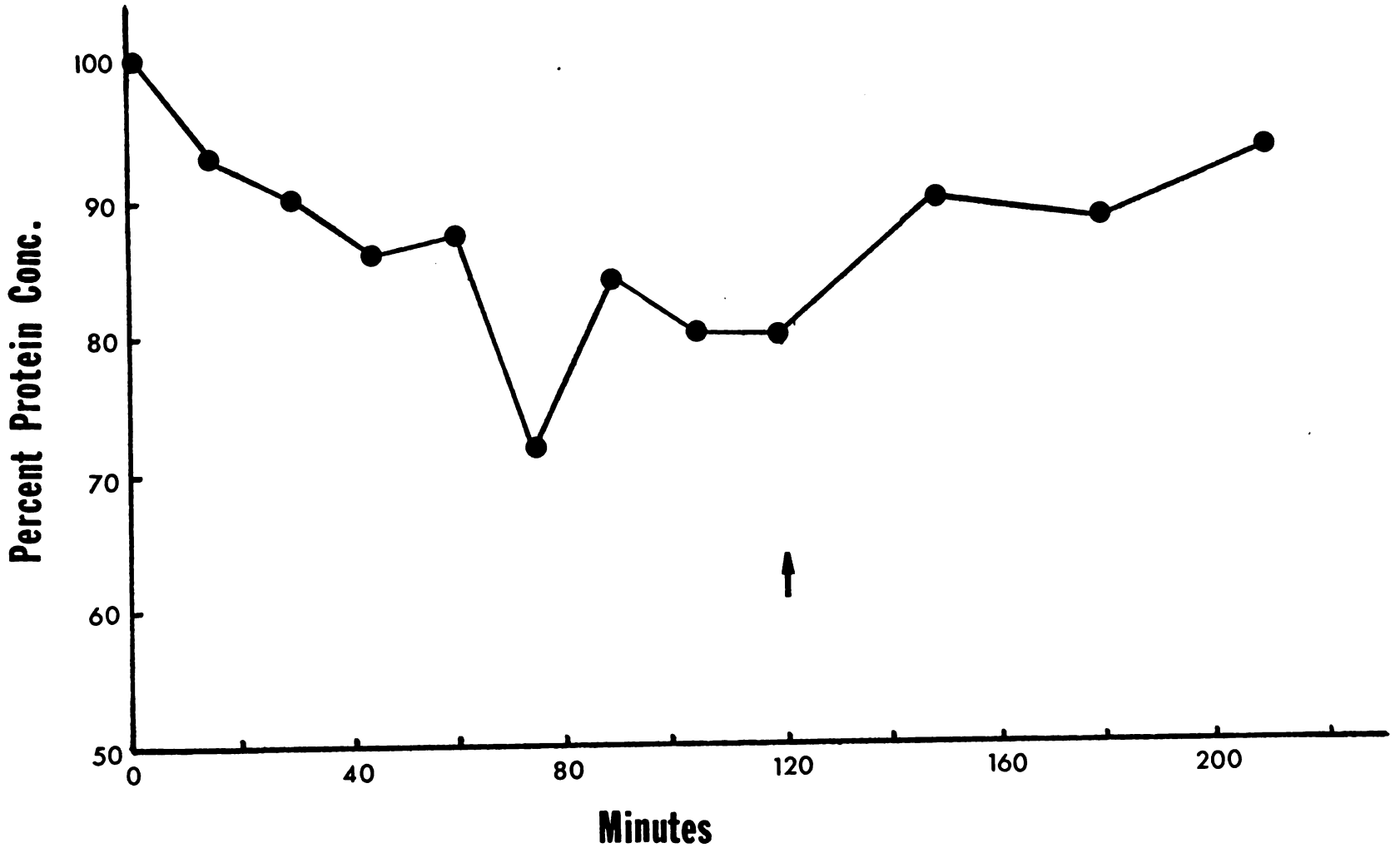
creases in the blood volume following the introduction of various agents which cause peripheral vasodilatation (146,147,148). The observation that quinidine plateau levels were restored upon cessation of dihydroquinidine infusion would lend support for blood volume changes as the mechanism responsible for this observation.

Modification of the blood volume was monitored by changes in plasma protein concentration. The maximum fall in protein concentration in this study corresponded to a 15 to 20% change in the volume following the infusion of dihydroquinidine (Fig. 29).

Under the conditions of this experimental protocol, the initial infusion of quinidine should also produce a changes in the plasma volume. Such an altered state would create an environment favoring an increase in the volume of distribution of dihydroquinidine. Thus, upon superimposition of dihydroquinidine, the post-infusion observations of a pronounced distribution phase and the increased elimination half-life may partially be explained by an apparent increase in the volume of distribution of dihydroquinidine.

In order to ascertain which pharmacokinetic parameter(s) is affected in the presence of dihydroquinidine, an intravenous bolus of quinidine was superimposed upon a plateau concentration in the plasma of dihydroquinidine. Dihydroquinidine was infused in animal A at a rate of 1.43 mg/kg/hr for a total of 420 minutes. After allowing sufficient time for steady state levels of the drug to be reached in the plasma (7.2 mcg/ml), at t = 250 minutes, a 2.7 mg/kg quinidine bolus was administered. A semilogarithmic plot of the concentration-time profile of

Figure 29 Plot of the percent plasma protein concentration versus time following the superimposition of an infusion of dihydroquinidine in animal A at a rate of 2.7 mg/kg/hr on steady state quinidine levels (see Fig. 28). The arrow indicates the time at which the infusion of dihydroquinidine was terminated.


Percent Protein Conc.
100
90
80
70
60
50
0
40
80
120
160
200
Minutes

quinidine is shown in Figure 30. The extrapolated Vd was 2.08 liters (0.46 L/kg). When compared with the volume of distribution (0.38 L/kg, see Appendix III) following an equivalent dose in the absence of dihydroquinidine, this change in Vd is approximately 21% and is consistent with the above study in which the concentration of quinidine appears to fall 15 to 20% during the period of dihydroquinidine infusion. The elimination half-life of quinidine in this study was 57 minutes. The computed plasma clearance is 25.17 ml/min (5.59 ml/min/kg). The decrease in the elimination rate constant of the drug in the presence of dihydroquinidine would also be explained by a decrease in quinidine clearance as well as an increase in Vd. A comparison of the pharmacokinetic parameters in the absence and presence of the impurity is summarized in Table 20.

As in the previous study, in which the two agents were administered concommitantly, immediately after the administration of the quinidine bolus, the steady state levels of dihydroquinidine were observed to drop off as presented in Figure 31. Plateau plasma levels of dihydroquinidine were restored 80 minutes after the initial fall in drug concentration. Presumably the mechanism of this observation is a temporary diminution of total plasma protein concentration. At this time, the plasma concentration of quinidine was 2.5 mcg/ml. Post-infusion of dihydroquinidine elimination was very slow ($t_{.5}$ = 130 minutes) when compared to that of an intravenous bolus (Fig. 26, p. 124).

The total volume of urine voided in 72 hours was collected and analyzed for the two compounds. No attempt was made to control the

Figure 30 The computer-simulated biexponential plasma level decay curve and experimental data points following the intravenous administration of 12 mg quinidine (2.7 mg/kg) to animal A during the infusion of dihydroquinidine at a rate of 1.43 mg/kg/hr. The upper time scale indicates the time following dihydroquinidine infusion. The lower time scale represents the time following the administration of the quinidine bolus. The distribution phase, obtained by feathering, is seen to be very rapid ($T_{.5}$ = 2.9 minutes). The slower elimination phase has a $T_{.5}$ = 57 minutes.

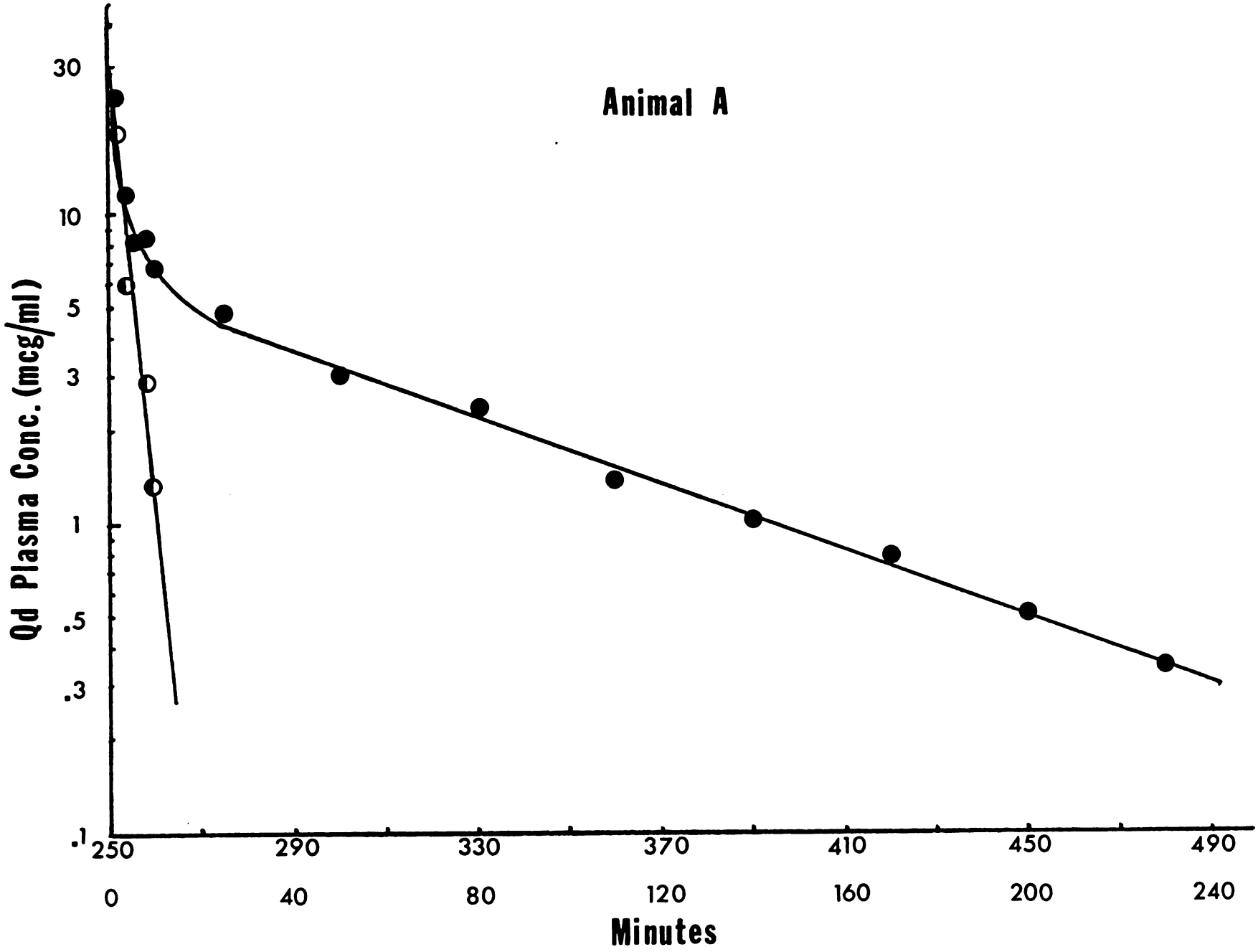
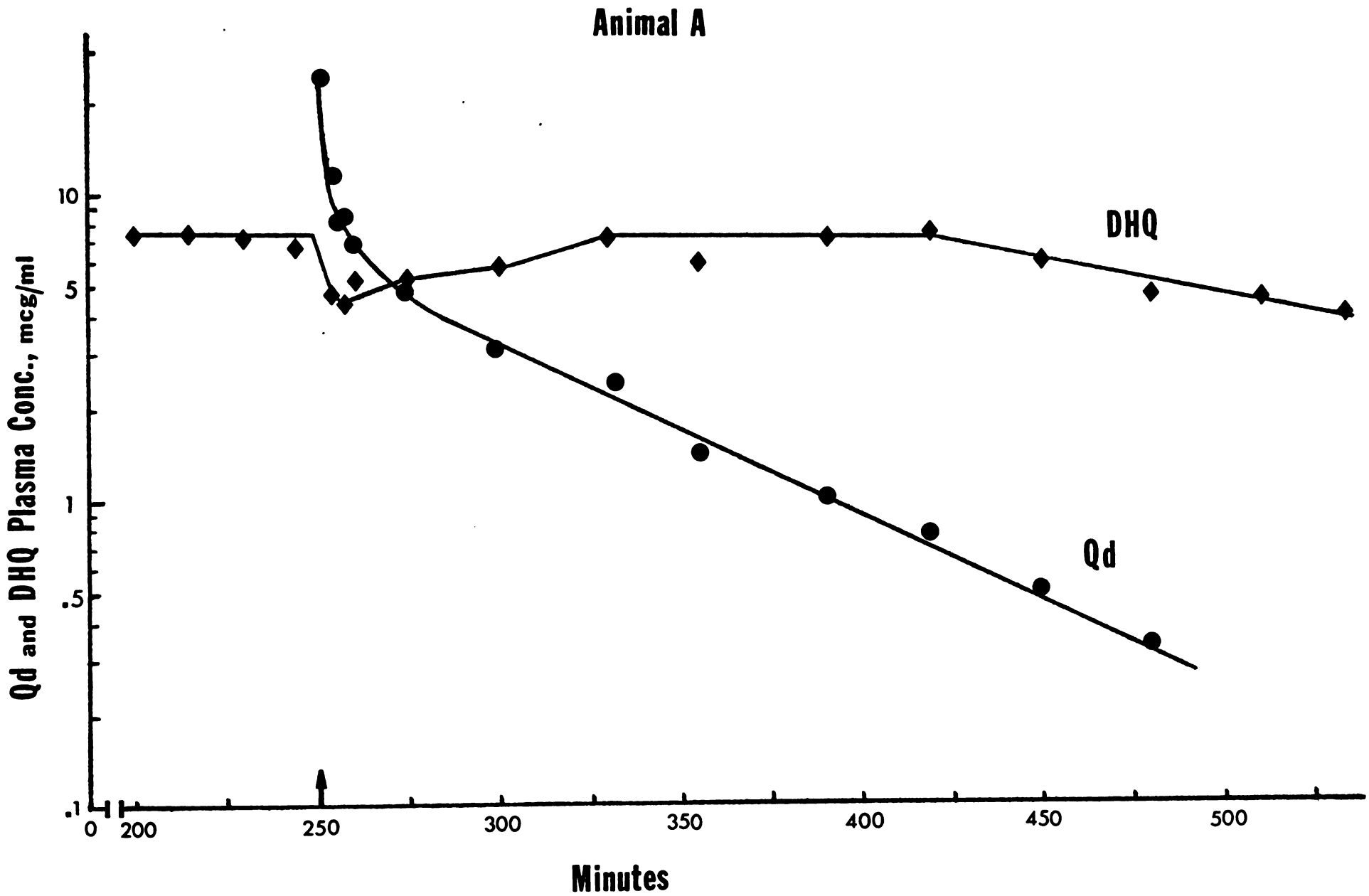

Animal A
Qd Plasma Conc. (mcg/ml)
30
10
5
3
1
.5
.3
.1
250
290
330
370
410
450
490
0
40
80
120
160
200
240
Minutes

Table 20

COMPARISON OF THE PHARMACOKINETIC CONSTANTS OF QUINIDINE AFTER INTRAVENOUS BOLUS ADMINISTRATION OF EQUAL DOSES IN ANIMAL A IN THE ABSENCE AND PRESENCE OF DIHYDROQUINIDINE

State	Date	A (mcg/ml)	B (mcg/ml)	α (min^{-1})	$t_{.5\alpha}$ (min)	β (min^{-1})	$t_{.5\beta}$ (min)	k_{12} (min^{-1})	k_{21} (min^{-1})	Kel (min^{-1})	Vd (L/kg)	Cl (ml/min/kg)
Absence	5.14.73	37.84	7.11	0.44	1.6	0.025	27.4	0.254	0.091	0.123	0.38	9.56
Presence	5. 2.73	20.29	5.76	0.24	2.9	0.012	57.0	0.141	0.062	0.047	0.46	5.59

Figure 31 Plasma level curves following the co-administration of quinidine and dihydroquinidine in animal A. Dihydroquinidine was infused at a rate of 1.43 mg/kg/hr for 420 minutes. At t = 250 minutes, a 2.7 mg/kg bolus of quinidine was superimposed upon a steady state dihydroquinidine concentration of 7.25 mcg/ml. The solid circles and diamonds represent quinidine and dihydroquinidine, respectively. The arrow indicates the time at which the quinidine bolus was administered. Note the fall in dihydroquinidine concentration upon administration of the quinidine bolus and that the post-infusion elimination half-life of dihydroquinidine was very slow ($T_{.5}$ = 130 minutes).


Animal A
DHQ
Qd
Qd and DHQ Plasma Conc., mcg/ml
10
5
1
.5
.1
0
200
250
300
350
400
450
500
Minutes

pH of the urine. The amount of quinidine and dihydroquinidine excreted during this 72 hour period was 3.2 and 3.5% of the doses administered, respectively (Table 21). In view of the fact that quinidine is primarily eliminated from the body by metabolism (approximately 1% of the administered dose is recoverable in the urine as unchanged quinidine (Table 22)), this increase in the total amount of unchanged drug appearing in the urine is consistent with a decrease in quinidine metabolic clearance in the presence of dihydroquinidine.

Alterations in both volume of distribution and plasma clearance of quinidine in the presence of dihydroquinidine indicate that these changes were induced by the latter agent. In the presence of dihydroquinidine, there was a three-fold increase in the fraction of quinidine excreted unchanged in the urine. In this same study the total plasma clearance of quinidine was observed to decrease by a factor of 1.71 (c.f., Table 20). These observations indicate that dihydroquinidine apparently induces changes in both metabolic and renal clearance of quinidine.

In man, the renal clearance of quinidine was observed to diminish with increasing urinary pH (27).. Although the author has seen no reports in the literature, an increase in the pH of the urine induced by quinidine and/or dihydroquinidine could possibly account for the changes in the elimination characteristics of quinidine.

An alternative explanation for the change in the fraction of quinidine excreted unchanged in the urine would be the influence of renal blood flow on quinidine renal clearance. In view of the acti-

Table 21

72 HOUR URINARY EXCRETION OF QUINIDINE AND DIHYDROQUINIDINE IN ANIMAL A

Compound	Dose	Urine Volume (ml)	Concentration (mcg/ml)	Amount (mg)	% Dose
Quinidine	2.70*	852	0.45	0.38	3.18
Dihydroquinidine	1.43†	852	1.88	1.60	3.54

* mg/kg

† mg/kg/hr

Table 22

VARIABILITY IN URINARY EXCRETION OF QUINIDINE IN ANIMAL A

Date	Total Dose (mg)	Urine Volume (ml)	Concentration (mcg/ml)	Amount (mg)	% Dose
3.12.73	30.00	538	0.46	0.25	0.83
3.22.73	142.92	815	0.98	0.80	0.56
3.31.73	182.28	940	0.95	0.90	0.49
5.30.73	72.90	800	0.75	0.60	0.82
9.25.73	18.00	1098	0.10	0.11	0.56
				Mean:	0.65

vities of quinidine and dihydroquinidine on the cardiovascular system, research in this area could perhaps provide an explanation for the alterations in quinidine elimination observed in these studies.

The decrease in the post-infusion elimination rate constant of dihydroquinidine might be attributed to an increase in its volume of distribution. In this study, the amount of unchanged drug excreted in the urine did not change.*

The observation that dihydroquinidine decreases the total plasma clearance of quinidine when co-administered has therapeutic implications. In view of the lower plasma clearance of dihydroquinidine as compared to that of quinidine and their similar volumes of distribution, administration of these drugs at the same rate would produce higher dihydroquinidine levels in the plasma. For quinidine preparations containing dihydroquinidine even in small quantities such as those found in commercial products (e.g., 10 to 20%), the levels of the latter agent may be quite significant (and possibly comparable to the levels of quinidine).

The recorded influences of dihydroquinidine on the pharmacokinetics of quinidine might explain the clinical observation of very slow recovery by patients in quinidine toxicity. The existence of high dihydroquinidine levels in the body would be suspected following multiple doses of commercial quinidine on its usual dosage schedule (200 to 400 mg four times a day). Since dihydroquinidine decreases the

* In the absence of dihydroquinidine, an unidentified compound is present in the area of the TLC plate normally occupied by dihydroquinidine (see Table 2, p. 40) when urine is analyzed. However, this would tend to over estimate the amount of dihydroquinidine in the urine in presence of quinidine.

elimination rate constant of quinidine, accumulation of quinidine to toxic levels might be anticipated if the dosage regimen was not modified. Clearly, the extent of dihydroquinidine influences on the disposition kinetics of quinidine would vary with the fraction of this impurity present in the quinidine preparation and with the amount in the body. The effects of quinidine on the disposition kinetics of dihydroquinidine in the body was not completely examined in this work. However, it appears that each drug influences the elimination of the other drug.

It was previously demonstrated that conventional methods utilized for the analysis of quinidine in plasma and other biological fluids are non-specific. In addition to quinidine, these methods also detect dihydroquinidine and their metabolites. Analytical procedures which fail to distinguish between quinidine and dihydroquinidine will result in erroneous interpretations of plasma level-response relationships. Both agents are pharmacologically active on the cardiovascular system. It is therefore important that the concentration of both quinidine and dihydroquinidine are measured when relating plasma drug levels to pharmacological effects. However, the relationships between drug plasma levels and cardiovascular effects have not been clearly established. Until such time that plasma level-response profiles are known for quinidine and dihydroquinidine, there are inherent problems in interpreting plasma level data using commercial quinidine dosage forms.

CONCLUSION

The therapeutic value of quinidine in the treatment of various cardiac arrhythmias is well established. Dihydroquinidine itself is not used as a chemotherapeutic agent. However, its presence in commercial quinidine preparations as an impurity stimulated interest in the pharmacokinetics of this compound together with the potential influences of dihydroquinidine on the disposition kinetics of quinidine in the body. Since quinidine is the principal agent in question, emphasis was given towards the elucidation of the pharmacokinetics of this drug.

The disposition kinetics of quinidine and dihydroquinidine were studied in the rhesus monkey. It was observed that the fate of both drugs in the body can be adequately described by a two-compartment body model. Both quinidine and dihydroquinidine exhibited rapid and shallow distribution phases. The slower elimination phase for quinidine had an elimination rate constant which was approximately twice that which was observed for dihydroquinidine following intravenous boluses ($t_{.5}$ quinidine = 30 to 35 minutes versus $t_{.5}$ dihydroquinidine = 62 minutes).

In the therapeutic plasma concentration range of 3 to 7 mcg/ml, quinidine was rapidly eliminated from the body. When toxic drug levels were approached and maintained in the plasma, concentrations exceeding 8 mcg/ml, there were noticeable changes in the disposition of quinidine. Quinidine is active on the cardiovascular system. In an

attempt to maintain a homeostatic state in the body, many compensatory mechanisms exist to offset disruptions of normal physiological functions. It therefore becomes apparent that agents which induce changes in the system by which they are distributed and eliminated in the body can influence their own disposition.

An increase in the apparent volume of distribution of quinidine was determined to be the cause of the diminished fractional rate of removal of quinidine at high concentrations. Several alternative explanations were discussed and some were examined in an attempt to ascertain the mechanism responsible for the increased volume of distribution of quinidine. Diminished plasma protein binding, either through saturation of the binding sites on the plasma constituents, drug displacement, or increased plasma volume were not demonstrated or were too insignificant in magnitude to explain the large change in the volume of distribution of quinidine.

A change in tissue pH was also examined as the explanation for the increase in Vd. A decrease in cellular or tissue pH induced by extracellular potassium depletion did not change the distribution volume of quinidine in the body.

It was demonstrated that dihydroquinidine is capable of altering the total plasma clearance and volume of distribution of quinidine in the body.

It was demonstrated that dihydroquinidine is present as an impurity in commercial quinidine preparations and that the conventional methods for quinidine analysis fail to distinguish between this drug,

dihydroquinidine, and their metabolites in the plasma. Thus, the observation that the plasma clearance of dihydroquinidine is considerably lower than that of quinidine and that dihydroquinidine decreases the plasma clearance of quinidine when co-administered are clinically significant.

Many unanswered questions were raised during the course of this work. The mechanism(s) responsible for the apparent increase in quinidine volume of distribution with time and dose remains the primary unanswered question. The pathway of quinidine (and dihydroquinidine) elimination in the body, the nature of the metabolites and their pharmacokinetics and the pharmacological activities of these metabolites are additional unanswered questions. Further studies are necessary in order to expand our knowledge of the pharmacokinetics of quinidine. The present work has laid the groundwork for future investigations.

APPENDIX

Appendix 1. Materials and Supplies

Diuril[R] (Sodium Chlorothiazide)	Merck Sharp and Dohme West Point, Pa.
Imferon[R] (Iron Dextran Injection)	Lakeside Labs. Inc. Milwaukee, Wisconsin
Intramedic[R] Luer Stub Adapters 18 guage	Clay-Adams Co. Parsippany, New Jersey
Intravenous Extension Tube 30 inches	McGaw Labs. Glendale, Ca.
Intravenous Set	McGaw Labs Glendale, Ca.
Lipo-Heparin[R] (Sodium Heparin Injection 1000 USP units/ml)	Riker Labs. Inc. Northridge, Ca.
Microcap[R] Micropipettes	Drummond Scientific Co. VWR Scientific Co. (Dist) San Francisco, Ca.
Microfuge[R] Micro Test Tubes	Beckman Instruments, Inc. Palo Alto, Ca.
Monojet[R] Disposible Syringes and Needles	Sherwood Medical Indust., Inc. Deland, Florida
Nembutal[R] (Sodium Pentobarbital 60 mg/ml)	Abbott Labs. No. Chicago, Illinois
Panalog[R] Ointment (Nystatin, Neo- mycin , Thiostrepton, and Triamcinolone Acetonide)	E.R. Squibb and Sons, Inc. New York, N.Y.
Phenol Reagent Solution (Folin-Ciocalteau Solution)	Fisher Scientific Co. Fair Lawn, New Jersey
Pre-Coated Kieselghur G TLC Plates, 0.25 mm	Analtech, Inc. Newark, Delaware
Pre-Coated Silica Gel TLC Plates 0.25 mm	E. Merck Reagents, Brinkman Instruments, Inc. Westbury, New York
Resinite Hi-Heat 105C Vinyl Insulating Sleeving	Borden Chemical Co. Compton, Ca.

Sodium Chloride Solution 0.9%, 1000 ml	McGaw Labs. Glendale, Ca.
Streptillin^{R} (Procaine Penicillin G 200,000 I.U. and Dihydrostreptomycin Sulfate Equivalent to 0.25 gm Streptomycin/ml)	Trico Pharmaceuticals San Carlos, Ca.
Thin Layer Chromatography Tank	Supelco Inc., Bellefonte, Penn.
Three-way Stopcock	Pharmaseal, Inc. Toa Alta, Puerto Rico
Trident Heparinized Capillary Tubes Red-Tip^{R}	Curtin Scientific Co. Houston, Texas
Vacutainer^{TM} (Sodium Heparin, 143 USP units/tube	Becton-Dickinson and Co. Rutherford, New Jersey
Vetalar^{R} (Ketamine Hydrochloride 100 mg/ml)	Parke-Davis and Co. Detroit, Michigan
Visking^{R} Cellulose Tubing 0.025 mm	VWR Scientific Co. (Dist.) San Francisco, Ca.

Appendix 2. Apparatus

Aminco-Bowman Spectrophotofluorometer, Model 8210	American Instrument Co. Inc. Silver Spring, Md.
Beckman Expandomatic pH Meter	Beckman Instruments, Inc. Fullerton, Ca.
Blak-Ray^{R} Ultraviolet Lamp (Long Wave UVL-22)	Ultra-Violet Products, Inc. San Gabriel, Ca.
Büchi Rotary Evaporator	Rinco Instrument Co., Inc. Greenville, Illinois
Cary Model 15 Spectrophotometer	Cary Instruments, Inc. Monrovia, Ca.
Dianorm^{R} Equilibrium Dialysis System	Innovativ-Medizin AG, CH-Esslingen, Switzerland
Hamilton Microliter Syringe	The Hamilton Co., Inc. Whittier, Ca.
Harvard Infusion Pumps Models 930 and 975	Harvard Apparatus Co. Millis, Mass.
International Centrifuge Model EXD	International Equipment Co. Needham Hts, Mass.
Millipore Filtration Apparatus	Millipore Corp. Bedford, Mass.
Reciprocating, Variable Speed Shaker	Eberbach Co. Ann Arbor, Michigan
Safe Guard Table Model Centrifuge	Clay-Adams Co., Inc. New York, N.Y.
Spinco Model 152 Microfuge	Beckman Instruments, Inc. Palo Alto, Ca.
Time It Electronic Timer	Precision Scientific Co. Chicago, Illinois
Uni-Melt^{R} Hoover Capillary Melting Point	Arthur H. Thomas Co. Philadelphia, Pa.
Vortex Jr. Mixer, Model K-500-J	Scientific Industries, Inc. Queens Village, N.Y.

Appendix III. Chronological Summary of the Experiments Performed in Animal A

Date	Experiment Number	Page	Compound*	Dose†	Duration (min)	Cp_{ss} (mcg/ml)	$t_{.5\beta}$ (min)	Vd (L/kg)	Cl (ml/min/kg)
3.12.73	2A	71	Qd	6.7	Bolus		35.0	0.67	13.5
3.22.73	5A	85	Qd	1.44	200	1.75	N.E.	N.E.	13.7
				2.71	200	3.00	N.E.	N.E.	15.1
				5.38	200	5.66	25.0	0.57	15.8
3.31.73	6A	87	Qd	1.44	200	1.75	30.0	0.59	13.7
				10.72	200	11.80	108.0	2.36	15.1
4.20.73	17C	133	Qd	2.71	420	7.20	Curve	N.E.	7.56
			DHQ	2.71	120	N.E.	280	N.E.	N.E.
5. 2.73	18C	137	Qd	2.7	Bolus		57.0	0.46	5.59
			DHQ	1.43	420	7.20	130.0	N.E.	N.E.
5.14.73	8A	92	Qd	2.7	Bolus		27.4	0.38	9.56
5.30.73	9A	95	Qd	2.7	360	6.30	Curve	N.E.	7.20

Appendix III. (continued)

Date	Experiment Number	Page	Compound*	Dose†	Duration (min)	Cp_{ss} (mcg/ml)	$t_{.5\beta}$ (min)	Vd (L/kg)	Cl (ml/min/kg)
6.10.73	11A	98	Qd	1.44	360	2.70	35.0	0.41	8.24
7.18.73	12A	108	Qd	10.72	178	10.40	252.0	6.18	17.17
9.14.73	13A	117	Qd	1.44	201	3.40	47.0	0.47	6.90
9.25.73	14A	118	Qd	4.00	Bolus		46.9	0.52	7.68

* Compounds: Qd = quinidine; DHQ = dihydroquinidine

† Dose: Bolus = mg/kg; Infusion = mg/kg/hr

N.E. = not estimated

Appendix IV. Chronological Summary of the Experiments Performed in Animal B

Date	Experiment Number	Page	Compound*	Dose†	Duration (min)	Cp_{ss} (mcg/ml)	$t_{.5\beta}$ (min)	Vd (L/kg)	Cl (ml/min/kg)
2. 9.73	1A	66	Qd	3.00	135		40.0	N.E.	N.E.
2.28.73	3A	72	Qd	3.33	Bolus		28.8	0.24	5.80
3.15.73	4A	83	Qd	8.77	180	7.50	N.E.	N.E.	19.50
				4.41	180	N.E.§	N.E.	N.E.	N.E.
				2.22	180	N.E.§	N.E.	N.E.	N.E.
				1.16	140	N.E.§	99.0	N.E.	N.E.
3.27.73	7A	91	Qd	4.41	110	13.20	91.0	0.76	5.56
5.12.73	15B	124	DHQ	2.20	Bolus		62.0	0.22	2.46
5.24.73	10A	96	Qd	2.22	360	8.60	Curve	N.E.	4.30
6. 6.73	16B	127	DHQ	1.76	240	10.70	160.0	0.63	2.75

* Compounds: Qd = quinidine; DHQ = dihydroquinidine
† Dose: Bolus = mg/kg; Infusion = mg/kg/hr
N.E. = not estimated
§ Does not appear that steady state is reached

Appendix V. Tabulation of the Experimental Data

Experiment 1A (Figure 7, p. 66)

Time (min)	Quinidine Concentration (mcg/ml)	
	Extraction Method	TLC-Fluorometric Method
0 (Control)	0.012	0.04
20	4.86	6.20
40	7.32	7.53
60	8.51	7.53
90	9.10	- -
120	9.56	- -
135	9.74	8.20
151	8.22	5.05
180	6.18	3.34
210	4.56	2.01
240	3.34	1.24
300	1.94	0.65
360	1.39	0.25
480	0.84	- -
600	0.58	- -
700	0.46	- -

Experiment 2A (Figure 9, p.71)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.024
2	12.50
4	10.91
6	9.84
8	9.04
15	7.34
30	5.43
60	2.87
90	1.70
120	0.96
150	0.49
180	0.28

Experiment 3A (Figure 10, p. 72)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.043
2	43.36
4	14.93
6	13.92
8	12.90
10	11.38
15	10.36
20	8.33
40	5.13
60	3.31
90	1.45
120	0.64
150	0.38
180	0.23

Experiment 4A (Figure 11, p. 83)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.032
120	7.56
150	7.57
180	7.56
225	5.94
270	4.83
300	4.96
330	4.75
360	5.13
405	4.45
450	4.07
480	3.84
510	3.64
540	4.09
585	3.61
630	3.46
680	3.31
720	2.04
765	1.41
810	1.01
840	0.81
870	0.59
900	0.49

Experiment 5A (Figure 12, p. 85)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.027
120	1.74
150	1.85
180	1.63
200	1.72
320	3.05
350	2.97
380	2.97
400	3.05
520	5.66
550	5.51
580	5.66
600	5.66
630	2.00
660	0.82
690	0.39
720	0.20

Experiment 6A (Figure 13, p. 87)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.03
120	1.79
150	1.79
180	1.77
200	1.82
220	1.20
240	0.79
260	0.45
280	0.29
400	11.65
430	11.88
460	12.11
480	11.88
510	9.58
540	8.20
570	7.74
600	5.45

Experiment 7A (Figure 15, p. 91)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.024
80	13.20
110	13.44
130	11.03
160	8.87
190	7.19
220	5.68

Experiment 8A (Figure 16, p. 92)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.045
2	24.08
4	12.02
6	8.93
8	7.02
10	5.76
15	5.43
30	3.91
60	1.41
90	0.68
120	0.33
150	0.15
180	0.09

Experiment 9A (Figure 17, p. 95)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.036
120	5.82
180	6.42
240	5.82
300	6.92
360	6.62
400	5.42
420	4.83
440	3.63
460	3.04
480	2.34
510	1.54
540	0.81

Experiment 10A (Figure 18, p. 96)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.03
120	8.84
180	9.14
240	8.53
300	8.53
360	8.23
390	7.31
420	5.65
450	4.64
480	3.08
510	2.53
540	1.95
570	1.34
600	1.03

Experiment 11A (Figure 19, p. 98)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.035
120	2.59
180	2.67
240	2.59
300	2.75
360	2.75
380	1.68
400	0.93
420	0.72
440	0.54
460	0.25
480	0.17
510	0.11

Experiment 12A (Figure 22, p. 108)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.03
112	9.82
137	10.45
160	10.52
178	10.52
210	9.92
240	8.53
270	7.32
300	7.59
330	6.65
360	6.56
420	5.87
480	5.39

Experiment 13A (Figure 24, p. 117)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.024
125	3.22
150	3.41
165	3.31
180	3.41
201	3.41
233	2.48
260	1.50
290	0.92
320	0.60
350	0.36
380	0.24
440	0.09

Experiment 14A (Figure 25, p. 118)

Time (min)	Quinidine Concentration (mcg/ml)
0 (Control)	0.02
2	9.52
4	7.84
6	7.23
8	6.48
10	6.09
15	5.13
30	4.26
60	3.04
90	1.82
120	1.20
150	0.73
180	0.45

Experiment 15B (Figure 26, p. 124)

Time (min)	Dihydroquinidine Concentration (mcg/ml)
0 (Control)	0.033
2	37.10
4	18.52
6	13.70
8	11.66
10	9.54
15	8.51
30	7.27
60	5.31
90	4.48
120	2.31
150	1.87
180	1.38
210	0.91
240	0.70

Experiment 16B (Figure 27, p. 127)

Time (min)	Dihydroquinidine Concentration (mcg/ml)
0 (Control)	0.028
120	8.90
180	10.52
240	10.70
270	9.94
300	8.76
330	7.69
360	6.61
390	5.53
420	5.21
480	4.67
540	3.17

Experiment 17C (Figure 28, p. 133)

Time (min)	Quinidine Concentration (mcg/ml)	Dihydroquinidine Concentration (mcg/ml)
0 (Control)	0.029	0.023
125	6.18	- -
150	6.50	- -
180	6.90	- -
195	7.30	4.52
210	7.22	5.25
225	6.74	5.66
240	6.66	6.18
255	6.82	6.59
270	6.10	6.69
285	6.42	7.31
300	6.02	7.00
330	6.42	5.56
360	6.98	5.25
390	6.98	4.73
410	6.90	4.42
440	6.26	4.21
470	5.54	4.11
500	4.91	- -
530	4.18	3.49

Experiment 18C (Figures 30 and 31, pp. 137 and 139)

Time (min)	Quinidine Concentration (mcg/ml)	Dihydroquinidine Concentration (mcg/ml)
0 (Control)	0.023	0.033
200		7.45
215		7.23
230		7.11
245		6.87
252	24.46	- -
254	11.67	4.76
256	- -	4.51
258	8.41	5.50
260	6.77	- -
275	4.79	5.40
300	3.07	5.75
330	2.42	7.11
360	1.36	5.88
390	1.03	6.96
420	0.79	7.23
450	0.51	5.87
480	0.34	4.39
510		4.51
535		3.90

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