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TISSUE ACCUMULATION OF OUABAIN AND PHENYTOIN, ALONE AND IN COMBINATION, AND THEIR RELATIONSHIP TO OUABAIN-INDUCED DYSRHYTHMIA.

THE OHIO STATE UNIVERSITY, PH.D., 1979
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TISSUE ACCUMULATION OF OUABAIN AND PHENYTOIN, ALONE AND IN COMBINATION, AND THEIR RELATIONSHIP TO OUABAIN-INDUCED DYSRHYTHMIA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by


***

The Ohio State University

1979

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ACKNOWLEDGMENTS

I wish to express my sincere gratitude and appreciation to my advisor, Dr. Robert L. Hamlin, for his guidance to fulfill my long term ambition to work in cardiovascular physiology and pharmacology.

I am grateful to Dr. S. Dutta for providing facilities and his particular encouragement for the improvement of this research.

During my stay at The Ohio State University, I have been very fortunate to associate with all the faculty members of the Department of Veterinary Physiology and Pharmacology and their friendly discussions in all aspects of my curricular activity throughout my post-graduate education. Their guidance and encouragement are highly appreciated.

The valuable assistance of Dr. Jean D. Powers for the statistical analysis is greatly acknowledged.

Last, but not least, my special thanks to my simple family who have been very patient and understanding in my long term ambition, especially my father for his encouragement all through my life.
TISSUE ACCUMULATION OF OUABAIN AND PHENYTOIN ALONE AND IN COMBINATION, AND THEIR RELATIONSHIP TO OUABAIN-INDUCED DYSRHYTHMIA

Ibrahim Ismail Ali

This study was designed to study the pharmacokinetics of both phenytoin and ouabain given individually and together in dogs. Concentrations were measured in plasma, cerebrospinal fluid, neuronal and non-neuronal portions of the nervous system, and other body tissues. Attempts were made to correlate the uptake of ouabain in various sites with the appearance of ventricular dysrhythmias, and to evaluate the effect of phenytoin on the uptake of ouabain by various tissues. In order to reach rapidly a therapeutic plasma level of 20 µg/ml and to maintain it at a constant level, a loading dose (12 mg/kg) and maintenance infusion (30 µg/kg/min) of phenytoin were calculated on the basis of pharmacokinetic parameters determined by injecting a single dose of phenytoin (10 mg/kg) in dogs. A one compartment model was assumed and average values for elimination rate constant (Ke) and apparent volume of distribution (aVd) were 0.16 hr⁻¹ and 0.57 l/kg, respectively.
The effectiveness of the above doses of phenytoin was assessed in the presence of dysrhythmia induced by an infusion of ouabain (.072 μg/kg/min) after a loading dose of 40 μg/kg. Results indicate that with this regimen of phenytoin, the plateau concentrations of plasma and cerebrospinal fluid were reached in 45 minutes and maintained steadily thereafter. Dogs that showed sustained reversal to sinus rhythm had a steady-state plasma concentration of 12.4±0.1 μg/ml of phenytoin. However, cerebrospinal fluid concentrations varied slightly (2-3 μg/ml) with an average of 2.3±0.57 μg/ml and were independent of plasma concentrations. The ouabain concentration in the area postrema was greater than in other neuronal tissues examined. This concentration was 6.3 times higher than the corresponding plasma concentration. Localization of ouabain may play a role in the centrogenic mechanism of cardiac glycoside-induced dysrhythmia apart from any direct myocardial effect. Although some regions of the central nervous system such as the medulla oblongata (deep portion) accumulate a relatively high concentration of phenytoin, the pharmacological effect of this drug in relation to its antiarrhythmic effect through neuronal tissue is not well understood. However, this study shows that phenytoin abolishes digitalis-induced dysrhythmia successfully. The direct myocardial effect of this drug in preventing ventricular tachycardia is very possible.
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<td>ADP</td>
<td></td>
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<td>Adenosine monophosphate</td>
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<tr>
<td>Ant.</td>
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<td>Anterior</td>
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<td>Ant. pit. gland</td>
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<td>Anterior pituitary gland</td>
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<td>ATP</td>
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dLVP/dt ................. Rate of rise of left ventricular pressure per unit time

dp/dt .................. Rate of rise of pressure per unit time

dpm ...................... Disintegration(s) per minute

EKG ........................ Electrocardiogram

4th V. choroid plexus .. Choroid plexus of the fourth ventricle

4th vent. med. ........... Fourth ventricle of the medulla oblongata

3H ........................ Tritium

Hg ........................ Mercury

HR ........................ Heart rate

Ke ........................ Disappearance rate

Kg ........................ Kilogram(s)

l ............................ Liter(s)

L.V. choroid plexus .... Choroid plexus of the lateral ventricle

LVP ........................ Left ventricular pressure

mEq/l ...................... Milliequivalent per liter

mg ............................ Milligram(s)

µg ............................ Microgram(s)

min ........................ Minute(s)

ml ............................. Milliliter(s)

mm ............................ Millimeter(s)

mV ............................ Millivolt(s)

nmole or nM .............. Nanomolar

xiii
$^{14}$C-phenytoin . . . . . . . $^{14}$C radioactive phenytoin
Pmole of PM . . . . . . . Picomolar(s)
post. . . . . . . . . . . . . Posterior
Post. pit. gland . . . . . . Posterior pituitary gland
R-C . . . . . . . . . . . . Resistance capacitance (differentiating circuit)
S-A . . . . . . . . . . . . Sinoatrial
S.A. . . . . . . . . . . . Specific activity
Sec . . . . . . . . . . . . Second(s)
S.E.M. . . . . . . . . . Standard error of the mean
t . . . . . . . . . . . . . . Time
T/CSF . . . . . . . . Tissue/cerebrospinal fluid
t$_0$ . . . . . . . . . . . Time at zero period
T/P . . . . . . . . . . . Tissue/plasma
Vent. port. med. . . . . Ventral portion of the medulla oblongata
CHAPTER I

INTRODUCTION

Since William Withering (1785) introduced digitalis nearly 200 years ago, there has been a great deal of interest in this group of drugs. There is much controversy over its clinical usefulness and the mechanism(s) of the positive inotropic action of cardiac glycosides. Digitalis is the fourth and most frequently prescribed drug by physicians in the United States today. A. J. Clark (1933) and Otto Loewi (1918) showed that the cardiac tissue is the site of the primary effect of digitalis.

At the beginning of this century, many investigators became extremely interested in the precise receptor site for digitalis effect on the heart. In 1913, Weizsacker was the first to note a correlation between the dose of digitalis and its myocardial activity. However, it was not until 1940 that Gold and Cattell first demonstrated that digitalis increased the force of isometric contraction under a constant rate of stimulation using a cat papillary muscle in a hypodynamic state. Subsequent studies (Sonnenblick
et al., 1966; Ellis and Dimond, 1966) confirmed the positive inotropic action of digitalis regardless of the functional state of the myocardium. Still, the molecular basis of digitalis induced inotropism is not understood (Marks, 1964; Langer, 1968). In recent years, in an attempt to understand the mechanism(s) of the positive inotropic action of the cardiac glycosides, the major cellular systems have been investigated by various workers. The possibility that digitalis may increase the contractile force through its effect on energy metabolism was extensively examined (Wollenberger, 1951; Lee et al., 1960; Furchgott and Gubareff, 1958). It is now generally agreed that the metabolic effects of digitalis appear to be secondary to its effects on the movement of cellular electrolytes (Lee and Klaus, 1971). The physicochemical or enzymatic effects of cardiac glycosides indicate that the positive inotropic action is probably not mediated by their direct interaction with the contractile protein (Katz, 1966; Luchi and Kritcher, 1967; Lee and Klaus, 1971). Other evidence suggests that the positive inotropic response to digitalis is due to its effect on the phasic changes of the available free calcium ion in the myocardial cell (Langer, 1965; Ebashi and Endo, 1968; Bailey and Dresel, 1968).

Repke (1963) hypothesized that the transport ATPase (Mg$^+$-dependent, Na$^+$ and K$^+$ activated adenosine triphosphatase)
is the receptor site for digitalis. This has attracted a
great deal of interest because of the positive correlation
between the susceptibility of (Na\(^{+}\)K\(^{+}\))-ATPase to cardiac
glycoside inhibition and their respective potencies for
different species. In addition, a second cause of interest
has been the relationship between the specific binding of
digitalis to the enzyme and the inhibition of the enzyme
in vitro.

Recently, Besch et al. (1970) studied the effect of
ouabain on three distinct cellular systems: mitochondrial
ATPase, calcium accumulating capability of the sarcoplasmic
reticulum fragments and the (Na\(^{+}\)K\(^{+}\))-ATPase in the dog
heart. The results of their study indicate that the effect
of ouabain was perhaps related only by the inhibition of
(Na\(^{+}\)K\(^{+}\))-ATPase activity, not by any other cellular systems
studied. Akera et al. (1970) were also able to demonstrate
the causal relationship between the inotropic response and
(Na\(^{+}\)K\(^{+}\))-ATPase inhibition induced by ouabain in canine
myocardium. However, Lee and Klaus (1971) pointed out that
one should be cautious in drawing any conclusions on the
basis of the comparison between an in vitro determination
of (Na\(^{+}\)K\(^{+}\))-ATPase activity and an arbitrary inotropic
response observed in vivo. Mason et al. (1971) suggested
that the inhibition of the (Na\(^{+}\)K\(^{+}\))-ATPase might be more
closely allied to the arrhythmia or toxicity induced by
cardiac glycosides than to the positive inotropic response. In spite of these works, direct evidence of the relationship between positive inotropy and inhibition of the \((\text{Na}^{+}\text{K}^{+})\)-ATPase activity has been convincing yet incontrovertible.

Apart from the direct action of cardiac glycosides upon the myocardium, evidence indicates the possibility that cardiac glycosides may produce effects on the heart through the central nervous system. Recently, results have been reported by Gillis and colleagues (Gillis, 1969; Gillis, Dionne and Standaerdt, 1972). By simultaneously recording sympathetic and phrenic nerve activity with electrocardiographs, they demonstrated a relationship between enhanced neuronal activity and cardiac disorders following systemic injection of digitalis in intact and spinal cats.

Clinically, digitalis demonstrates an exceedingly narrow therapeutic range, therefore, arrhythmias are commonly observed. This is unfortunate because a high percentage of the population requires digitalis therapy at one time or another. Such use has led to a high incidence of intoxication (2.5 to 14-fold over that of 30 years ago), with death occurring in 3-39% of the patients receiving digitalis.

On the other hand, phenytoin was shown to be very effective in treating digitalis induced tachyarrhythmia. At the time of the early clinical studies of phenytoin in treatment
of epilepsy, certain cardiovascular effects were observed. As early as 1942, Finkelman et al. noted that administration of phenytoin resulted in electrocardiographic changes. However, Harris and Kokernot (1950) found that phenytoin prevents ectopic contractions in dogs subjected to acute operative coronary occlusions. Meanwhile, Mosey and Tyler (1954) showed that phenytoin reversed ouabain-induced ventricular tachycardia in 12 dogs. These investigators produced ventricular tachycardia with ouabain poisoning in 12 dogs. Phenytoin was given intravenously; in a short time, normal sinus rhythm was restored and heart rate improved with cessation of ectopic activity in all cases. Phenytoin has been successfully shown to antagonize ouabain-induced arrhythmias; however, the correlation between the antiarrhythmic mechanism of phenytoin and ouabain-induced dysrhythmias still is not understood. It is essential, therefore, that an earnest effort be made to study the electrophysiological properties and molecular mechanisms of this group of drugs at subcellular levels. The basic pharmacological as well as clinical problem is to identify the mechanism of digitalis-induced arrhythmias. In an attempt to understand the mechanism of these arrhythmias, it is proposed that an attempt be made to identify in dogs the structural and kinetic characteristics
of the binding site of phenytoin in heart and brain tissue, while this drug is successfully antagonizing ouabain-induced arrhythmias. Attempts will be made to determine time dependent changes in the appearance of this drug in the plasma and CSF.
CHAPTER II

REVIEW OF THE LITERATURE

In 1785, Withering was the first to successfully treat a dropsy woman with an herbal mixture and thus began his studies which led to the clinical introduction of digitalis. Since that period, the digitalis group of drugs has been established as the primary therapeutic agent in the treatment of cardiac diseases. The study of cardiac glycosides continues to fascinate both clinicians and laboratory scientists as a very interesting subject of investigation.

Information regarding the source and structural activity relations of cardiac glycosides has remained relatively static over the past 10 years. As generally used, the term "digitalis" refers to any of the steroid glycoside compounds that exert typical positive inotropic and electrophysiologic effects on the heart. All of these compounds, of which more than 300 are known to exist, contain a steroid nucleus with a five-membered or six-membered \( \alpha,\beta \) unsaturated lactone ring which characterizes the classes known as cardenolides or bufadicolides respectively, and are attached at the C\textsubscript{17}
position. The double bond of the lactone ring appears to be necessary for cardiac action and saturation results in either partial or complete loss of activity. A hydroxyl group in the \( \beta \) configuration at the \( C_{14} \) and \( C_{15} \) fusion of the C and D rings of the steroid nucleus is an additional requisite for cardiac activity. All structural requirements for activity are present in an aglycone (such as digitoxigenin), and the addition of one or more sugar residues at the \( C_3 \) position modifies potency and duration of action, but not the fundamental pharmacologic properties of this class of compounds. Henderson (1969) has reviewed the chemistry and biological activity of the cardiac glycosides in a concise fashion.

Ouabain is a cardiac glycoside which was discovered and named by Arnaud in 1888 (Jacobs and Bigelow, 1932). However, it was Sir Thomas Fraser who, in 1890, discovered its digititis-like action while studying African arrow poisons made with Strophanthus and introduced into medicine (Moe and Farah, 1967). Ouabain may be obtained from the seeds of Strophanthus gratus (Wall and Hook), Baillon or from the wood of Acokanthera schimperi (A. DC.), Schwf, (Claus and Tyler, 1965).
Jacobs and Bigelow (1932) were credited with establishing the preliminary structure of ouabain (Fig. 1).

Figure 1. Structure of Ouabain

Ouabain is a cardenolide with a steroid nucleus with the C-D rings in the cis configuration and a five-membered unsaturated lactone ring at C₁₇ and the glycone or sugar moiety rhamnose attached at the three position of the aglycone.

Although the action of cardiac glycosides has been under investigation since 1785, the use of digitalis in any form still involves many risks, primarily frequent toxicity. This is attributed mainly to the following reasons. First, there is inadequate information regarding the digitalis mechanism of action. Second, electrophysiologic, hemodynamic and pathophysiologic changes are still not completely understood. Finally, there is a lack of information about its effects on its metabolism, its excretion and its toxic effects.

A large amount of work has been done in these areas, and although there are few definite answers, a great deal has
been learned. Some of this information is of great help to the practicing clinician and some has enabled us to use these drugs on a more rational basis.

There are two important questions which, hopefully, will be answered in the next few years. One of these questions is what digitalis actually does. The second question is how digitalis produced its beneficial effects. The two favorable effects are (1) improvement in congestive heart failure; and (2) its effect on the electrical activity of the heart. The increase in electrical activity has been of great importance in helping to control a number of arrhythmias. Whether these two major effects are two distinct mechanisms or more, or are two aspects of the same effect is still unresolved. The action of digitalis on the electrical function of the heart might be attributed to changes in the fluxes or the concentration of the electrolytes which produce these electrical events, while the action of digitalis in improving congestive heart failure in a patient without arrhythmia is probably mediated through a change in mechanical rather than electrical effects, and through an increase in force of contraction of the cardiac muscle, i.e., an increase in inotropic state.

The effects of digitalis compounds have been shown to involve complex properties of cardiac muscle (contractility,
excitability), alterations of the refractory period, and control of impulse formation and control of impulse conduction. In addition, such cardiac glycosides affect renal function and the tone of peripheral vasculature.

Subcellular Mechanisms of Action

The positive inotropic augmentation of the action of digitalis could result from the following effects:

1. A favorable effect on the metabolism of myocardial tissue resulting in improved production and conversion of energy.
2. A change in the intracellular milieu which indirectly improves the contractility of cardiac actomyosin.
3. A direct effect on the mechanical efficiency of the contractible proteins.

Burdette (1952) and Crevasse et al. (1962) have shown that myocardial oxygen consumption increases prior to an increase in contractility when slices of cardiac tissue (not homogenates) are exposed to digitalis glycosides. The effect has been attributed (Crevasse, et al., 1962) to the uncoupling of oxidative phosphorylation, i.e., interference with the transfer of energy produced by oxidation or glycolysis to high energy phosphates. Evidence suggests that the
increased contractility following the administration of digitalis causes a secondary increase in oxygen consumption. However, investigations of Blain et al. (1956) and Goksel et al. (1963) have shown no change in $O_2$ consumption although increased contractility was recorded in the heart (where the heart work was kept constant).

There is experimental evidence to show that slices of cardiac tissue manifest an increase in oxygen consumption prior to an increase in positive inotropism of cardiac glycosides. However, Crevasse and others argue against the existence of any effect of digitalis on oxygen consumption or on energy metabolism. The argument is that in most instances of acute congestive heart failure, there is no change or defect in oxidation metabolism, no uncoupling of oxidative phosphorylation, and there is an adequate supply of high energy phosphates (Crevasse et al., 1966). Moreover, Blain et al. (1956) showed no alteration in the supply of high energy phosphates after effective treatment with digitalis. Lastly, Sonnenblick et al. (1966) have shown that there is an increase in contractility in normal hearts produced by digitalis where there is no defect in energy metabolism.

Investigators have shown that cardiacactive glycosides affect the contractility of proteins by affecting the
viscosity of actomyosin solution. Meanwhile, Luchi and Cown (1965) have indicated that the shortening of glycerol-extracted actomyosin strips could be increased by glycosides in some situations. However, Benson (1955) and Kay and Green (1964) designed similar studies which demonstrated the previous effect but failed to show this effect of cardiac glycosides on the viscosity of actomyosin solution. Where positive results were observed, systems far removed from the physiological environment were used. Holland, in 1964, attributed other positive results of glycosides to the action of digitals on the electrolytes in contaminating cell fractions. Investigators have also demonstrated that tritiated digoxin does not bind to carefully purified actin and myosin (Dutta et al., 1968; Fozzard and Smith, 1965; Sonnenblick et al., 1964).

An important observation known by workers for many years is that changes in the internal milieu of cardiac cells could be produced by digitalis. However, in cells and, in particular in cardiac muscle cells, it has been shown that toxic levels of digitalis consistently reduce the intracellular concentration of potassium ions. This reduction in intracellular potassium ion results from the slowing of its influx into the cell. In 1964, Holland demonstrated an increase in sodium concentrations and water in the cell.
The decreased influx after administration of cardiac glycoside could be due to inhibition of an active ion pump or to interference with passive, but carrier-mediated, ion transport. Recently, Albers (1967), Besch et al. (1970), Schwartz et al. (1968 and 1969) reported that cardiac glycosides inhibit a cell membrane ATPase (Na\(^+\)K\(^+\))-ATPase which supplies energy for active transport of ions across the cell membrane.

There is evidence showing a close relationship between (Na\(^+\)K\(^+\))-ATPase inhibition and the cardiac action of digitalis, specifically between membrane ATPase inhibition and cardiac activity (increased inotropism). Repke (1965) has shown that although inhibition of this enzyme system was well described, enzyme activity has been shown to be affected by changing electrolyte concentration; changes in the distribution of sodium and potassium ions by digitalis-induced interference with a carrier system might inhibit the enzyme secondarily. In recent years, workers have demonstrated that digitalis interferes with some other substances which are not actively transported, such as chloride ion and organic molecules, e.g., glucose. Luchi and Cown (1965) state that this effect might suggest the support of competitive interference by glycosides. Palmer et al. (1966) have also suggested that potassium ion could not only obliterate
the toxic manifestation of digitalis, but could also restore
normal activity of the membrane ATPase, supporting the latter
interpretation. However, since low doses of digitalis
appear to stimulate the enzyme and actually increase the
influx of potassium ion, strong support was demonstrated by
Repke (1963), Lee and Yu (1963) and Palmer and Nechay (1964)
for a direct effect on the enzyme rather than a mechanism
involving competition for carrier sites.

Studies performed by measuring A-V differences of potas-
sium ion have shown that a loss of potassium ion from the
heart is consistently associated with the increased contrac-
tility produced by digitalis. Holland et al. (1954) and
Carslake and Weatherall (1962) have shown that the increased
contractility produced by digitalis is not related to a loss
of potassium ion.

Clarke and Mosher (1952) have indicated that hearts in
failure, but compensated by digitalis, show no decrease and,
in certain cases, there is an increase in intracellular
potassium ion as compared to the potassium ion-depleted state
associated with congestive heart failure. Other workers
(Gonlubol et al., 1956) indicated no change in the concentra-
tion of potassium ion flux in ventricular muscle at thera-
peutic rather than toxic doses of digitalis. Some studies
(Lee et al., 1961) have observed that digitalis increases the
force of contraction before any change in potassium ion flux
occurs.
From all the evidence which has been mentioned, it is clear that the inotropic action of digitalis cannot be related to its effect on potassium ion alone. Because of that, there has been a search for other possible intracellular changes. However, several works have been done and many reports of either an increase in intracellular calcium ion or an increase in calcium ion flux at therapeutic doses of digitalis have been made, such as reported by Lee and Klaus (1971).

Klaus (1967) shows that there is probably a small pool of labile calcium ion when flux rate is increased by therapeutic doses of digitalis. However, the mechanism of how this increased lability of calcium ion affects contractility is not well understood. It is known that the immediate mechanism for cardiac contraction is the sliding and perhaps folding of actin molecules along the myosin molecules, resulting in shortening of the sarcomere. The energy source is ATP, which releases its high energy phosphate under the influence of myosin ATPase which can be found on bridges between the actin and myosin molecules. These bridges also contain calcium ion and magnesium ion. Calcium ion increases contraction and magnesium ion depresses it.

Since digitalis can be found in these approximate areas of the cell, it would seem that the action of myosin ATPase might be similar to the action of the membrane ATPase.
However, no report has demonstrated such an effect of digitalis on myosin ATPase. There is evidence that rather than stimulating myosin ATPase directly, digitalis acts by interfering with physiologic inhibitors of contraction. This inhibition is called the relaxing factor.

The relaxing factor has a high affinity for calcium ion and has been found to be a portion of the sarcoplasmic reticulum which surrounds every myofibril. In the polarized state, the relaxing factor and the Z-lines contain a high concentration of calcium ion. Upon depolarization, some of the calcium ion shifts to actomyosin, and shifts back to the relaxing factor during repolarization which permits relaxation. Digitalis might reduce ATPase and thus facilitate mobilization of calcium ion to actomyosin at the time of depolarization.

There are many other observations that are not related to the aforementioned explanation for the cellular action of digitalis on the heart. One of these is the repeatedly demonstrated greater tolerance of the female to toxic effects of digitalis as observed by Grinnell and Smith (1957) and Grinnell et al. (1961). The second is the observation affirmed by some but denied by others, that reserpineization to the point of near complete depletion of cardiac catecholamines but not denervation or beta blockade, will block the
positive inotropic effect of digitalis. If this is correct, it would suggest that the effect of digitalis on contraction is mediated through catecholamines. This has been suggested by Lee and Klaus (1971).

Among these observations, workers have shown the increased force of contraction produced by digitalis is more or less dependent upon the heart beating. The time of onset of increased positive inotropic action is inversely proportional to the heart rate. Vincenzi (1967) has noted that if cardiac tissue is incubated with digitalis for one-half hour and then washed, minimal to no significant increase in contractility is seen. Koch-Weser (1971) showed that although the development of myocardial effects of digitalis compounds do not require cardiac activity, frequency dependent changes in the functional state of the myocardium significantly affect the magnitude of the positive inotropic action of these drugs. How this dependence on heart beats fits with the effect of digitalis is not well known. While revealing many quantitatively important factors involved in activity, studies of speed of onset, and duration of effect have likewise failed to yield an answer to the mechanism of action.

In the last ten years, studies concerned with biologic activities of the cell and cellular substructure have begun to provide some insight into the basic mechanism of cardiac
function and have added some information in answering ques-
tions on the precise mode of drug effects. The ionic exchange
across cell membranes and their influence on excitability and
conduction, the behavior of contractible mechanisms in health
and disease and the interaction between these two (excitation-
contraction, coupling) have led to a remarkable though still
incomplete concept of the mechanism and site of glycoside
action.

Cardiac Glycoside Uptake and Subcellular Localization

According to a review of its long history, cardiac gly-
coside site of action as previously mentioned was determined
from various biochemical and biophysical responses to those
agents in vitro. This is particularly true of proposals re-
garding the effect of digitalis on the (Na\(^+\)+K\(^+\))-ATPase system
or calcium ion binding property of the sarcoplasmic reticulum
membranous fraction. Further investigation about the mechan-
isms of action of cardiac glycosides will require investiga-
tion of whether the heart, in general, or certain subcellular
fractions of the heart, in particular, bind digitalis prefer-
entially. Furthermore, if the response to digitalis is a
consequence of its binding to its receptor, it was logical
that as soon as supportive techniques to measure intracellular
concentration of digitalis became available, various workers
investigated whether digitalis underwent interaction with
any particular component of the cardiac cell.
As Okita et al. (1955) report, the liver accommodates about three times as much digitoxin and ten times as much digitoxin metabolites as does the heart. This confirmed earlier findings by Fischer et al. (1952) who showed that the heart possesses no greater affinity for cardiac glycosides. In a later study, Dutta and Marks (1966) showed a similar result with 16 organs of both guinea pigs and rats, though there are species' differences. Despite the lack of specific affinity of heart muscle for digitalis, it is the heart which is chiefly benefited by digitalis.

Although Harvey and Pieper (1952) found only 57% of digitoxin radioactivity in the supernatant fraction in isolated guinea pig heart, after intravenous administration of digitoxin to the rat, St. George et al. (1953) identified about 89% of it in supernatant fraction of heart, measured by the sensitive bioassay method of embryonic duck heart. Spratt and Okita (1958) obtained similar results using radioactive digitoxin in rats. With perfused bullfrog and canine heart-lung preparations, Fozzard and Smith (1965) could identify 60% of tritiated digoxin over the A-bands of the sarcomere, similar to the observation made by Tubbs et al. (1964). Sonnenblick et al. (1964) could identify the uptake of tritiated digoxin in the transverse tubular system of
myocardium. Conrad and Baxter (1964) attempted to correlate the change of myocardial contractility and the intracellular distribution of radiolabeled digoxin in the rat and dog heart. But, the significance of these studies is questionable.

In isolated guinea pig hearts, Dutta et al. (1968a and 1968b) showed the highest digitalis concentrations in the microsomal fractions. They proposed that the digitalis receptor existed in the light particulates derived chiefly from plasma membrane and sarcoplasmonic reticulum. This observation is significant because of a recently growing body of experimental evidence which indicates the (Na\(^+\)+K\(^+\))-ATPase which is prepared from the microsomal fraction has many remarkable characteristics as a possible receptor substance of cardiac glycosides.

In isolated guinea pig hearts, Dutta and Marks (1969) studied the characteristics of digitalis accumulation, including saturability of the ouabain uptake system, dependency of sodium and potassium ion concentrations in the perfusion medium and competition between pharmacologically active, structurally related compounds. Also, digitalis accumulation was affected profoundly by chemical agents which affect cellular energy metabolism. More recently, Baskin (1971) has tested whether the antiarrhythmic action of phenytoin is accomplished by a decrease of ouabain uptake.
in isolated guinea pig hearts which is true with only high doses of phenytoin.

**Electrophysiologic Effects of Cardiac Glycosides**

Haas (1972) suggested that the transmembrane resting potential of cardiac cells (80-90 mV) is maintained by sodium and potassium ion gradients (particularly the latter), which are in turn dependent upon the activity of the sodium-potassium ion pump. Therefore, it is appropriate to consider that agents such as cardiac glycosides that inhibit the pump mechanism have significant effects on the electrophysiology of the intact heart as well as isolated muscle preparations. Now there is a general agreement that inhibition of (Na⁺+K⁺)-ATPase underlies toxic effects on the cardiac rhythm rather than the inducing inotropism due to cardiac glycosides.

Despite major advances indicated by many workers in the field of cardiac electrophysiology, many unanswered questions remain concerning therapeutic and toxic effects of digitalis on the electrical activity of the normal and diseased heart. Currently, most investigators agree that cells in various parts of the heart show differing sensitivities to digitalis, and both direct and neurally mediated effects must be dissected before final conclusions are reached concerning the mechanism of action of cardiac glycoside.
Hoffman and Singer (1964) were able to demonstrate that within the specialized conduction tissues of the heart, the refractory period is increased by digitalis and conduction velocity is diminished, tending to slow the ventricular response to atrial fibrillation and atrial flutter, or to prolong the P-R interval in the presence of normal sinus rhythm. In atrial and ventricular myocardium, on the other hand, the refractory period tends to be shortened and the more rapid recovery time is reflected in a shortening of the Q-T interval of the electrocardiogram.

Studies concerning the effects of digitalis glycosides on transmembrane action potentials in vitro have been subjected to investigation by many workers. These studies have been directed for the most part at the mammalian ventricular Purkinje fiber and the effects observed are generally assumed to have relevance to electrophysiologic changes occurring elsewhere. Vassalle and Hoffman (1962) and Kassebaum (1963) showed that electrophysiologic effects of cardiac glycosides vary with concentration, duration of exposure, and rate of stimulation. Hoffman et al. (1972), Dreifus et al. (1972) and Hoffman et al. (1969) demonstrated that at low concentrations, the resting potential, action potential amplitude and time course of depolarization and repolarization remain unchanged at the time when inotropic effects are first apparent.
On the other hand, at higher cardiac glycoside concentrations, and particularly at more rapid stimulation rates, there is progressive loss of resting potential. Changes occur in the time course of depolarization and repolarization, including decreased slope of the upstroke of the action potential, shortening of the plateau phase and increased rate of spontaneous diastolic depolarization. Because of the controversial points of view regarding the effective cardiac glycoside concentration in the conduction system of normally digitalized patients, it has been difficult to tell which of these effects are part of "therapeutic" effects of digitalis and which represent "toxic" effects that would occur only at doses incompatible with life.

Recently, Rosen et al. (1973) showed experimental data on isolated canine Purkinje fibers which were perfused with the blood of infant donor dogs, and ouabain-induced changes in the donor dog's electrocardiogram were correlated with changes in the Purkinje fiber transmembrane potential. At the time of onset of early ouabain toxicity in the donor dog, defined as junctional or ventricular premature contractions or junctional tachycardia, Purkinje fiber recording showed decreases in action potential amplitude, resting membrane potential, and maximum velocity of the upstroke of the action potential. Slowing of conduction was also
apparent, often varying in extent from cycle to cycle. With further progression of toxicity to ventricular tachycardia in the donor dog, changes in transmembrane potential became still more marked. In further experiments, increased automaticity (increased slope of Phase 4 spontaneous diastolic depolarization) was found to occur at the time of early toxicity at plasma potassium ion concentrations below 4 mEq per liter. Also of interest in these studies was the occurrence of slow, graded depolarizations (low amplitude potentials) during Phase 4, more or less simultaneous with onset of ouabain-induced junctional or ventricular rhythm disturbances in the donor dog.

Increased automaticity was more frequent when Purkinje fibers had been stretched and in the presence of hypokalemia, which correlates well with clinical observations of increased frequency of digitalis toxicity in the dilated failing heart as well as in the presence of hypokalemia. There is, as yet, no unequivocal answer to the question of whether ectopic beats and sustained tachyarrhythmias arising as a clinical manifestation of digitalis toxicity occur as a result of increased automaticity (i.e., increase spontaneous diastolic depolarization) or re-entry. It may well be that both mechanisms are operative at different times.
A new area of significant progress in recent years has involved neuronal-mediated effects of cardiac glycosides. Although workers have indicated certain evidence of neuronal effects of few cardiac glycosides, this phenomenon is still controversial among others and more evidence is required to support this mechanism of neuronal mediation of cardiac glycosides.

Moe and Farrah (1970) have demonstrated that vagal effects have long been known to be involved in the influence of digitalis on atrioventricular conduction, although many investigators have a controversial opinion concerning sympathetic effects of cardiac glycosides. According to Tanz (1964), sympathetic nerve activity plays an important role in cardiac muscle contraction. Meanwhile, Gillis et al. (1969 and 1972) correlated sympathetic nerve activity in cardiac dysrhythmia and cardiac glycoside toxicity, while Daggett et al. (1965) and Mason (1964) showed evidence pointing to a digitalis-induced withdrawal of sympathetic tone, particularly with amelioration of the state of congestive heart failure. Gillis et al. (1969 and 1972) have provided direct evidence from nerve recordings that ouabain can influence preganglionic cardiac sympathetic nerve activity in allobarbital-urethane anesthetized cats. At the toxic dosage, sympathetic nerve
activity was substantially augmented and high intensity activity was temporarily correlated with ventricular tachyarrhythmias, including fatal ventricular fibrillation.

Additional brainstem effects were manifested as ouabain-induced enhancement of traffic in vagus and phrenic nerves, the latter resulting in hyperventilation. Furthermore, Gillis (1972 and 1973) demonstrated that spinal cord section prevented all of these effects and increased the dose of ouabain required to produce ventricular arrhythmias. Propranolol administration reduced ouabain-induced neuronal hyperactivity and usually converted ventricular arrhythmias to normal sinus rhythm. These studies support the concept that neuronal activation by cardiac glycosides may play an important role in the development of cardiac rhythm disturbances and hyperventilation. Furthermore, Wallace et al. (1967) and Kellihcr et al. (1972) demonstrated, through animal studies, that sympathetic ablation by surgical or chemical methods increased the dose of cardiac glycoside required to cause rhythm disturbances. This also tends to change the mode of death from ventricular tachycardia followed by ventricular fibrillation to atrioventricular block and cardiac standstill.

As shown by Gillis (1972) and Wallace (1967), propranolol and other β adrenergic blocking agents cause marked changes in cardiac response to toxic doses of cardiac glycosides,
but interpretation of these experiments is complicated by the existence of direct antiarrhythmic effects of these agents in addition to their adrenergic blocking properties. Also, according to Kelliher (1973), this alteration of sympathetic neural function may also be dependent on the particular cardiac glycoside. Dutta et al. (1977) indicated in cats that no specific localization of ouabain in any of the neural areas could provide evidence for local accumulation of digitalis glycosides that might account for a central nervous system origin of digitalis-induced cardiac arrhythmias.

**Digitalis Toxicity**

Rodensky (1961) has shown that although cardiac glycosides remain as one important inotropic agent in cardiac therapy, then toxicity remains to be considered a serious cardiac emergency when it causes ectopic arrhythmias, conduction defects and suppression of sinus pacemaking functions. Guiffra (1952) has estimated that 6%-20% of patients receiving these glycosides develop digitalis toxicity because of the narrow range that exists between the therapeutic and toxic dose. In fact, it has been shown by Lown (1954) that the therapeutic dose is approximately 60% of the toxic dose. According to Szent (1952) and Hellems (1955), there are a number of factors which precipitate digitalis toxicity: (1) overdose, (2) individual idiosyncracies, (3) electrolyte
imbalance following potassium ion loss due to diarrhea or gastric intubation, etc., and (4) calcium ion administration to the digitalized patient causing a sudden potassium ion exodus from cardiac muscle. However, Wedd (1939) and Corday (1961) suggest a common cause of digitalis toxicity in hypokalemia resulting from use of saluretic drugs in conjunction with digitalis.

Other workers agree that there are no specific arrhythmias due to digitalis toxicity. When the glycosides are used in excess, or if there is an associated loss of potassium ion, ectopic arrhythmias are apt to appear. These may consist of premature atrial systoles, atrial tachycardia, flutter or fibrillation, ventricular tachycardia and fibrillation, arrest in pacemaking function in the S-A node, or depression of the normal conduction system in the atria, A-V node or ventricle, resulting in sinoatrial, intra-atrial, atrioventricular and bundle branch block, interference, dissociation of idioventricular rhythm.

Among drugs used in the treatment of ectopic arrhythmias caused by digitalis toxicity are potassium salts. Although it has been proven by many workers that these salts are valuable in the treatment of digitalis toxicity, on occasion, the administration of potassium may be ineffective. It is thought that digitalis can prevent potassium ion from
re-entering the cell during the resting period by blocking the permeability of the cell membrane. It has now become increasingly apparent that potassium, as shown by Page (1955) and Fisch (1960), must be administered with great caution because excessive or too rapid administration may result in serious, and often fatal disturbances in conduction and rhythm. Guber (1957) and Bernstein (1959) demonstrated that the calcium chelating agent, trisodium edetate (sodium versenate) can be effective in correcting cardiac arrhythmias. Its undesirable toxicity and adverse effects, such as profound hypotension, have deterred clinicians from using this agent in the treatment of digitalis intoxication. Recently, Corday and Barbieri (1964) have shown that the citrate compounds, which also chelate calcium ion, are able to correct digitalis-induced cardiac arrhythmias. Also, it has been suggested that these compounds may be useful both as a diagnostic test for arrhythmias associated with digitalis intoxication and for its treatment.

The high morbidity and mortality of digitalis toxicity make it necessary to search for other effective agents to correct digitalis-induced arrhythmias. Phenytoin has been shown by Bernstein (1965) to be effective in the prevention and treatment of acute and recurrent cardiac arrhythmias.
Phenytoin and Digitalis Toxicity

Chemistry

Biltz (1908) synthesized phenytoin (Fig. 2), also known as Dilantin which was later introduced by Merritt and Putnam (1938). Toman (1965) noted that phenytoin is related to other hydantoins such as ethotoin and mephenytoin, as well as being related to barbiturates such as phenobarbital. The only difference is that the hydantoins have a five membered structure in contrast to the six membered barbiturates and also that the point of difference lies in the absence of a C=O (carbonyl group).

Figure 2. Structure of Phenytoin

Anticonvulsant Effect of Phenytoin

Phenytoin is highly effective in the treatment of grand mal, focal motor and psychomotor epilepsy and, in addition, has been found useful in several other neurologic conditions unrelated to epilepsy, but is of no value in petit mal
epilepsy. In their experiments, Putnam and Merritt (1938) electrically induced convulsions in cats. They demonstrated that phenytoin was much more effective in controlling these convulsions than were the bromides and phenobarbital. They also confirmed the earlier findings that phenytoin was not a sedative. These authors applied their laboratory findings to clinical use with phenytoin, treated a group of 118 patients with chronic grand mal attacks who had not responded to treatment with bromides and phenobarbital. The results were dramatic in that 58% of these intractable cases became free of attacks and 27% showed marked improvement without sedation. The mechanism of action whereby phenytoin depresses neuronal excitability is not well known. But Toman (1949) proposed that phenytoin acts as a stabilizer of excitable cellular membranes including peripheral nerves, skeletal, and cardiac muscle membrane as well as in the central neurons.

**Antiarrhythmic Effect of Phenytoin**

In the time of the early clinical studies of phenytoin in the treatment of epilepsy, certain cardiovascular effects were observed. As early as 1942, Finkelman *et al*. noted that administration of phenytoin resulted in electrocardiographic changes. Harris and Kokernot (1950) found that phenytoin prevents ectopic contractions in dogs subjected to acute
operative coronary occlusion. By using a method of gradual coronary occlusion, they showed that cardiac ischemia suppressed ectopic discharges in these hearts. Confidence in the interpretation of the results was gained by the observation of quick diminution of frequency of ectopic complexes almost immediately after injection of phenytoin. When phenytoin was not present, these ectopic complexes returned.

Slow intravenous administration of phenytoin produced little or no change in blood pressure or respiration. The authors found in their experiments that it was possible to control all ventricular tachycardia by adequate amounts of phenytoin. Mosey and Tyler (1954) showed that phenytoin reversed ouabain-induced ventricular tachycardia in 12 dogs. The authors noted that Harris (1950) had made the observation that tissues of the heart and those of the central nervous system both responded to phenytoin insofar as ectopic activity was concerned. They decided that it would be valuable to determine if phenytoin would be useful in the regulation of ventricular tachycardia. The authors induced ventricular tachycardia with ouabain poisoning in 12 dogs and gave phenytoin intravenously. In a short time, in all cases, normal sinus rhythm was restored and heart rates improved with cessation of ectopic activity.
Leonard (1958) demonstrated the beneficial effect of phenytoin in controlling ventricular hyperirritability complicating myocardial infarction in the patient. This appears to be the first recorded experience of the use of phenytoin in the treatment of cardiac disorders in humans. Dreifus et al. (1964), reviewing newer agents in the treatment of cardiac arrhythmias, stated that phenytoin appeared to be effective in both supraventricular and ventricular mechanisms and possessed properties which made it effective against digitalis-induced arrhythmias. It has been found successful in preventing paroxysmal atrial tachycardia when usual antiarrhythmic agents have failed. The authors stated that when used for the treatment of rapid supraventricular or ventricular tachycardia, it can be injected slowly, intravenously. Further, phenytoin can also be administered orally for suppression of ectopic beats or as a prophylaxis against recurrent paroxysmal tachycardia.

The authors pointed out that phenytoin exerted its activity in the nervous system without general depression. It was also noted that the action of phenytoin on peripheral nerves was to stabilize the neuronal membrane and to decrease the intracellular content of sodium ion. They noted further that it was possible that the mechanisms of action of phenytoin on the heart muscle and the nervous tissue were basically similar.
Bernstein (1965) used oral phenytoin prophylactically in the prevention of recurrent cardiac arrhythmias. The authors defined the overall response as excellent. They found it of particular significance that the entire group consisted of patients who were proved refractory to or intolerant of conventional modes of prophylactic antiarrhythmic therapy. Conn (1965) found that phenytoin administered intravenously to 24 patients with a variety of cardiac arrhythmias was particularly effective in supraventricular and ventricular arrhythmias resulting from digitalis excess. It was also beneficial in controlling paroxysmal atrial and ventricular arrhythmias. In three cases of atrial fibrillation and two with atrial flutter, no therapeutic effect was noted. Toxicity consisted of transient bradycardia and hypotension in one patient and short-term atrioventricular block with bradycardia in another. The author stated that phenytoin appears to be a significant addition to the drug therapy of cardiac arrhythmias. Ruthen (1965) reviewed some examples of the effectiveness of phenytoin in cardiac arrhythmias and drew attention to the electrophysiologic evidence that phenytoin stabilizes cell membranes.

Lugo et al. (1966) reported the therapeutic effectiveness of oral phenytoin in chronic cardiac disease with multifocal extrasystolic ventricular arrhythmias. Phenytoin was
used in 11 cases of this type in the dose of 100 mg four times a day. The authors state that the use of phenytoin is a contribution to the treatment of this particular arrhythmia. Helfant et al. (1967) studied the effects of intravenous phenytoin on atrioventricular conduction in man at constant heart rates in the digitalized and undigitalized state. It was suggested that when digitalis excess is manifested, phenytoin would have special utility since, in contrast to the commonly used antiarrhythmic agents, it enhances A-V conduction in addition to suppressing ectopia.

Conn (1967), in a review, refers to phenytoin as one of the new antiarrhythmic agents along with propranolol and lidocaine. He discussed the efficacy of these agents and their toxicity. The author stated that phenytoin has been found to have predictable antiarrhythmic effects and that it has been used in the treatment of a great many patients with cardiac arrhythmias. It is suggested that antiarrhythmic effects are consistent with the evidence from studies of the basic mechanisms of action. These indicated that phenytoin is a stabilizing agent against repetition stimuli, the result of which is an "antispreading effect" from an area of abnormal discharge. In comparison with other antiarrhythmic agents, Lieberson et al. (1967) pointed out that two of the most commonly used antiarrhythmic agents, quinidine
and procainamide, often produce undesirable hemodynamic effects. One reported effect is depressed myocardial function; the other well known effect is significant systemic hypotension during intravenous administration. The effect of phenytoin given intravenously on left ventricular function was determined during cardiac catheterization in nine patients with heart disease. The authors stated that although this study demonstrated that phenytoin depresses myocardial function, the effect may have limited clinical significance since it was relatively short-lived and did not reduce cardiac output or greatly elevate ventricular end-diastolic pressure. Furthermore, the absence of a significant systemic hypotensive effect with phenytoin is of distinct clinical importance. It is the opinion of the authors that phenytoin is a safer medication for cardiac arrhythmias when given intravenously than is quinidine or procainamide.

Karliner (1967) confirmed the usefulness of phenytoin in a variety of cardiac arrhythmias, especially those which appear to be related to digitalis excess. Rapidity of action and relative paucity of side effects, when used properly, make phenytoin an effective antiarrhythmic agent.

Mercer (1967) reviewed the literature, including the safety, toxicity and basic mechanisms of action, and
reported on his own experience in the treatment of ventricular arrhythmias associated with anesthesia, cardioversion, cardiac catheterization, and cardiac surgery. On the basis of his experience, he also considered phenytoin to be superior to quinidine and procaainamide. Also in this respect, Scherlag (1968) compared the effects of phenytoin and procaainamide on A-V conduction in digitalis-intoxicated and normal hearts. Phenyltoin consistently converted more acetylsalicylic acid-induced ventricular tachycardia to sinus rhythm than did procaainamide and completely restored A-V conduction to control values. In contrast, procaainamide, in doses necessary to counteract digitalis-induced ventricular tachycardia, invariably exacerbated the A-V conduction prolongation produced by the glycosides.

Droph (1968) reported that phenytoin is useful in arrhythmias due to digitalis intoxication; a dilute solution of 100 mg given slowly, intravenously, had been used with very good success. Wehrmacher (1969) recommended oral administration of phenytoin (100 mg) every six to eight hours and indicated that oral maintenance could be carried on for months or years for treatment of arrhythmias. Furthermore, the author stated that phenytoin is particularly valuable for the control of arrhythmias which occur as a result of digitalis intoxication, myocardial infarction and cor pulmonale.
Cardiovascular Mechanism of Phenytoin in Digitalis-Induced Dysrhythmias

Direct Myocardial Mechanism of Action

Bigger (1970) studied the effects of phenytoin on excitability and automaticity in the canine heart. The author reported that phenytoin (1) shortened the refractory period, particularly of ventricular muscle, (2) increased the multiple response and fibrillation thresholds in the atrium and ventricle, (3) had little effect on diastolic threshold, (4) slightly enhanced the conduction velocity of ventricular muscle that was stimulated either in diastole or in its relative refractory period, and (5) produced slight decreases in automaticity in the ventricular specialized conducting system in vivo. Also, in reporting on clinical experience, the author states that this experiment suggests that phenytoin differs from quinidine in its effect on fundamental electrophysiological processes in the human heart. Phenytoin does not prolong the Q-R-S interval, but does shorten the Q-T interval. These observations indicate that the drug does not prolong intraventricular conduction and suggests that it shortens the refractory period.

Goldstein et al. (1973) stated that the efficacy of phenytoin in treating digoxin toxicity is well established. In reporting the results of an extensive study of isolated
dog hearts, the authors stated that phenytoin, when added to digoxin, enhanced the inotropic benefits of digoxin. The authors found that before toxic digoxin levels were reached, phenytoin ultimately produced greater increases in contractile force, greater potassium ion efflux and greater inhibition of (Na\(^+\)+K\(^+\))-ATPase activity. The authors suggest that these are mechanisms which enable phenytoin to enhance the inotropic benefits of digoxin. Hansen (1974), by using both isolated atrium and barbiturate-damaged heart/lung preparations of guinea pigs, showed that the toxic, arrhythmic side effects of digoxin are prevented by phenytoin without affecting its inotropic benefits. By the addition of phenytoin, glycoside dosages can be beneficially increased. The authors conclude that phenytoin offers new aspects for the treatment of cardiac failure.

Scherlag et al. (1968) demonstrated in dogs that phenytoin consistently converted digitalis-induced ventricular tachycardia to sinus rhythm with a corresponding reversal of the digitalis-induced potassium ion efflux. Although the toxicity of digitalis was markedly delayed and myocardial contractility improved, increased dosage of digitalis was not affected by phenytoin. Baskin, Dutta and Marks (1973), in a study of the guinea pig heart, showed that phenytoin and
potassium significantly prevent ouabain intoxication without preventing the inotropic benefits of ouabain.

Watson et al. (1973), studying ouabain intoxication in guinea pigs, observed that phenytoin prevented ouabain-induced electrolyte changes and prevented the development of ouabain-induced arrhythmias. Under some conditions, pretreatment with phenytoin reduced the lethality of ouabain from 90% to 34%. The authors concluded that these results were consistent with the concept that the antiarrhythmic effect of phenytoin is due to an action on the transport of electrolytes across cell membranes, since plasma potassium ion concentration was normalized by phenytoin pretreatment.

It has been shown by many electrophysiologists that the primary mechanism of phenytoin in preventing arrhythmias is due to its pronounced effect on the conduction system of the heart. For instance, Bissett et al. (1974) directly measured conduction in the His Purkinje system in 14 patients and found that phenytoin improved intraventricular conduction. The purpose of their study was to determine the effect of phenytoin on intraventricular conduction in man. Utilizing the introduction of premature atrial beats before infusion of phenytoin, they showed that His Purkinje conduction delay occurred with right bundle branch block in nine patients, and with left bundle branch block in five patients. After
infusion of phenytoin (5 mg/kg at a rate of 50 mg/min), the onset or degree of His Purkinje delay was improved in all patients. The results of this study significantly demonstrate that phenytoin does improve intraventricular conduction in man.

Anderson et al. (1973) studied the effect of phenytoin on cardiac conduction in patients who suffered from myotonic dystrophy. They found that phenytoin was beneficial, not only for the myotonia, but also for cardiac conduction defects common in this disease. In five of the eight patients treated with oral phenytoin, the P-R interval was shortened by 5-35 msec. This was in marked contrast to quinine and procainamide. Quinine produced P-R interval prolongation in four of ten patients, and procainamide produced P-R interval prolongation in nine of ten patients. The authors' studies indicate diffuse involvement of the His Purkinje conduction system with procainamide and quinine, but not with phenytoin. Furthermore, Benaim et al. (1972) reported on 15 patients with arrhythmia who were injected with doses of 5-10 mg/kg of phenytoin. Recordings of the His potential achieved during the therapeutic test showed that (1) the drug does not usually alter the frequency of the sinus node, (2) it definitely improves atrioventricular conduction; (3) in most cases, it does not alter
intraventricular conduction and H-V remains constant. The authors suggested that phenytoin is valuable in arrhythmias, where these are accompanied by atrioventricular conduction defects.

Damato et al. (1970) showed that in 13 patients, phenytoin enhanced atrioventricular conduction (i.e., shortened the P-H interval) over various paced heart rates. Also, phenytoin did not prolong intraventricular conduction as measured by H-Q intervals.

In spite of these observations concerning the anti-arrhythmic effects of phenytoin on the myocardium, the exact molecular mechanism of action in correcting cardiac arrhythmias has not been elucidated.

Recently, a number of investigations have been concerned with elucidating the mechanism of the interaction of phenytoin with the cardiac glycosides. Scherlag et al. (1968) and Helfant et al. (1968) suggested that phenytoin exerts its antiarrhythmic effect on cardiac glycoside intoxication through a specific antagonism of the toxic actions of digitalis. Meanwhile, these investigators reported a reversal of the myocardial K⁺ venoarterial difference induced by toxic doses of the cardiac glycoside. However, in contrast to the latter observations, Miller and Gilmore (1970) have reported that phenytoin fails to alter K⁺ efflux induced by acetylstrophanthidin. Also, they explain that the myocardial K⁺
loss demonstrated by Scherlag and Helfant (1968) was mainly secondary to the tachycardia-induced by digitalis and that reversal by phenytoin was due to abolition of the tachycardia and not specific reversal of the digitalis effect. Spain and Chidsey (1971) showed that phenytoin administration did not reverse (Na⁺+K⁺)-ATPase inhibition in animals made toxic with ouabain. Nor did phenytoin reverse enzyme activity where it was added in vitro to ouabain-inhibited enzyme. In addition, Gibson and Harris (1969) found no effect of phenytoin on (Na⁺+K⁺)-APTase activity in the microsomal fraction from human myocardium or any reversal of the inhibitory effects of ouabain. More recently, Goldstein et al. (1973) reported that perfusion of the isolated canine heart preparation with phenytoin combined with digoxin delayed the development of toxic arrhythmias but resulted in an even greater degree of inhibition of (Na⁺+K⁺)-ATPase. McCans et al. (1973) also demonstrated in man lack of effect of phenytoin on ouabain-induced K⁺ loss. Based on these observations, we can safely say that phenytoin alters digitalis arrhythmias but by some mechanism other than stimulation of (Na⁺+K⁺)-ATPase.

Central Nervous System Action of Phenytoin

Previously, several reports have drawn attention to the possibility that cardiac glycosides may produce effects on
the heart through the central nervous system (CNS) apart from any direct action on the myocardium. This centrogenic effect of phenytoin in digitalis-induced arrhythmias is still controversial. Some investigators believe now that at least part of the antiarrhythmic effect of phenytoin in digitalis-induced arrhythmias may be due to altered nervous system function. Gillis et al. (1971, 1972) have shown that activation of the sympathetic nervous system may be involved in the arrhythmogenic effects of the cardiac glycosides. Also, they have provided direct evidence from nerve recordings that ouabain can influence preganglionic cardiac sympathetic nerve activity in allobarbital-urethane anesthetized cats. At toxic doses, sympathetic nerve activity was substantially augmented and high intensity activity was correlated temporally with ventricular tachyarrhythmias. Administration of phenytoin depressed the glycoside-induced enhanced nerve activity and converted ventricular tachycardia to sinus rhythm. Raines et al. (1970) reported that phenytoin increased the lethal dose of ouabain in spinal cats with high adrenergic neural activity, but did not alter it in animals in which spinal cord transsection resulted in a substantial reduction in adrenergic activity. According to Ferrier et al. (1973) and Rosen (1973), phenytoin reduces adrenergic neural activity in digitalis-induced arrhythmias when this activity
is enhanced. However, digitalis glycosides, in larger doses, can produce arrhythmias in animals in which the sympathetic response is removed. In spite of these observations, the question of cardiac glycosides inducing arrhythmia through neuronal activation is still an important, controversial point among many investigators. The relative importance of the neuro-depressant effects as opposed to direct myocardial effects require further investigation.

**Pharmacokinetics of Phenytoin**

**Distribution, Absorption and Excretion.** Noach et al. (1958) comprehensively studied the absorption and excretion of phenytoin. Rats of 300 gm body weight were injected intravenously with 22 mg of 4-C\(^{14}\) labeled phenytoin. Within five minutes after injection, high concentrations of free phenytoin were found in the liver, kidney and salivary glands with lower concentrations found in the brain, body fat, and skeletal muscle. All tissue concentrations were higher than the plasma concentrations. Phenytoin concentrations in all organs decreased to zero within 24 hours. A second peak occurred one and one-half hours after drug administration. Comparison of radioactivity measurements with the values for free phenytoin showed that alteration of phenytoin takes place mainly in the liver, in agreement with the conclusion of Swinyard et al. (1952). After 24 hours, 50% of the
administered radioactivity was present in the gastrointestinal tract, and the remainder was found in the urine. Since 93.7% of the radioactivity appeared in the urine with 48 hours, it was concluded that phenytoin and its metabolites are excreted into the gastrointestinal tract due to biliary excretion of these compounds. Phenytoin is also secreted by the salivary gland in cats; in rats, such secretion was suggested by the high concentration of phenytoin in these glands. Since the major part of the radioactivity in the bile was present in acidic extracts, whereas neutral urine extracts contained most of the radioactivity, it was assumed tentatively that a conversion of phenytoin metabolites found in the gastrointestinal tract takes place before their urinary excretion. Toman (1965) showed that the principal urinary metabolite in dogs and man results from parahydroxylation of a single phenyl group which is sufficient to destroy the anticonvulsant actions of phenytoin. Meanwhile, Maynert (1960) showed that hydroxylation of the same site or of the other phenyl group can also occur and may be followed by glucuronide formation. Maynert (1960) and Loeser (1961) reported that more than 48 hours are required for excretion of N\(^{15}\) labeled phenytoin in feces and urine in dog, rat, and man. Butler (1957) reported that about one-half of
administered phenytoin appears in the urine in man as 5-(p-hydroxyphenol), 5-phenylhydantoin (HPPH); also, variations in excretion have been reported among different ages in dog and man.

Petty (1965), comparing the anticonvulsant effect of phenytoin in young and old animals, found a difference in peak time of action for phenytoin. In young mice, the peak time was 30 minutes; however, in old mice, peak time was 10 hours. Also, Svensmark et al. (1960) noted individual variations in the metabolism of phenytoin after oral or intravenous administration as reflected in human spinal fluid, saliva, and blood levels. Jensen et al. (1966) examined individual variation in the excretion of phenytoin after intravenous injection. The elimination after a single intravenous injection of phenytoin was nonexponential and showed individual variation, but was constant in the individual patient and independent of the urinary pH. Cucinell (1966) observed, in agreement with Jensen, that where the same amount of phenytoin is given, the rate of disappearance of the drug from plasma is variable from individual to individual. However, Cucinell stated that this rate can vary over a wide range in the same person under different conditions. The rate of decay was found to depend upon prior or concomitant administration of other chemical substances to the individual. For instance phenobarbital was found capable of increasing the rate of metabolism of phenytoin. Also, Frey et al. (1968), studying
the metabolism of phenytoin with and without phenobarbital in dogs, found considerable individual variation in the degree of interaction of the two agents. Variations in excretion and toxification of phenytoin among diseased animals have also been reported.

Kutt et al. (1964) observed the metabolism of phenytoin in 15 patients with liver disease. At normal daily dosages, phenytoin intoxication was observed in five patients. They concluded that a lowering of the ability to metabolize phenytoin may be present in liver disease. Triedman et al. (1960) drew attention to the manner in which the route of administration and dosage schedule of phenytoin affects the level of phenytoin in the plasma. To maintain a uniform maximum level, repeated oral doses of phenytoin at 12 hour intervals were required. The concentration of phenytoin in the cerebrospinal fluid was found to be equal to the fraction of plasma phenytoin not bound to plasma protein.

**Entry and Binding of Phenytoin in the Brain.** Firemark et al. (1963) reported that phenytoin penetrated the brain rapidly and concentrations two to three times greater than plasma levels were found in all brain regions for 24 hours after injection. Phenytoin was found in the brain and plasma. The drug concentration in cerebrospinal fluid (CSF) was equivalent to the concentration in ultrafiltrates of the
brain and plasma. That was consistent with the findings of Noach et al. (1958) that the brain and plasma of rats contained predominantly unchanged phenytoin. According to Firemark et al., phenytoin stands in sharp contrast to all other compounds except thiopental, another fat soluble drug. The prolonged delay in penetration of phenytoin into adult white matter could not be attributed solely to blood flow since the white matter did not attain its highest concentration until one hour after injection while adult cerebral cortex, for example, contained its highest concentration of the drug within two minutes. In addition, the magnitude of the accumulation in each brain area did not vary directly with lipid content. Also, Firemark et al. found that circulation was a determining factor in the entry of phenytoin into gray matter.

Cerebral gray matter was penetrated more quickly than white matter. The concentration of phenytoin in white matter increased during the first hour following a single injection, but rose only slightly thereafter. At 3, 6, and 24 hours, the concentration of phenytoin in white matter exceeded that in cerebral cortex. The concentrations of phenytoin in all areas of brain tissue at 24 hours were only lower than the levels at 3 hours, illustrating unusual retention of this drug. Furthermore, between 30 minutes and 24 hours following
injection, the concentrations of phenytoin in all brain areas were two to three times greater than the total concentration in plasma. Equilibrium was rapidly achieved between the unbound concentration of phenytoin in plasma, CSF and brain water.

In the kitten brain, the components which govern total accumulation of phenytoin increase in the course of maturation. The progressive increase of binding in brain with increasing age, as demonstrated by ultrafiltration, was considered to be an additional manifestation of the change due to neurochemical and structural maturation. These studies indicated that the myelin lipid compounds are not the prime factor involved in the accumulation of phenytoin. The authors speculated that the affinity for phenytoin in the brain may be due to binding to protein constituents of brain. The authors expressed the opinion that the exceptionally high binding of phenytoin in the brain correlates with the persistence of the drug in this organ after a single dose and serves to explain its long pharmacological action. The authors feel it was possible that the interaction of phenytoin with protein, lipoprotein, or other constituents of the brain could be a reflection of interaction with neural membranes or with enzymes which function in maintaining the neuronal ion permeabilities and polarization on which normal neuronal activity depend.
Nakamura et al. (1966) showed that the oral administration of extremely large doses (100 mg/kg/day) of phenytoin to dogs and cats for more than one year resulted in preferential accumulations of the drugs in the superior and inferior colliculus, amygdala and hippocampus as compared to 16 other cerebral areas examined without any manifestation of neurotoxicity. Also, Nakamura (1967) found phenytoin concentrated in the pituitary and adrenal glands. In addition, Rosenblum and Stein (1963) demonstrated that phenytoin localizes preferentially in human primary brain tumors when compared to adjacent normal brain tissue, while on the other hand, in experimental subcutaneous mouse ependymomas and adenocarcinomas this preferential localization did not occur. Kemp and Woodbury (1962) studied the intracellular distribution of C\textsuperscript{14} phenytoin in rats' brains at intervals between 15 and 240 minutes. Initial fixation of phenytoin in the cell nucleus was suggested followed by transfer to microsomes for fixation or metabolism.

**Plasma Concentration and Half-Life of Phenytoin.** Arnold et al. (1970) and Svensmark et al. (1960) reported in man that phenytoin is slowly absorbed from the gastrointestinal tract with peak blood levels 8-12 hours after oral administration. After intravenous injection, the immediate peak blood level declines rapidly over a 20-40 minute interval.
due to distribution of the drug to the body tissues. According to VanGelder et al. (1971) the apparent volume of distribution (aVd) in normal individuals has been estimated at 0.64 to 0.72 l/kg. Svensmark et al. (1960) also reported that phenytoin has a high affinity for fat and protein and it is concentrated in the liver, kidney, salivary glands, brain, adipose tissue, and muscle. Noach et al. (1958) reported that following sustained oral or parenteral administration, the blood concentration declines exponentially at a rate of 35-55% per day, with a plasma half-life of approximately 24 hours.

Very few studies have been done concerning the plasma level and the antiarrhythmic effect of phenytoin and more investigation is required to establish the reasonable therapeutic levels of phenytoin and antiarrhythmic effect. Bigger et al. (1968) and Kutt et al. (1964) have indicated that a close correlation exists between the successful control of arrhythmias and serum levels between 10-18 mg/liter, while toxic effects occur when serum levels exceed 20 mg/liter in human. Also, Bigger et al. reported that in order to obtain therapeutic blood levels rapidly, they recommended that 50-100 mg of phenytoin be given intravenously every five minutes until the arrhythmias is abolished (until 1,000 mg are given) or until undesirable effects appear. Arnold et al.
(1970) suggested an oral loading dose of 1,000 mg on day one, 500 mg on the second and third days, and maintenance doses of 300-400 mg daily, thereafter. Dreifus et al. (1964) advised one intravenous dose of 5-19 mg/kg of body weight slowly over a 15-minute period with possible repetition in two or three hours. Conn (1967) advised a single intravenous dose of 3.5-5.0 mg/kg of body weight administered in one to three minutes. However, Bigger et al. (1968) have shown that a single intravenous dose of 300 mg of phenytoin will maintain a therapeutic blood level for only a few minutes. These clinical observations confirm the fact that phenytoin has a long half-life of about 24 hours and initial loading doses are required to establish rapidly a therapeutic blood level.

**Effect of Phenytoin on Cardiac Parameters.** The effect of phenytoin on the physiological cardiac parameters is still controversial among investigators. Puri (1971) reported that phenytoin, when administered intravenously in therapeutic doses, is associated with some depression in myocardial contractility and a reduction in the peripheral vascular resistance. Lieberson (1969) reported an increase in left ventricular end-diastolic pressure during phenytoin administration in patients with heart disease. Also, Mixter et al. (1966) reported a reduction in cardiac output and a fall in
left ventricular dp/dt after phenytoin administration. These effects are quite transient and rarely last more than 15-20 minutes after intravenous administration. Similar hemodynamic abnormalities have not been documented during maintenance of oral phenytoin therapy. In comparison with the above studies, where propylene glycol was used as a solvent for phenytoin, Gillis (1971) was able also to demonstrate that phenytoin has some depressory effect on the force of contraction, blood pressure and heart rate in a solution where phenytoin was dissolved in 0.85% sodium chloride and brought to pH 12 with NaOH. Furthermore, he indicated that this depressory effect is dose dependent and it is significant with a larger dose such as 8-16 mg/kg phenytoin.
CHAPTER III

OBJECTIVES AND RATIONALE

Digitalis remains one of the most important positive inotropic drugs in clinical use today since its introduction into clinical therapy by Withering in 1785. However, cardiac glycoside-induced arrhythmias are common because digitalis demonstrates an exceedingly narrow therapeutic range. In fact, it is now recognized that digitalis intoxication is among the most common of adverse drug reactions and may provoke arrhythmias and conduction disturbances in as many as 25% of patients receiving this drug for treatment of congestive heart failure (Mason et al., 1971b, Massumi et al., 1972a). These cardiac toxic manifestations result from the electrophysiological actions of digitalis of (1) increasing automaticity and excitability, (2) shortening the refractory period, and (3) prolonging conduction velocity and diminishing responsiveness in atrial, junctional, and Purkinje tissues. Each of the digitalis preparations is equally capable of producing digitalis toxicity and no special advantage is achieved by the use of one glycoside over another in terms of extracardiac reactions or toxicosis.
Three potential mechanisms involving ionic alterations for cardiac glycoside-induced arrhythmias are related to calcium, sodium, and potassium ions. However, sodium-potassium-activated ATPase has been implied by Matsui and Schwartz (1968), Besch et al. (1970), Akera and Brody (1970) and Repke and Portius (1963) to be the pharmacological receptor for digitalis. As a consequence of the effect of cardiac glycosides on (Na\(^{+}\)+K\(^{+}\))-ATPase ionic changes might occur and result in arrhythmogenic effects. Repke (1963) hypothesized that the inhibition of (Na\(^{+}\)+K\(^{+}\))-ATPase by cardiac glycosides is causally related to the positive ionicotropic effect of digitalis. This is still controversial among many investigators. In fact, a number of investigators (Spain and Chidsey, 1971; Goldstein et al., 1973; Rhee et al., 1976) have attempted to answer the questions of (1) whether there is a parallelism between onset of digitalis-induced arrhythmia and inhibition of (Na\(^{+}\)+K\(^{+}\))-ATPase activity, and (2) whether the reversal of glycoside arrhythmia is associated with parallel recovery of (Na\(^{+}\)+K\(^{+}\))-ATPase activity. These investigators were able to demonstrate a marked inhibition of (Na\(^{+}\)+K\(^{+}\))-ATPase prepared from hearts of digitalis-induced arrhythmic dogs. However, they were unable to show any recovery of enzyme activity when the arrhythmia was
abolished with phenytoin. However, we can precisely say that at the time of this investigation, the data necessary to conclusively link (Na+K+)-ATPase with an arrhythmogenic site in the myocardium is missing.

Although phenytoin has been successfully used in the treatment of cardiac arrhythmia which has been primarily induced by digitalis toxicity (Bigger et al., 1968; Helfant et al., 1967a; Jensen and Katzung, 1970), the relationship between digitalis-induced arrhythmia and antiarrhythmic effect of phenytoin is still unresolved. Considerable studies have been made on phenytoin's antiarrhythmic action, but the precise mechanism of the action is still unknown. As suggested by Entman et al. (1972) and Mason et al. (1971), if the digitalis-induced toxicity is due to the inhibition of (Na+K+)-ATPase, the immediate experimental approach is to test the effect of phenytoin on the activity of (Na+K+)-ATPase.

Akera et al. (1970) studied the effect of antiarrhythmogenic KCl infusions on (Na+K+)-ATPase activity in dogs. It is of interest that KCl was able to reverse the arrhythmia-induced by ouabain to normal sinus rhythm without relieving the inhibitions of (Na+K+)-ATPase activity induced by ouabain. In this aspect, Spain and Chidsey (1971) could not demonstrate a stimulatory effect of phenytoin on (Na+K+)-ATPase
prepared from dog myocardium although the medium of high
Na\textsuperscript{+}/K\textsuperscript{+} (50 mM/0.2 mM) ratio stimulated (Na\textsuperscript{+}+K\textsuperscript{+})-ATPase
activity significantly. However, Woodbury (1955) and Watson
and Woodbury (1972) suggested that the antiarrhythmic pro-
property of phenytoin might be due to the stimulation of
(Na\textsuperscript{+}+K\textsuperscript{+})-ATPase activity.

One of the fundamental questions related to pharmacology
is the localization of the drug with relation to biological
actions (Leake, 1961). Since the accumulation of a drug at
a biological site may be related to an ionic mechanism of
action, it might prove fruitful to investigate this aspect
of cardiac glycosides.

Spratt and Okita (1955) showed that from in vivo studies
over 90% of unchanged \textsuperscript{3}H digitoxin was found in the soluble
supernatant fractions of liver, heart, and kidney cells of
the rat. For the particular fractions of the rat heart,
5.4%, 1.9%, and 1.2% were present in the microsomes, mito-
chondria and nuclei, respectively.

The subcellular distribution of \textsuperscript{3}H digoxin was further
studied (Dutta et al., 1968b) in guinea pig and rat hearts.
Dutta and his coworkers found in both rats and guinea pigs
that the subcellular fraction which accumulated the greatest
\textsuperscript{3}H digoxin ng/g of tissue was the microsomal fraction. The
concentration of atrial $^3$H digoxin was approximately two-thirds that of the ventricle in the guinea pig heart at all times. They suggest from this study that microsomal binding may be directly related to the differences seen in the biological activities in the rat and guinea pig. In further investigations into the nature of accumulation of cardiac glycosides, Dutta and his coworkers (1968b) provide evidence, based on distinct differences in the effect of K$^+$ on the uptake of different cardiac glycosides, for the existence of more than one "receptor site" in reticulum fragments.

Baskin (1971) tested the possibility that antiarrhythmic effects of phenytoin and KCl were produced by reducing the accumulation of ouabain in isolated perfused guinea pig heart. He was able to conclude that the antiarrhythmic effect of KCl and phenytoin are mediated by the reduction of accumulation of ouabain because there was a significant decrease of ouabain content in myocardial fraction when the antiarrhythmic effect of KCl and phenytoin were evident. However, the effective phenytoin concentration was as high as $10^{-4}$ M in the perfusion medium.

Previous reports have drawn attention to the possibility that cardiac glycosides may produce effects on the heart
through the central nervous system (CNS), apart from any
direct action upon the myocardium. Gillis (1969) and
McLain (1969) demonstrated that administration of ouabain
results in dose related increments in sympathetic, para-
sympathetic and phrenic nerve activity. Meanwhile, Gillis
et al. (1972) demonstrated that the increments in nerve
activity are forerunners of ventricular rhythm disorders
and death. They further observed that spinal cord trans-
section prevents neural hyperactivity induced by ouabain
and increases the dose of ouabain needed to produce death.
Levitt et al. (1973) demonstrated that exclusion of the
sympathetic nervous system by spinal cord transsection
increases the dose, serum level and tissue level of ouabain
needed to produce ventricular fibrillation or asystole.
Also, Cagin et al. (1974) observed that the tissue level of
ouabain needed to produce "death" in vitro in the absence
of neural influence was substantially greater than that
needed to produce death in vivo. On the basis of this
work, it appears that digitalis toxicity in the intact
animal and presumably in man is largely a neural phenomenon
whereas intoxication in the isolated heart probably results
from the direct myocardial effect of the drug.

Moreover, drugs with the capacity to depress sympathe-
tic nervous activity have been demonstrated to be effective
in the blockage of digitalis toxicity. Thus, Levitt et al. (1970) demonstrated that propranolol, reserpine, n-isopropyl-p-nitro-phenylethanolamine (INPEA), guanethidine, and phenytoin among others have been demonstrated to increase the dose of digitalis needed to produce toxic rhythm disorders and death.

Dutta et al. (1977) were unable to demonstrate in cats any preferential localization of cardiac glycosides, namely ouabain and digitoxin in any specific neuronal tissue examined. Recently, however, Dutta and his coworkers (unpublished observations), through a careful examination of the central nervous system as a possible site of digitalis induced dysrhythmia, have demonstrated that certain areas of the brain localize ouabain preferentially (such as choroid plexus, area postrema, and pituitary gland). Moreover, Firemark et al. (1963) reported previously an "unusual and sustained accumulation" of phenytoin in the brain of the cat. Those studies indicated CNS localization of cardiac glycosides implicating the CNS in the dysrhythmogenic effect of digitalis. Although phenytoin was efficacious in converting dysrhythmias induced by cardiac glycosides, the mechanism of its antiarrhythmic effect is not well understood. This study, then, is a systemic evaluation to answer the following questions:
1. What is the localization of ouabain in various organs and brain regions in dogs?

2. Does the antiarrhythmic dosage of phenytoin affect the preferential localization of ouabain by the various organs and brain regions?

3. Upon administration of phenytoin to reverse ouabain-induced dysrhythmia in the dog, what is the steady-state level of the drug in plasma and CSF?

4. What is the localization of antiarrhythmic dosage of phenytoin in the various organs and brain regions?
CHAPTER IV

MATERIALS AND METHODS

Animal Preparation and Physiological Measurements

Both male and female mongrel dogs weighing 10-24 kg were used for all experiments. Animals were anesthetized intravenously with pentobarbital sodium (25 mg/kg) without supporting medication. After induction of anesthesia, an endotracheal tube was inserted and dogs were artificially respirated with room air (Harvard Apparatus Co., Dover, MS, Model 607). The dogs were ventilated at a rate of 20 strokes per minute with an appropriate tidal volume. Radiopaque catheters were inserted into the right femoral artery and right femoral vein (Fig. 3) for the purpose of measuring blood pressure and administering drugs, respectively. Drugs were administered through a polyethylene catheter at an appropriate infusion rate.

The left ventricular pressure was recorded through the left femoral artery into the lumen of the left ventricular chamber. The end of the catheter was connected to a pressure transducer (Statham P23AC transducer, Becton, Dickinson
Figure 3. A schematic diagram for the animal preparation showing the catheters in the right femoral artery and left femoral artery for the purposes of measuring the blood pressure and left ventricular pressures, respectively. A polyethylene catheter was inserted into the left femoral vein for administering the drug with a Desaga peristaltic pump. A 22-gauge spinal needle was inserted into the cisterna magna for serial collection of CSF.
and the ventricular pressure was recorded on one channel of the Beckman Type R Dynograph (Beckman Instruments Inc., Schiller Park IL). The rate of change of the left ventricular $dp/dt$ was recorded by means of a resistance-capacitance (R-C) differentiator and displayed on another channel of the dynograph. End-diastolic pressure (EDP) was also measured by increasing the sensitivity of the amplifier of the left ventricular pressure. Since the EDP did not change significantly in most of the experimental procedures, $dp/dt_{\text{max}}$ was employed as an estimate of myocardial contractile performance. Maximum $dp/dt$ was measured in millimeters of positive deflection and expressed as percent change from the equilibrium period ($t_0$). Systemic arterial blood pressure was recorded on one channel of the dynograph. A 22-gauge spinal needle was inserted into the Cisterna magna for serial collection of cerebrospinal fluid (CSF) as shown in Fig. 3. Arterial blood samples were collected serially through the femoral catheter. Electrocardiogram lead II and sometimes leads II and III simultaneously were monitored on the Beckman Dynograph using one of three different paper speeds (10 mm/sec, 25 mm/sec or 50 mm/sec).

In this study, nonlabeled ouabain was purchased from Nutritional Biochemicals Corp., Cleveland OH, and tritiated
ouabain (S.A. 13 curies/mM) was obtained from New England Nuclear Corp., Boston MS. Unlabeled phenytoin was purchased from Parke-Davis, Detroit MI. Radioactive phenytoin (\(^{14}\)C-phenytoin) was obtained from New England Nuclear Corp., Boston MS, and Amersham/Searle, Arlington Heights IL with S.A. 235.4 \(\mu\)Curies/mg for both sources. Radiochemical purity was 98%.

Effects and Tissue Distribution of \(^{3}\)H-Ouabain Administered Alone

In a group of seven preliminary experiments, a series of control measurements were recorded after 30-60 minutes of control period to assure stabilization of physiological parameters. After that, blood pressure, peak systolic left ventricular pressure, rate of change of the left ventricular pressure (\(dp/dt_{\text{max}}\)) and electrocardiogram lead II were recorded. Simultaneously, control samples of arterial blood and CSF were taken for the measurement of plasma and CSF concentrations of ouabain as a control value. At zero time, a 40 \(\mu\)g/kg loading dose of \(^{3}\)H-ouabain in 5 ml of saline was injected via the femoral vein over a one minute period. Following the loading dose, ten minutes later, an infusion of \(^{3}\)H-ouabain at .072 \(\mu\)g/kg/min was administered by a Desaga peristaltic pump through a polyethylene tube into the femoral vein. A series of measurements of cardiac parameters (peak systolic left ventricular pressure, systemic
arterial pressure, rate of change of the left ventricular pressure $dp/dt_{\text{max}}$, and electrocardiograph lead II) were recorded after the administration of ouabain and for the entire experimental duration. Blood samples were collected at 15, 45, 90, and 120 minutes, after the administration of the bolus dose. Upon the termination of the experiment at 120 minutes, the animal was sacrificed by using Uthol$^R$ intravenously. Through a midline sternotomy, the chest was exposed and 30-50 mg of the myocardium were obtained for radioactive measurement. The abdomen was also exposed and samples from the liver, kidney, spleen, adrenal and skeletal muscle were obtained for radioactive measurement.

The cranial cavity was exposed gently through a complete circular line around the occipital, temporal, and frontal bones on both sides of the cranial cavity, using a special cutting edge drill as shown in Fig. 4. The whole brain was elevated from the cranial cavity and taken out for specific dissection. Figure 5 shows a simple diagramatic illustration of the mid portion of the brain with the most common areas of interest. Specifically in this set of experiments, certain areas of the brain such as the pons, medulla oblongata (deep, fourth ventricle, and ventral portions of the medulla oblongata), area postrema and choroid plexus only
Figure 4. Illustrating the procedure of exposing the cranial cavity using an electrical cutting drill through a circular line around the occipital, temporal, and frontal bones of both sides of the cranial cavity.
Figure 5. A schematic illustration of brain after exposure of the cranial cavity.
were selected for determination of $^{3}H$-ouabain. Peripheral, neuronal and non-neuronal tissues were dissected very carefully. Instruments were washed in saline solution between dissection to prevent contamination of radioactivity from tissues. Tissue weight was measured with electronic balance and expressed in gram tissue wet weight.

In an additional group of dogs, a loading dose of 40 $\mu$g of ouabain/kg body weight and an infusion dose of 0.072 $\mu$g/kg/min were administered for five hours. A series of cardiac hemodynamic parameters, blood and CSF samples were taken for control measurement as described in the previous experiment. Blood and CSF samples were obtained at 15, 30, 45, 90, 120, 150, 180, 240, and 300 minutes for measurement of $^{3}H$-ouabain. Peripheral tissues were obtained as described for the previous experiment. The following brain regions were taken for measurement of $^{3}H$-ouabain: cerebellum, optic chiasm, lateral ventricle, and fourth ventricle of the choroid plexi, visual cortex, medulla oblongata (ventral, fourth ventricle and deep portions of medulla oblongata), area postrema, pons, mesencephalon, olfactory bulb, anterior and posterior hypothalamus, anterior and posterior pituitary gland and pineal gland. Samples of 30-50 mg of each section of brain, myocardium, liver, kidney, spleen, adrenal, skeletal muscle, and gum
were taken to determine their radioactive content. A commercially available phase combining system (Amersham/Searle) was used as the scintillation fluid. Radioactivity in the tissue, blood plasma and cerebrospinal fluid (CSF) was determined by a liquid scintillation spectrometer. Sufficient net counts were taken to assure a counting error within a permissible range as determined by Loevinger and Berman (1951). All samples were counted for a period of time sufficient to limit the counting error to 5% or less. All counts were corrected for quenching by means of a quenching chart prepared using a series of quenched standard samples compared with the external standard counts. From the specific activity of ouabain, disintegrations per minute (dpm) were converted into picomoles of ouabain and that data were expressed as picomoles of ouabain per gram of wet tissue. Since there are no known metabolites of ouabain in this species, the radioactivity in each fraction can be assumed to be due to unchanged $^{3}$H-ouabain (Rhee, 1976).

Determination of Pharmacokinetic Parameters of Phenytoin

A single bolus injection of 10 mg $^{14}$C-phenytoin/kg was given. Control measurements of hemodynamic parameters, blood, and CSF were obtained as described previously. Over a period of five hours, physiological and hemodynamic
parameters were recorded at different time intervals after administration of $^{14}$C-phenytoin for the purpose of comparison with control values. In this set of experiments, blood samples were obtained at 2, 5, 10, 20, 30, 45, 90, 120, 150, 180, 210, 240, 270, and 300 minutes after administration of phenytoin. However, cerebrospinal fluid (CSF) was obtained at 3, 9, 15, 35, 50, 80, 110, 140, 170, 200, 230, 260, and 300 minutes following administration of phenytoin. At 300 minutes, the experiment was terminated and samples of peripheral, neuronal and non-neuronal tissues were obtained for the determination of radioactive phenytoin content. Plasma and CSF concentrations of phenytoin at each time were plotted on semilog paper against time for the purpose of calculation of pharmacokinetic parameters.

The apparent volume of distribution (aVd) was calculated by using an equation of aVd equal to total dpm injected/dpm/l. The dpm/l was obtained by extrapolating concentration to time (0) from the log phenytoin plasma concentration time curve. The elimination rate constant of phenytoin ($K_e$) was obtained by using the equation

$$K_e = \frac{0.693}{t_{\frac{1}{2}}}$$

where $t_{\frac{1}{2}}$ equals the time for $C_T/C_0 = \frac{1}{2}$. In order to administer phenytoin so that it maintained a constant plasma level throughout the experimental period,
it was necessary to administer phenytoin at the rate it was being eliminated during the time interval under study. In kinetic terms, this is expressed by effective plasma concentration and elimination rate \((K_e)\) times the apparent volume of distribution \((aVd)\). The loading dose was calculated based on the equation: 

\[
\text{loading dose} = \frac{\text{infusion rate}}{.0693/T_1^1}
\]

The following peripheral organs were selected for determination of \(^{14}\text{C}-\text{radioactivity}: \text{myocardium, liver, kidney, skeletal, adrenal, spleen and gum, and sections of the brain; cerebellum, optic chiasm, lateral and fourth ventricles of choroid plexi, visual cortex, medulla oblongata (ventral, deep and fourth ventricle), area postrema, pons, mesencephalon, olfactory bulb, anterior and posterior hypothalamus. Non-neuronal areas of brain, anterior and posterior pituitary, and pineal gland were also taken for determination of radioactive content.}

**Tissue Distribution of Phenytoin in the Presence of Ouabain**

Experiments were conducted to determine the tissue distribution and effectiveness of phenytoin in competing ouabain induced dysrhythmia. In this group of animals, a loading dose of 40 \(\mu\text{g/kg}\) and an infusion of \(.072 \mu\text{g/kg/min}\) of unlabeled ouabain were administered intravenously by a Desaga peristaltic pump through a catheter into the femoral
vein. This loading and infusion dose of ouabain produced a sustained dysrhythmia up to five hours. After the confirmation of 130 minutes of sustained dysrhythmia, a loading dose of 12 mg/kg and an infusion dose of 30 μg/kg/min of 14C-phenytoin were administered for 120 minutes. The protocol was such that following ouabain infusion, the persistence of dysrhythmia was confirmed for up to three hours, then the desired dose of phenytoin was administered for the next two hours in the presence of ouabain infusion. Control physiological and hemodynamic parameters were recorded before and after ouabain and phenytoin administration. Blood samples were obtained throughout the two hour period of 14C-phenytoin administration at 10, 20, 30, 45, 60, 75, 90, 105, and 120 minutes after the start of the bolus dose. Meanwhile, CSF samples were taken over a period of 120 minutes at 15, 30, 45, 90, 105, and 120 minutes, respectively, after administration of 14C-phenytoin. Upon the termination of the five hour experiments, peripheral, neuronal and non-neuronal tissues were obtained as described for the previous experiment. Blood, CSF, and tissue samples were prepared for determination of 14C-phenytoin as described previously for ouabain.
Effects and Distribution of $^{14}$C-Phenytoin When Administered Alone

In this group of dogs, an experiment was conducted to determine effects, plasma, CSF and tissue distribution of $^{14}$C-phenytoin when administered alone. These values were obtained for the purpose of comparison with values when phenytoin was administered in the presence of ouabain. A loading and infusion dose of $^{14}$C-phenytoin which was established in the previous experiment to combat ouabain induced dysrhythmia were administered over a period of two hours. Physiological and hemodynamic parameters were recorded before and after $^{14}$C-phenytoin administration. Blood samples were obtained at 10, 20, 30, 45, 60, 75, 90, 105, and 120 minutes after the start of the bolus dose. Cerebrospinal fluid was obtained at 15, 30, 45, 60, 75, 90, 105, and 120 minutes after administration of radioactive $^{14}$C-phenytoin. At the termination of the experiment, peripheral, neuronal and non-neuronal tissues were taken for determination of $^{14}$C-phenytoin.

Effect of Phenytoin on the Distribution of Ouabain

This group of experiments was performed for the purpose of determining tissue distribution of ouabain in the presence of phenytoin. Control measurements were recorded prior to injection of either drug. At zero time, a loading dose of
40 ug $^3$H-ouabain/kg body weight and an infusion dose of 0.072 ug/kg/min was administered. After confirmation of three hours of sustained dysrhythmia, a loading dose of 12 mg phenytoin/kg of body weight was followed by an infusion of 30 ug/kg/min for the next two hours. This dose produced sustained sinus rhythm up to the end of the experiment where peripheral, neuronal, and non-neuronal tissues were obtained for radioactive ouabain determination.

Blood and CSF samples were taken at 15, 30, 90, 120, 180, 240, and 300 minutes for measuring plasma concentration of $^3$H-ouabain.

All statistical analyses were carried out using Hotellings $T^2$ multivariants comparison test. All values which gave $p$ greater than 0.05 were considered to be not significant in this study.
CHAPTER V

RESULTS

Hemodynamic Effects and Tissue Distribution of $^3$H-Ouabain When Given Alone

A single intravenous bolus dose of 40 µg of ouabain per kg of body weight followed by a constant infusion of 0.072 µg/kg/min produced steady-state blood concentrations of 67±22 pmole/ml at 45 minutes after onset of infusion (Table 1). Highest blood levels were measured in the first blood sample collected (i.e., 15 min). Concentrations of ouabain in cerebrospinal fluid varied between 12.6±4 and 11.3±3 pmole/ml.

After 15 minutes of ouabain infusion, left ventricular contractility and mean systemic arterial pressure rose significantly ($p < 0.05$); the heart rate slowed, but the decrease was not statistically significant (Fig. 6). Ventricular dysrhythmias were not observed at this time. By 30 minutes, myocardial contractility and mean systemic arterial pressure were significantly higher ($p < 0.05$) than at 15 minutes (Fig. 7). The mean systolic arterial pressure remained constant, and sinus rate decreased. At 60 minutes, all dogs manifested sustained ventricular dysrhythmia, many
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Plasma (Pmole/ml ± S.E.M.)</th>
<th>CSF (Pmole/ml ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>108±22</td>
<td>8.2±3</td>
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<tr>
<td>30</td>
<td>66±23</td>
<td>12.3±5</td>
</tr>
<tr>
<td>45</td>
<td>67±22</td>
<td>12.6±4</td>
</tr>
<tr>
<td>90</td>
<td>51±23</td>
<td>11.5±4</td>
</tr>
<tr>
<td>120</td>
<td>53±26</td>
<td>13.6±5</td>
</tr>
<tr>
<td>150</td>
<td>55±24</td>
<td>12.2±4</td>
</tr>
<tr>
<td>180</td>
<td>58±29</td>
<td>13.1±4</td>
</tr>
<tr>
<td>240</td>
<td>69±33</td>
<td>11.3±4</td>
</tr>
<tr>
<td>300</td>
<td>44±19</td>
<td>11.3±3</td>
</tr>
</tbody>
</table>
Figure 6. The effect of arrhythmogenic doses of ouabain on the dp/dt, BP, LVP and ECG lead II and 15 minutes' duration.
CONTROL 15 MINUTES AFTER QUABAIN ADMINISTRATION
Figure 7. The effect of arrhythmogenic doses of ouabain on the dp/dt, BP, LVP and ECG lead II at 30 and 60 minutes.
manifesting ventricular tachycardia. This tachycardia was sustained for up to 300 minutes (Fig. 8). In all instances, as judged by the predominantly negative QRS complex in lead II, the focus for dysrhythmia was within the conduction system of the left ventricle.

In a preliminary study on seven dogs at two hours after administration, ouabain was highly concentrated in the heart, choroid plexus, and area postrema (Fig. 9), achieving concentrations of 18±3, 7±7, and 2±0.2 times that of plasma, respectively. The pons, deep portions of the medulla, ventral portion of the medulla, and the fourth ventricular portion of the medulla achieved levels of only a fraction (approximately 25%) of that in plasma.

In three additional dogs, tissues were removed after five hours of ouabain infusion. All tissue concentrations were expressed as pmole/g and as a ratio of concentrations in both plasma and CSF (Table 2). Arbitrarily, any organ containing greater than five times that in plasma was defined as having a "relatively high affinity" for ouabain; while organs containing concentrations greater than 10 times that found in plasma were defined as having an "exceptionally high affinity" for ouabain. Using these definitions, it
Figure 8. The effect of arrhythmogenic doses of ouabain on dp/dt, BP, LVP and ECG lead II and 180 and 300 minutes.
180 MINUTES AFTER QUABAIN ADMINISTRATION

300 MINUTES AFTER QUABAIN ADMINISTRATION
Figure 9. Accumulation of $^3$H-ouabain in the heart, choroid plexus, area postrema (A. post.), plasma (PLAS), fourth ventricle of the medulla (Med. F.V.), deep portion of the medulla (Med. Dp.) and pons.
follows that the kidney, heart, and non-neuronal tissues (choroid plexi of the lateral and fourth ventricle) had an exceptionally high affinity while peripheral tissues (adrenal gland) and neuronal tissues (area postrema) had a relatively high affinity for ouabain (Table 2).

Pharmacokinetic Parameters and Dosing for Phenytoin

Figure 10 shows the average time course of disappearance of phenytoin from blood and CSF from four dogs following intravenous doses of 10 mg/kg. Table 3 shows the mean values plus the standard error of the mean for each time interval. The apparent volume of distribution (aVd) was 0.57±0.06 L/kg; the elimination rate constant (Ke) was 0.16/hr and the biological half-life (T½) for the terminal phase of elimination was 265 minutes. Based upon these parameters and a desired blood level of 20 µg/ml, a loading dose of 12 mg/kg followed by a constant infusion dose of 30 µg/kg/min was calculated.

\[
C_{pss} = \frac{\text{infusion rate}}{\text{total body clearance}} = \frac{\text{infusion rate}}{K_eV_d}
\]

Therefore, infusion rate = \(C_{pss} K_eV_d\)

\[
= (20 \, \mu g/ml) \times (0.0027 \text{min}^{-1}) \times (570 \, ml/kg)
\]

\[
= 30.4 \, \mu g/kg/min
\]

Loading dose (L.D.) = \(\frac{\text{infusion rate}}{0.693/T_{\frac{1}{2}}} = V_d C_{pss}\)

\[
= 11.69 \, mg/kg
\]
<table>
<thead>
<tr>
<th>Samples</th>
<th>Pmole/g ± S.E.M.</th>
<th>T/P ± S.E.M.</th>
<th>T/CSF ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>453± 40</td>
<td>15.2± 5.0</td>
<td>54.6±22.0</td>
</tr>
<tr>
<td>Liver</td>
<td>152± 44</td>
<td>4.2± 1.0</td>
<td>20.7±12.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>1365±778</td>
<td>65.0±45.0</td>
<td>98.7±52.0</td>
</tr>
<tr>
<td>Skeletal</td>
<td>90± 25</td>
<td>2.5± 0.8</td>
<td>12.1± 6.0</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>300± 76</td>
<td>8.0± 1.0</td>
<td>46.0±30.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>87± 15</td>
<td>2.6± 0.8</td>
<td>11.0± 5.0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>74± 33</td>
<td>2.5± 1.0</td>
<td>6.9± 1.0</td>
</tr>
<tr>
<td>Optic chiasm</td>
<td>68± 28</td>
<td>1.5± 0.4</td>
<td>11.4± 8.0</td>
</tr>
<tr>
<td>L.V. choroid plexus</td>
<td>369± 70</td>
<td>14.0± 7.0</td>
<td>39.9±11.0</td>
</tr>
<tr>
<td>4th V. choroid plexus</td>
<td>358± 6</td>
<td>14.5± 8.0</td>
<td>37.2± 9.0</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>41± 6</td>
<td>1.2± 0.5</td>
<td>2.3± 0.5</td>
</tr>
<tr>
<td>Vent. port. med.</td>
<td>48± 24</td>
<td>1.5± 0.8</td>
<td>4.8± 1.0</td>
</tr>
<tr>
<td>Deep port. med.</td>
<td>51± 5</td>
<td>1.7± 0.6</td>
<td>6.1± 2.0</td>
</tr>
<tr>
<td>4th vent. med.</td>
<td>56± 7</td>
<td>1.6± 0.4</td>
<td>7.9± 4.0</td>
</tr>
<tr>
<td>Area postrema</td>
<td>187± 23</td>
<td>6.3± 2.0</td>
<td>21.8± 8.0</td>
</tr>
<tr>
<td>Pons</td>
<td>63± 8</td>
<td>2.1± 0.8</td>
<td>7.2± 2.0</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>59± 15</td>
<td>2.0± 0.7</td>
<td>6.4± 2.0</td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>76± 25</td>
<td>2.5± 1.0</td>
<td>7.8± 2.0</td>
</tr>
<tr>
<td>Ant. hypothalamus</td>
<td>96± 44</td>
<td>3.2± 1.0</td>
<td>9.1± 2.0</td>
</tr>
<tr>
<td>Post. hypothalamus</td>
<td>69± 7</td>
<td>2.2± 0.7</td>
<td>8.6± 4.0</td>
</tr>
<tr>
<td>Ant. pit. gland</td>
<td>386±113</td>
<td>14.8± 6.0</td>
<td>35.7± 2.0</td>
</tr>
<tr>
<td>Post. pit. gland</td>
<td>1633±578</td>
<td>58.1±24.0</td>
<td>158.0±31.0</td>
</tr>
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<td>Pineal gland</td>
<td>77± 12</td>
<td>2.2± 1.0</td>
<td>10.5± 1.0</td>
</tr>
<tr>
<td>Gum</td>
<td>57± 15</td>
<td>2.2± 1.0</td>
<td>6.1± 1.0</td>
</tr>
</tbody>
</table>
Figure 10. A plot of phenytoin plasma and CSF concentrations (ordinate) against time (abscissa) after a single bolus injection of 10 mg/kg
PHENYTOIN KINETICS

AVd .57 L/kg
K e .16/hr
TABLE 3

Plasma and CSF Concentrations of Radioactive Phenytoin After a Single Bolus Injection of 10 mg/kg

<table>
<thead>
<tr>
<th>Time minutes</th>
<th>Plasma nmole/ml ±S.E.M.</th>
<th>Time minutes</th>
<th>CSF nmole/ml ±S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>77±17</td>
<td>3</td>
<td>1.2±0.7</td>
</tr>
<tr>
<td>10</td>
<td>109±27</td>
<td>9</td>
<td>4.2±1.0</td>
</tr>
<tr>
<td>20</td>
<td>33±14</td>
<td>15</td>
<td>7.9±3.0</td>
</tr>
<tr>
<td>30</td>
<td>71±11</td>
<td>35</td>
<td>12.6±4.0</td>
</tr>
<tr>
<td>45</td>
<td>64±3</td>
<td>50</td>
<td>13.5±5.0</td>
</tr>
<tr>
<td>60</td>
<td>60±7</td>
<td>20</td>
<td>8.9±2.0</td>
</tr>
<tr>
<td>90</td>
<td>55±7</td>
<td>110</td>
<td>3.8±1.0</td>
</tr>
<tr>
<td>120</td>
<td>50±6</td>
<td>140</td>
<td>5.2±1.0</td>
</tr>
<tr>
<td>150</td>
<td>47±5</td>
<td>170</td>
<td>3.0±1.0</td>
</tr>
<tr>
<td>180</td>
<td>44±4</td>
<td>200</td>
<td>13.5±5.0</td>
</tr>
<tr>
<td>210</td>
<td>41±5</td>
<td>230</td>
<td>12.3±5.0</td>
</tr>
<tr>
<td>240</td>
<td>40±5</td>
<td>260</td>
<td>14.3±7.0</td>
</tr>
<tr>
<td>270</td>
<td>37±5</td>
<td>300</td>
<td>14.2±7.0</td>
</tr>
<tr>
<td>300</td>
<td>33±4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The above equations are standard equations for determination of pharmacokinetic parameters (Gibaldi et al., 1975).

The hemodynamic effects of a single bolus injection of 10 mg phenytoin/kg body weight at 2, 120, and 190 minutes are shown in Figures 11, 12, and 13, respectively. No significant alteration in any of the parameters measured was observed at two minutes. At 120 minutes and 190 minutes, however, \( \frac{dp}{dt_{max}} \), left ventricular peak systolic pressure, and heart rate were all depressed significantly \((p<0.05)\), but not to lethal levels.

In a few cases upon rapid administration of phenytoin, hemodynamic parameters were decreased significantly (below 60% of control values) and simultaneous changes in the electrocardiogram were observed with these hemodynamic changes. There was a marked augmentation of the amplitude of Q, R, and S waves and a marked amplification of T waves in all electrocardiographic leads. These alterations in hemodynamic parameters and electrocardiographic changes upon rapid administration of phenytoin resulted in the death of two dogs. These dogs were not included in the statistical analysis of the data.

The tissue distribution of \(^{14}\)C-phenytoin was determined at five hours following intravenous administration of a dose
Figure 11. Effect of saline and a single bolus injection of 10 mg/kg phenytoin on the dp/dt, LVP, BP, and electrocardiogram leads I and II after two minutes' duration.
CONTROL

10 mg/kg $^{14}$C-Phenytoin I.V.
2 minutes after injection
Figure 12. Effect of a single bolus injection of 10 mg/kg phenytoin on the $\frac{dp}{dt}$, LVP, BP, and electrocardiogram leads I and II after 120 and 190 minutes' duration.
10 mg/kg $^{14}$C-Phenytoin I.V. 190 minutes after $^{14}$C-
120 minutes after injection Phenytoin injection
Figure 13. The effect of a single bolus injection of 10 mg/kg of phenytoin on the dp/dt, LVP, BP, and electrocardiogram leads I and II after 220, 270, and 300 minutes.
220 minutes after 14C-Phenytoin injection
270 minutes after 14C-Phenytoin injection
300 minutes after 14C-Phenytoin injection
of 10 mg/kg of body weight. Table 4 shows the tissue concentration and tissue:plasma ratio of phenytoin at the end of the experiment. Highly vascular tissues such as the adrenal gland and the liver accumulated the highest levels of phenytoin. These levels were about twice those of plasma. However, neuronal tissues such as the deep and fourth portion of the medulla oblongata, pons, mesencephalon, and posterior hypothalamus all accumulated equal concentrations of phenytoin.

Hemodynamic Effects and Tissue Distribution of Phenyltoin When Given Alone

The left ventricular peak systolic pressure, $\frac{dp}{dt}_{\text{max}}$, and the heart rate were all depressed significantly ($p<0.05$) after administration of a loading dose of 12 mg/kg and a maintenance dose of 30 $\mu$g/kg/min as shown in Table 5. A slight decrease in blood pressure was also observed, but it was not significant.

The concentration of phenytoin in myocardium, liver, kidney, and adrenal were slightly greater than in plasma (2:1). The spleen and the skeletal muscle accumulated the same concentrations of phenytoin as the plasma. The gum tissue accumulated the least amount of phenytoin concentration. The concentration of phenytoin in the medulla oblongata, mesencephalon and pons were twice that of plasma concentration.
<table>
<thead>
<tr>
<th>Samples</th>
<th>nmole/g</th>
<th>T/P</th>
<th>Samples</th>
<th>nmole/g</th>
<th>T/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>47±9</td>
<td>1.4</td>
<td>4th V port. med.</td>
<td>66±16</td>
<td>2.3</td>
</tr>
<tr>
<td>Liver</td>
<td>68±13</td>
<td>2.0</td>
<td>Area postrema</td>
<td>57±14</td>
<td>1.6</td>
</tr>
<tr>
<td>Kidney</td>
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<td>1.4</td>
<td>Pons</td>
<td>73±17</td>
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<td>Skeletal</td>
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<td>1.1</td>
<td>Mesencephalon</td>
<td>72±19</td>
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<td>Adrenal</td>
<td>78±18</td>
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<td>Olfactory bulb</td>
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</tr>
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<td>Spleen</td>
<td>31±6</td>
<td>1.0</td>
<td>Anterior hypothalamus</td>
<td>56±13</td>
<td>1.6</td>
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<td>Cerebellum</td>
<td>67±19</td>
<td>1.9</td>
<td>Posterior hypothalamus</td>
<td>74±22</td>
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<td>Optic chiasm</td>
<td>65±14</td>
<td>1.9</td>
<td>Ant. pit. gland</td>
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<td>1.5</td>
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<td>LV choroid plexus</td>
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<td>1.5</td>
<td>Post. pit. gland</td>
<td>52±14</td>
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</tr>
<tr>
<td>4th V choroid plexus</td>
<td>52±13</td>
<td>1.4</td>
<td>Pineal gland</td>
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<td>1.8</td>
</tr>
<tr>
<td>Visual cortex</td>
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<td>1.6</td>
<td>Gum</td>
<td>24±2</td>
<td>0.7</td>
</tr>
<tr>
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<td>67±11</td>
<td>2.0</td>
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<tr>
<td>Deep port. med.</td>
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<td></td>
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<tr>
<td>Parameter</td>
<td>Control ± SEM</td>
<td>30 minutes ± SEM</td>
<td>60 minutes ± SEM</td>
<td>120 minutes ± SEM</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>dp/dt</td>
<td>71.6±12</td>
<td>53.3±10*</td>
<td>51.6±7*</td>
<td>49.3±1*</td>
<td></td>
</tr>
<tr>
<td>LVP</td>
<td>191.6±24</td>
<td>140.0±10*</td>
<td>140.0±10*</td>
<td>125.0±15*</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>120.0±10</td>
<td>98.3±37+</td>
<td>101.6±44+</td>
<td>99.0±45+</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>166.6±11</td>
<td>146.6±11*</td>
<td>140.0±17*</td>
<td>136.6±20*</td>
<td></td>
</tr>
</tbody>
</table>

* significant p < 0.05
+ not significant
The concentration of phenytoin in these neuronal tissues was slightly greater than in plasma, although this difference was statistically insignificant (p>0.05).

Hemodynamic Effects and Tissue Distribution of Ouabain and Phenyltoin during Concomitant Administration

After ventricular dysrhythmia was produced with ouabain, a loading dose of 12 mg phenytoin/kg body weight followed by a constant infusion of 30 µg/kg/min was given. This caused the return of sinus rhythm within 5-10 minutes after onset of infusion, and the sinus rhythm was maintained for 300 minutes (Fig. 14) at which time the experiments were terminated. Return to sinus rhythm occurred at plasma levels of phenytoin between 51±11 and 49±6 nmole/ml (Fig. 15, Table 6) with an average value of 49±0.58 nmole/ml (12.4±0.1 µg/ml). In one dog in which the level was only 25 nmole/ml, only transient reversal of dysrhythmia was observed.

At return to sinus rhythm, the cerebrospinal fluid concentration of phenytoin was between 8.7±0.7 and 9.2±.6 nmole/ml with an average of 9.12±0.57 nmole/ml (2.3 µg/ml) and was not linearly proportional to the plasma concentration. Table 6 shows the absolute values for concentrations of phenytoin in plasma and CSF in the presence of ouabain. When phenytoin was given alone, concentrations of the drug in plasma and CSF were slightly lower than when given in the presence of ouabain (Fig. 15,16, Tables 6,7), but the differences were not significant (p>0.05).
Figure 14. The effect of antiarrhythmogenic doses of phenytoin when given after 180 minutes of ouabain-induced dysrhythmia. Note sinus rhythm occurred within five minutes after phenytoin administration.
Figure 15. A plot of the plasma (---.) and CSF (--.--.) phenytoin concentration (on the ordinate) against time (on the abscissa) during ouabain infusion.
OUABAIN + $^{14}$C-PHENYTOIN

PLASMA

CSF

PHENYTOIN (ng/ml)

TIME (minutes)

0 10 20 30 40 50 60 70 80 90 100 110 120
### TABLE 6

Plasma and CSF Concentrations of 14C-Phenytoin During Ouabain Infusion

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Plasma Concentration (nmole/ml ± S.E.M.)</th>
<th>CSF Concentration (nmole/ml ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>94±18</td>
<td>4.6±.8</td>
</tr>
<tr>
<td>20</td>
<td>53±11</td>
<td>6.5±.4</td>
</tr>
<tr>
<td>30</td>
<td>57±13</td>
<td>8.1±.8</td>
</tr>
<tr>
<td>45</td>
<td>51±11</td>
<td>8.7±.7</td>
</tr>
<tr>
<td>60</td>
<td>48±4</td>
<td>9.0±.6</td>
</tr>
<tr>
<td>75</td>
<td>47±9</td>
<td>8.9±.5</td>
</tr>
<tr>
<td>90</td>
<td>49±9</td>
<td>9.8±.4</td>
</tr>
<tr>
<td>105</td>
<td>50±8</td>
<td>9.2±.6</td>
</tr>
<tr>
<td>120</td>
<td>49±6</td>
<td></td>
</tr>
</tbody>
</table>
Figure 16. A plot of plasma (---) and CSF (-----) phenytoin concentration (on the ordinate) against time (on the abscissa) when this drug was administered alone.
PHENYTOIN ALONE

- - - - - - - - PLASMA
- - CSF

TIME (minutes)

PHENYTOIN (mg/ml)
<table>
<thead>
<tr>
<th>Time minutes</th>
<th>Plasma nmole/ml ± S.E.M.</th>
<th>Time minutes</th>
<th>CSF nmole/ml ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>91±14</td>
<td>15</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>20</td>
<td>52±8</td>
<td>45</td>
<td>7.2±0.6</td>
</tr>
<tr>
<td>30</td>
<td>44±2</td>
<td>60</td>
<td>7.1±0.2</td>
</tr>
<tr>
<td>45</td>
<td>43±2</td>
<td>75</td>
<td>7.3±0.2</td>
</tr>
<tr>
<td>60</td>
<td>41±1</td>
<td>90</td>
<td>7.8±0.2</td>
</tr>
<tr>
<td>75</td>
<td>43±1</td>
<td>105</td>
<td>8.2±0.4</td>
</tr>
<tr>
<td>90</td>
<td>41±3</td>
<td>120</td>
<td>8.2±0.3</td>
</tr>
<tr>
<td>105</td>
<td>43±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>46±1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The presence of phenytoin at a plasma concentration of 12 μg/ml had no significant effect (p > 0.05) on the plasma concentration of ouabain. However, the concentration of ouabain in CSF was elevated in the presence of phenytoin (Table 8), but this slight increase was not significant. Furthermore, the peak systolic left ventricular pressure, dp/dt_max, systemic blood pressure, and heart rate were returned to normal within 10-15 minutes after phenytoin administration.

A comparison of ouabain concentration per unit of organ weight or as tissue to plasma or tissue to CSF ratios did not reveal statistically significant differences produced by the coadministration of phenytoin (p > 0.05) (Table 9) and ouabain.

The concentration of phenytoin in the mesencephalon and deep portion of the medulla were slightly greater than in plasma (2:1), although this difference was statistically insignificant (p > 0.05). When phenytoin was given with ouabain to four additional dogs, no significant differences in tissue:plasma or tissue:CSF concentration of phenytoin were observed (Table 10). Although the deep portion of the medulla appeared to accumulate greater concentrations of phenytoin when this drug was given with ouabain than when given alone, the differences were not statistically significant (p > 0.05).
### TABLE 8

Concentrations of $^3$H-Ouabain in Plasma and CSF in Presence of Phenytoin

<table>
<thead>
<tr>
<th>Time minutes</th>
<th>Plasma Pmole/ml ± S.E.M.</th>
<th>CSF Pmole/ml ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>111±4</td>
<td>19±5</td>
</tr>
<tr>
<td>30</td>
<td>100±15</td>
<td>20±3</td>
</tr>
<tr>
<td>45</td>
<td>56±14</td>
<td>20±4</td>
</tr>
<tr>
<td>90</td>
<td>57±14</td>
<td>20±4</td>
</tr>
<tr>
<td>120</td>
<td>60±14</td>
<td>22±5</td>
</tr>
<tr>
<td>150</td>
<td>62±18</td>
<td>23±3</td>
</tr>
<tr>
<td>180</td>
<td>48± 4</td>
<td>23±4</td>
</tr>
<tr>
<td>240</td>
<td>53±13</td>
<td>23±4</td>
</tr>
<tr>
<td>300</td>
<td>47±12</td>
<td>21±4</td>
</tr>
</tbody>
</table>
TABLE 9

Comparison of Ouabain Uptake When Administered Alone or in the Presence of Antiarrhythmogenic Doses of Phenytoin

<table>
<thead>
<tr>
<th></th>
<th>Ouabain Alone</th>
<th>Ouabain and Phenytoin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dysrhythmogenic Dog; N=3</td>
<td>Recovered Dog; N=4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pmole/g</td>
<td>T/P</td>
<td>T/CSF</td>
</tr>
<tr>
<td>Heart</td>
<td>453±40</td>
<td>15.2±5.0</td>
<td>54.6±22.0</td>
</tr>
<tr>
<td>Liver</td>
<td>162±44</td>
<td>4.2±1.0</td>
<td>20.7±12.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>1365±778</td>
<td>65.0±45.0</td>
<td>98.7±52.0</td>
</tr>
<tr>
<td>Skeletal</td>
<td>90±25</td>
<td>2.5±0.8</td>
<td>12.1±6.0</td>
</tr>
<tr>
<td>Adrenal</td>
<td>300±76</td>
<td>8.0±1.0</td>
<td>46.0±30.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>87±15</td>
<td>2.6±0.6</td>
<td>11.0±5.0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>74±33</td>
<td>2.5±1.0</td>
<td>6.9±1.0</td>
</tr>
<tr>
<td>Optic chiasm</td>
<td>68±26</td>
<td>1.5±0.4</td>
<td>11.4±8.0</td>
</tr>
<tr>
<td>L.V. choroid plexus</td>
<td>369±70</td>
<td>14.0±7.0</td>
<td>39.9±11.0</td>
</tr>
<tr>
<td>4th V. choroid plexus</td>
<td>368±6</td>
<td>14.5±8.0</td>
<td>37.2±9.0</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>41±6</td>
<td>1.2±0.5</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td>Vent. port. med.</td>
<td>48±24</td>
<td>1.5±0.8</td>
<td>4.8±1.0</td>
</tr>
<tr>
<td>Deep port. med.</td>
<td>51±5</td>
<td>1.7±0.6</td>
<td>6.1±2.0</td>
</tr>
<tr>
<td>4th vent. med.</td>
<td>56±7</td>
<td>1.6±0.4</td>
<td>7.9±4.0</td>
</tr>
<tr>
<td>Area postrema</td>
<td>187±23</td>
<td>6.3±2.0</td>
<td>21.0±8.0</td>
</tr>
<tr>
<td>Pons</td>
<td>63±8</td>
<td>2.1±0.8</td>
<td>7.2±2.0</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>59±15</td>
<td>2.0±0.7</td>
<td>6.4±2.0</td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>76±25</td>
<td>2.5±1.0</td>
<td>7.3±2.0</td>
</tr>
<tr>
<td>Ant. hypothalamus</td>
<td>96±44</td>
<td>3.2±1.0</td>
<td>9.1±2.0</td>
</tr>
<tr>
<td>Post. hypothalamus</td>
<td>69±7</td>
<td>2.2±0.7</td>
<td>8.6±4.0</td>
</tr>
<tr>
<td>Ant. pit. gland</td>
<td>386±113</td>
<td>14.8±6.0</td>
<td>35.7±2.0</td>
</tr>
<tr>
<td>Post. pit. gland</td>
<td>1633±528</td>
<td>58.1±24.0</td>
<td>158.0±31.0</td>
</tr>
<tr>
<td>Pineal gland</td>
<td>77±12</td>
<td>2.3±0.6</td>
<td>10.5±5.0</td>
</tr>
<tr>
<td>Gum</td>
<td>57±15</td>
<td>2.2±1.0</td>
<td>6.1±1.0</td>
</tr>
</tbody>
</table>
TABLE 10
Comparison of Phenytoin Uptake When This Drug Is Administered Alone and When Administered to Abolish Ouabain Induced Dysrhythmia

<table>
<thead>
<tr>
<th>Samples</th>
<th>Control Dogs (Phenytoin Alone)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmole/g ± S.E.M.</td>
<td>T/P</td>
<td>T/CSF</td>
</tr>
<tr>
<td>Heart</td>
<td>84± 7</td>
<td>1.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Liver</td>
<td>108± 4</td>
<td>2.3</td>
<td>13.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>85± 6</td>
<td>1.8</td>
<td>9.6</td>
</tr>
<tr>
<td>Skeletal</td>
<td>60± 6</td>
<td>1.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Adrenal</td>
<td>124± 8</td>
<td>2.6</td>
<td>15.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>52± 4</td>
<td>1.1</td>
<td>6.5</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>80± 6</td>
<td>1.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Optic chiasm</td>
<td>61±10</td>
<td>1.3</td>
<td>7.8</td>
</tr>
<tr>
<td>L.V. choroid plexus</td>
<td>51±11</td>
<td>1.0</td>
<td>7.2</td>
</tr>
<tr>
<td>4th V choroid plexus</td>
<td>66± 8</td>
<td>1.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>69± 7</td>
<td>1.4</td>
<td>9.2</td>
</tr>
<tr>
<td>Vent. port. med.</td>
<td>82± 6</td>
<td>1.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Deep port. med.</td>
<td>87± 8</td>
<td>1.8</td>
<td>11.3</td>
</tr>
<tr>
<td>4th vent. port. med.</td>
<td>95± 8</td>
<td>2.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Area postrema</td>
<td>72± 0</td>
<td>1.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Pons</td>
<td>94±10</td>
<td>2.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>100± 2</td>
<td>2.1</td>
<td>12.5</td>
</tr>
<tr>
<td>Olfactory</td>
<td>75±13</td>
<td>1.5</td>
<td>10.7</td>
</tr>
<tr>
<td>Ant. hypothalamus</td>
<td>76± 6</td>
<td>1.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Post. hypothalamus</td>
<td>67±15</td>
<td>1.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Ant. pit. gland</td>
<td>77± 5</td>
<td>1.6</td>
<td>9.8</td>
</tr>
<tr>
<td>Post. pit. gland</td>
<td>74± 8</td>
<td>1.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Pincal gland</td>
<td>74± 7</td>
<td>1.6</td>
<td>9.3</td>
</tr>
<tr>
<td>Gum</td>
<td>37± 8</td>
<td>0.8</td>
<td>4.6</td>
</tr>
</tbody>
</table>

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CHAPTER VI

DISCUSSION

Adequacy of the Experimental Approaches and Methods Used

Animal preparation and measurement of control parameters were usually completed within 30 minutes without causing any appreciable loss of blood. Neither heart rate nor temperature changed significantly in these experiments. Moderate doses of ouabain used in this study did not significantly affect the end diastolic pressure (EDP) so that monitoring percent change of LV $dp/dt$ instead of $dp/dt_p$ was justified as representing the true increase or decrease of inotropic state in response to ouabain. Similar observations have been reported by Albert et al. (1971) and Williams et al. (1966) in dogs with comparable doses of ouabain.

Hemodynamic Effects and Tissue Distribution of $^3$H-Ouabain Given Alone

Considerable effort has been expended to correlate plasma levels of ouabain with the occurrence of ventricular dysrhythmia. It would be ideal if a specific dysrhythmia could be maintained for a prolonged period to test for potential anti-arrhythmics. This study demonstrated that for ouabain, an
intravenous loading dose of 40 ug/kg followed by a constant infusion of 0.072 ug/kg/min produced just such a response; that is, a steady-state ventricular tachydysrhythmia that persisted for up to five hours. Such dysrhythmia correlated with steady-state plasma concentrations of ouabain. These concentrations existed after 15 minutes of infusion. Similar observations have been reported by Rhee (1976), Albert et al. (1971), and Williams et al. (1976). Also, in agreement with the previous workers, the dysrhythmia was composed mainly of left ventricular tachycardia. Evidence for concluding that the dysrhythmia originated from the left ventricle is based on the following criteria: the QRS complex in lead II for ectopic beats was bizarre in contour and was prolonged above the normal limit of duration (65 msec), and the QRS complex was predominantly negative.

Changes in left ventricular myocardial contractility, mean systemic arterial pressure, heart rate, and automaticity upon administration of ouabain doses have been observed by others (Rhee, 1976). These changes were attributed to the negative chronotropic, positive inotropic, and positive bathmytopic effects of digitalis glycosides.

It is hypothesized that preferential localization of a drug at a biological site is related to its mechanism of
action. This research was designed to measure quantities of ouabain at specific sites within the body to ascertain if perhaps a relation could be established between site of action and uptake.

This study demonstrated a relatively great affinity of the myocardium for ouabain, and is consistent with the inotropic and chronotropic effects of ouabain. Others, Harvey and Pieper (1952) and St. George et al. (1953) have demonstrated the distribution of ouabain in specific microsomal fractions of myocardium. In particular, the myocardium supernatant has the highest affinity for ouabain. Although ouabain may be concentrated in this fraction, there is no evidence that such concentration is responsible for pharmacologic effects of ouabain.

This study demonstrated that various tissues in addition to the myocardium had relatively great affinity for ouabain. Some investigators believe that toxic and perhaps therapeutic actions of digitalis may be exerted through the nervous system since experimental animals require greater amounts of digitalis to manifest toxicity if they have undergone sections of the brainstem.

The concentration of ouabain in the area postrema was six times that of the plasma. It is tempting to suggest that such concentration may indicate that this organ is at least in part
the target site for the effect of ouabain. Other neuronal tissues such as the anterior hypothalamus accumulate a relatively high amount of ouabain, but much lower than for the area postrema. The other neuronal tissues such as olfactory bulb, cerebellum, posterior hypothalamus, mesencephalon, medulla oblongata, and visual cortex accumulate the least amount of ouabain. At the present time, there is no evidence of any of these tissues for a centrogenic mechanism of ouabain-inducing cardiovascular effects.

Area postrema is one of a pair of small rounded eminences on either side of the fourth ventricle just rostral to the apex formed by the junction of the ventricle and the central canal (Fig. 17) (Borison 1974). It is found in all mammalian species, as well as in many other vertebrates, astride the opening of the spinal canal into the fourth ventricle. The area postrema is one of an uncertain number of midline structures that is in contact with the cerebrospinal fluid (CSF) and whose capillaries, like those in the choroid plexuses, are permeable to protein bound vital dyes. In 1906, Wilson attempted to rename area postrema the "nucleus postremus" because he identified neuronal elements in human specimens. Until 1950, based upon anatomical studies, area postrema was thought to perform a neurosecretory and/or blood flow control function, neither of which has been substantiated
Figure 17. Dorsal view of the area postrema in the cat. From Herbert L. Borison.
Upper left: Dorsal view of the area postrema (AP, shown hatched) in the cat. Note line of attachment of the tela choroidea (TC) to the margin of the fourth ventricle (VENT. IV). Lower left: Cross-section of the medulla at level of dashed line above. Area bounded by square is enlarged in photomicrograph on right. Silver stain shows nerve fibers in region subjacent to TC. Note labyrinthine spaces associated with blood vessels beneath the ventricular surface of AP. The choroid plexus (CP) is considerably removed from this region of AP. MVN - medial vestibular nucleus; IVN - inferior vestibular nucleus; CUN - cuneate nucleus; GN - gracile nucleus; FS - fasciculus solitarius; X - motor nucleus of vagus; XII - hypoglossal nucleus; IO - inferior olive.
according to Cammermeyer (1947). On the basis of an extensive series of physiological experiments, Borison and Wang (1951 and 1953) suggested that the ala cinerea next to area postrema was the chemoreceptor trigger zone for vomiting. Other functions that have been postulated for the area postrema are: (1) epinephrine-induced hyperglycemia (Distefano, 1961); (2) respiratory regulation of carbon dioxide (Yamamato, 1962); (3) control of food intake (Reynolds et al., 1961); (4) serotonin-induced synchronization of the electroencephalogram (Czicman, 1966); (5) cardiovascular effects of angiotensin (Joy, 1970); and (6) control of renal function (Ganong, 1960).

It is possible that additional functions of area postrema may be relevant to mediating effects of ouabain since serotonin and angiotensin can mediate their effects through this organ. However, the actions of serotonin and angiotensin command special interest since these agents are believed to act also on the subfornical organs so as to produce balancing or reinforcing influences vis-a-vis their effect on area postrema. Further experiments supporting these intriguing postulations must be undertaken.

Evidence that supports a hypothesis that the area postrema may be the site of action for ouabain-induced dysrhythmia includes the work of Joy and Lowe (1970) who were able to
demonstrate that bilateral ablation, by thermocoagulation, of the area postrema in greyhounds resulted in abolishing the cardiovascular response to vertebral infusion of angiotensin. The central effects of low concentrations of angiotensin were dependent on the integrity of the area postrema or regions immediately adjacent. Because ablation of the area postrema neither changes the resting heart rate nor abolishes several other control autonomic effects, it seems likely that the area postrema is relatively specific for angiotensin. Furthermore, Dutta (1977) demonstrated that in cats in which the area postrema was ablated by electrocoagulation, ouabain-induced dysrhythmia was prevented. They also indicated that the distribution of ouabain in CNS differed significantly \( p < 0.05 \) between intact cats and those with ablation of the area postrema.

Borison and Wang (1953) have shown that a highly vascular area postrema lacks a blood-brain barrier and mediates the emetic effects of digitalis. Preliminary studies by earlier workers demonstrated that the area postrema may affect circulatory hemodynamics, presumably through the autonomic nervous system. Recently, Somberg and Smith (1979) demonstrated that, in the cat, digitalis-induced cardiac arrhythmias were mediated through neuronal activation in the medulla. Furthermore, they located this neuronal region as the area postrema.
It can be postulated that drugs such as cardiac glycosides induce certain cardiovascular effects via this neuronal tissue. Localization of toxic amounts of ouabain in neuronal tissues such as area postrema, might indeed produce certain cardiovascular disturbances. However, the pharmacological basis of this localization requires further investigation to indicate the cellular and biochemical sequences of this specific accumulation. Possibly, localization of ouabain in the area postrema induces arrhythmia by activation of sympathetic discharges originating in nuclei of the area postrema. Furthermore, if this is true, this supports the centrogenic effect of ouabain-induced cardiac rhythm disturbances through sympathetic action (which was suggested originally by Gillis et al., 1969, 1972).

The site of action of these drugs may be in some non-neuronal structure in the brain such as the choroid plexus or pituitary gland which also accumulated substantial amounts of ouabain. However, until now, there is very little information available suggesting that the CNS effect of ouabain can be mediated by the pituitary gland. That the preferential accumulation of both ouabain and digitoxin is in the choroid plexus suggests the possibility that the drugs may influence active transport of ions and/or amine metabolites into the CSF. Dutta et al. (1977) were unable
to find any changes in the cation content of CSF. Other workers have recently found preferential accumulation of digoxin in the CSF and choroid plexus of man (Neblett et al., 1972; Bertler et al., 1973), dog (DasGupta and Binnion, 1974) and cat (Dutta et al., 1977). Although it is clear from this study, as well as from previous studies, that there is a preferential localization of cardiac glycosides in the choroid plexus, the pharmacological implications of this binding have yet to be established.

**Pharmacokinetic Parameters of Phenytoin**

If phenytoin is to be used in dogs as an antiarrhythmic, it must be administered several times a day because the biological half-life (t½) measured in this study was so brief (265 minutes). This is in contradistinction to the half-life of phenytoin in man which has been measured as greater than 24 hours (Noach et al., 1958) following sustained oral or parenteral administration of phenytoin. Following oral administration of this compound, peak blood concentrations are not achieved before 8-12 hours because of the slow absorption from the gastrointestinal tract of both man and dog as was demonstrated by Arnold et al. (1970) and Svensmark et al. (1960). Thus, although the half-life may be short, blood levels may be sustained longer for oral administration than would be predicted from intravenous studies because of the slow rate of absorption.
The apparent volume of distribution (aVd) for phenytoin varies within members of a species and between species. For dogs in this study, aVd varied between 0.56 and 0.72 L/kg with a mean volume of 0.57±0.06 L/kg. VanGelder et al. (1971) reported similar values for the volume of distribution for phenytoin between 0.64 and 0.72 L/kg with no mean given.

Based upon the apparent volume of distribution and the dose of phenytoin given, the plasma concentrations were lower than expected. This discrepancy may have been caused by one or many factors. First, it is possible that the anesthetic (sodium pentobarbital) used in these studies altered the apparent volume of distribution via changes in circulatory hemodynamics that were not detectable by systemic blood pressure, peak left ventricular pressure, or dp/dt max measurements. Secondly, sodium pentobarbital may compete for serum protein binding sites of phenytoin. However, there is no evidence that indicates this interaction. Thirdly, it has been shown that sodium pentobarbital induces hepatic microsomes that are responsible for metabolism of phenytoin. Cucinell et al. (1965) reported in mice and dogs that phenobarbital lowers the serum concentration and shortens the half-life of phenytoin appreciably. However, in the present study, anesthetic was used for a short period (5-8 hours) in contrast to the long interval between barbiturate treatment and phenytoin.
injection as was reported in Cucinell's study. Thus, hepatic microsomal induction by pentobarbital would unlikely be the cause of serum phenytoin concentration reduction. Finally, in the present study, a one compartment model has been assumed which perhaps was not sufficient to define precisely the pharmacokinetic parameters for phenytoin. However, it is logical to indicate higher doses of phenytoin may be required to abolish dysrhythmia when this drug is used with anesthetic drugs such as sodium pentobarbital.

Generally, phenytoin concentrations in all tissues except for gum were higher than plasma concentrations. These results are in agreement with those of Noach et al. (1958). The reason for the apparent affinity of some tissues for phenytoin may be related to blood flow, that the organs are excretory for the compound, or that the organ contains proteins to which phenytoin is bound. This agrees with observations of Swinyard et al. (1952). This study demonstrates very low concentrations of phenytoin in gum tissue. This is in contradistinction to observations of high concentrations in gum of cat, man, and rat; and correlates with the clinical absence of gingival hyperplasia in dogs receiving phenytoin for long periods. It has been suggested that salivary excretion of phenytoin is a factor in the etiology of gingival hyperplasia, a frequent side effect of phenytoin in humans.
Species' variation has been observed also by others. For instance, King and coworkers (1952) reported that ferrets are not susceptible to phenytoin-induced gingival hyperplasia. They indicate that the gum surface in closest contact with the salivary duct appears to be more susceptible. In the present study, a very low accumulation of phenytoin in gum tissue was found. Perhaps the sample of gum was taken too far from the region in contact with saliva.

Generally, neuronal tissues accumulated a relatively high amount of phenytoin. This is in agreement with Firemark et al. (1963) who reported an "unusual and sustained accumulation of phenytoin in the brain of the cat". Also, Firemark reported that the brain of the cat accumulated phenytoin two to three times the level of plasma which is in agreement with the present study. Similar localization of phenytoin accumulation in certain neuronal tissues such as superior and inferior colliculus, amygdala, and hippocampus has been observed by Nakamura (1966). Furthermore, preferential localization of phenytoin in human primary brain tumors in relation to adjacent normal tissues has been reported by Rosenblum and Stein (1963). From the present study and previous work of others, preferential localization of phenytoin in the central nervous system is significant to its
antiepileptic and perhaps some of its antiarrhythmic effects apart from its direct myocardial action.

Hemodynamic Effects and Tissue Distribution of Phenytoin When Given Alone

The cardiac depressant effects of phenytoin observed during this research have also been reported by other investigators. Puri (1971) reported in dogs that phenytoin when administered intravenously in therapeutic doses of 5 mg/kg is associated with depression of myocardial contractility and reduction in peripheral vascular resistance. Higher doses of 10 mg/kg decreased these parameters significantly.

Similar observations by Lieberson (1969) have been reported in patients with heart disease. Furthermore, Mixter (1966), in agreement with the present observation, indicated a fall in left ventricular dp/dt max and significant reduction of cardiac output with doses up to 30 mg/kg in dogs.

Following a rapid, intravenous injection, of 12 mg/kg and 30 µg/kg/min infusion of phenytoin to dogs in this study (whether to dogs with ouabain-induced tachydysrhythmia or to dogs otherwise untreated), a precipitous fall in blood pressure and left ventricular pressure, dp/dt max occurred. The more rapid the administration of phenytoin, the more severe and the more prolonged were these decreases in ventricular function. Simultaneous with decreased contractility were
periods of cardiac arrest manifested by p waves; and abberation of ventricular activation manifested by bizarre QRS complexes. Immediately following this, alteration in ST-T segment persisted until greater hemodynamic stability was observed. During periods of cardiac arrest, exaggerated ventilatory motions were made, no doubt because of cerebral hypoxia. These observations were reported, initially, by Harris et al. (1950) in dogs.

These profound changes resulted probably not from phenytoin, but from the solvent, propylene glycol. The Parke-Davis preparation utilized in this study was phenytoin dissolved in a 40% solution of propylene glycol. Louis et al. (1968) injected propylene glycol, alone, and observed alterations identical with those of this study. This is not to imply that phenytoin cannot produce myocardial depression and sinus nodal arrest. Gillis et al. (1971) reported both myocardial depression and sinus arrest by injecting phenytoin dissolved in a solvent known to not affect myocardial contractility. It is also possible that synergism between sodium pentobarbital anesthesia and phenytoin or phenytoin and propylene glycol may have resulted in the depression noted.

Nevertheless, the most likely explanation for both sinus arrest and myocardial depression following rapid intravenous administration of phenytoin was the solvent, propylene glycol.
The optimal ratio of solute (phenytoin) to solvent (propylene glycol) in the treatment of cardiac rhythm disturbances has not been determined, but excessive dilution in the propylene glycol solvent is known to be hazardous. This study confirms that when given intravenously with the solvent, propylene glycol, phenytoin should be given slowly to prevent acute propylene glycol toxicosis. That the electrocardiographic and hemodynamic consequences of rapid administration are short-lived, may represent both redistribution of propylene glycol and a beneficial effect of phenytoin.

The observation that phenytoin is taken up in rather great concentrations by adrenal may indicate that it has an antiadrenal effect; while this study does not prove the compound exerts its effect via the adrenal, at least it identifies this compound as having affinity for that organ.

Neuronal tissues, specifically the deep portion of the medulla oblongata, manifested greater affinity for phenytoin than did plasma, and affinity of neuronal and non-neuronal tissue except gum were comparable. It is tempting to assert that phenytoin may have at least, in part, antiarrhythmic effects due to uptake by neuronal tissues. This would concur with statements by Gillis et al. (1969) that digitalis induced arrhythmias have a "strong" centrogenic basis. However, results of this study neither proved or disproved that possibility.
Hemodynamic Effects and Tissue Distribution of Ouabain and Phenytoin During Concomitant Administration

The left ventricular peak systolic pressure, myocardial contractility, systemic blood pressure, heart rate, and the electrocardiogram in dogs given ouabain were completely returned to normal within a short period of time (10-15 min) after phenytoin administration. This observation is in agreement with Musey and Tyler (1954), Droph (1968), and Rhee (1976). That phenytoin successfully reversed ouabain-induced ventricular dysrhythmia may have been attributed to the direct effect of phenytoin on the myocardium.

By comparing plasma ouabain concentrations in dogs receiving phenytoin to dogs denied that compound, it is clear that phenytoin did not alter plasma concentrations of ouabain. However, it is interesting to note that concentrations of ouabain in cerebrospinal fluid were actually elevated in dogs receiving phenytoin above those in dogs without phenytoin. It was noted by others that ouabain decreased CSF production; however, in this study, CSF production was elevated. No explanation has been offered for either of these observations. It is interesting to conjecture that phenytoin may interact with ouabain at the choroid plexus, since others have reported a relatively high concentration of digoxin in that organ of dogs (DasGupta and Binnion, 1974). Perhaps ouabain inhibited \((\text{Na}^+\text{K}^+)\)-ATPase in the choroid plexus. This will slow production of CSF while phenytoin prevented this inhibition.
The primary objective of this study was to ascertain whether phenytoin altered the uptake and/or distribution of ouabain in any of the various tissues examined. In particular, it was desired to examine the effects on uptake by neuronal tissues, since it was in these tissues where Gillis et al. (1969) suspected digitalis may trigger dysrhythmia. In fact, Dutta and Marks (1969) suggested that phenytoin may alter distribution of digitalis. Further, Gillis et al. (1971) and Garone et al. (1974) have suggested that phenytoin may reduce sympathetic efferent discharge to the myocardium.

In studies on guinea pig myocardium, Baskin et al. (1971) showed that massive doses of phenytoin reduced both ventricular dysrhythmia and concentration of ouabain within the myocardium. They suggested that phenytoin may exert, at least in part, some antidysrhythmic effect on ouabain-induced dysrhythmia by reducing the uptake of ouabain by the myocardium.

Data from this study suggest that although concentrations of ouabain in various tissues were depressed generally when given with phenytoin, that depression did not achieve levels of statistical significance. In some dogs, it appears that phenytoin may have actually caused increased uptake of ouabain in the posterior pituitary gland; however, this increased uptake did not reach levels of statistical significance.
Although this study showed that the abolition or prevention of ouabain-induced dysrhythmias with phenytoin was not due to gross reduction in uptake of ouabain, it does not preclude the possibility that other antiarrhythmics exert their effects by such means. For example, Craig and Jacobs (1943) were the first to suggest that the cardiac action of the digitaloids was due to specific steriochemical configuration of the steroid nucleus itself. Lefer and Sayer (1965) suggested that aldosterone prevents inotropy of ouabain due to its steriochemical similarity with ouabain, and therefore, may bind to a digitalis receptor site. This has been challenged by Levy and Richards (1965, 1964) who stated that aldosterone and ouabain do not compete for the same receptor sites; but that, perhaps aldosterone exerts its antidigitalis effect by countering ionic fluxes produced by ouabain. Selye et al. (1969) have shown that spironolactone and norbolthone - both aldosterone antagonists - increase the dose of digitalis required to produce lethal dysrhythmia.

Cosmides and his coworkers (1956) reported that tetrahydrofurfuryl alcohol (THRA), a lactone-like compound, was capable of preventing cardiac glycoside-induced arrhythmias. They implied that such blockade was via a competitive mechanism at a locus of "toxicologic action". However, Shafer and Adicott (1970) were unable to confirm that THFA was an
antagonist. Moore et al. (1961) suggested that the effect may have been mediated via erythrolysis and subsequent release of potassium ion. This latter suggestions is untenable since the canine erythrocyte possesses relatively small concentrations of potassium ion.

In summary, this study shows that phenytoin abolished or prevented digitalis-induced dysrhythmia; but that such alterations could not be attributed to gross changes in ouabain uptake by specific tissues or organs.

Studies conducted on specialized conductile tissues of isolated perfused heart support the concept of a direct action of phenytoin on both electrophysiologic and mechanical properties. Bigger et al. (1968) demonstrated that phenytoin shortens action potential duration, decreases automaticity, and increases diastolic membrane potential in partially depolarized Purkinje fibers. Strauss et al. (1968) demonstrated that phenytoin enhanced membrane responsiveness and increased conduction velocity. Both concluded that phenytoin exerted its beneficial effects via action on the heart.

In disagreement with the above studies, Jenson (1970) demonstrated in atrial muscle (not in Purkinje fibers as did Bigger) depressed membrane responsiveness and reduced conduction in response to phenytoin. Nevertheless, he concluded that its antiarrhythmic effect probably resulted from these actions on the heart.
When given with ouabain, no differences in uptake of phenytoin were observed when compared with uptake without concomittant ouabain therapy. Thus, it appears that phenytoin and ouabain are taken up independently of each other. Although the medulla manifested slightly greater affinity for phenytoin when given with ouabain, this difference did not achieve statistical significance.

In as much as this study demonstrated a high accumulation of phenytoin by certain neuronal tissues, there is no direct evidence to indicate any centrogenic mechanism of this drug. Moreover, ouabain was unable to change significantly any specific localization of phenytoin by neuronal tissues. Further study is required to confirm this centrogenic mechanism of phenytoin in preventing dysrhythmia.

Others suggested that action of phenytoin on the nervous system might contribute to its antiarrhythmogenic action. For instance, Gillis et al. (1971) tested the effect of phenytoin on cardiac sympathetic nerve activity in preganglionic sympathetic nerve fibers. These investigators suggested that phenytoin has the capacity to depress cardiac sympathetic activity and that this neurodepressant action is the basis for its effect on the cardiovascular system. Lisander et al. (1975) stimulated the posterolateral hypothalamus in cats to evoke cardiac arrhythmias of varying
severity both during and after stimulation. The mean effective dose of phenytoin required to prevent arrhythmia via intravenous routes was 11.9 mg/kg, while via the vertebral artery route and via fourth ventricular route, it was only 1.9 and 1.4 mg/kg, respectively. They suggested that phenytoin has identifiable antiarrhythmic action on the heart itself, but it has a strong antiarrhythmic effect via the central nervous system as well.

However, this centrogenic mechanism of phenytoin in abolishing dysrhythmias has been contradicted by two groups of investigators. Lang et al. (1965) induced arrhythmia in autotransplanted heart by administering digitalis intravenously and by applying aconitine topically. They found that phenytoin converted these arrhythmias when it was administered intravenously, but not when administered into a restricted cerebral circulation. Also, Strauss et al. (1968) administered phenytoin to spontaneously beating right atrial preparations and observed slowing of sinus rate. Pretreatment with either atropine or propranolol did not modify the response of the sinus rate to phenytoin and they concluded that the decrease in sinus rate resulted from a direct effect of phenytoin on sino-atrial (S-A) nodal cells.

This study has shown that phenytoin is concentrated in those neuronal tissues that when electrically stimulated
produce ventricular dysrhythmias identical with those produced by toxic concentrations of digitalis. Whether or not the phenytoin uptake by those neuronal tissues is responsible for the antiarrhythmic effect of that compound is yet equivocal. That phenytoin is identified in those tissues supports the contention that it may exert its antiarrhythmic effect partially due to localization in neuronal tissues apart from its concentration in the myocardium and specialized conductile tissue. Furthermore, this study refuted suggestions that phenytoin may exert its antiarrhythmic effects due to gross alterations of the uptake of ouabain at least in the tissues examined.
CHAPTER VII

SUMMARY AND CONCLUSIONS

Digitalis glycosides are in common use in both human and veterinary medicine for the purpose of increasing the force of ventricular contraction, for extinguishing supraventricular tachydysrhythmia, and for slowing the ventricular rate in instances of atrial fibrillation. One of the factors limiting the usefulness of digitalis is the toxic effect which often occurs at blood levels very close to therapeu­tic effects. As a matter of fact, digitalis toxicity is among the most common of adverse drug reactions and may cause arrhythmias and conduction disturbance in as many as one of five patients receiving it. Particularly responsible are the electrophysiological properties of digitalis in increasing the automaticity of subsidiary pacemakers, reducing the refractory period, and prolonging conduction velocity in the atria and ventricles, and delaying conduc­tion in the atrioventricular node. Digitalis can provoke every type of cardiac arrhythmia and no specific disorder of rhythm can be considered absolutely pathognomonic of
digitalis toxicity. However, if these toxic effects of cardiac glycosides could be minimized, no doubt even greater therapeutic effects could result from higher doses.

Phenytoin has been used as an anticonvulsant since its introduction in 1938 by Merritt and Putnam. In recent years, it was discovered that phenytoin has antiarrhythmia properties, particularly those arrhythmias due to digitalis toxicity. In the present study, attempts were made to examine the inter-relationship between phenytoin and ouabain in the genesis and abolition of ventricular dysrhythmia and to examine further the relation between doses of phenytoin and levels in serum, CSF, and various peripheral, neuronal, and non-neuronal tissues. Also, this study demonstrates a dosing regimen to achieve in the dog, a plasma and CSF level adequate to abolish ouabain-induced dysrhythmia. Furthermore, an in-depth study was undertaken to investigate the relationship between the tissue accumulation of ouabain and the occurrence of dysrhythmia as well as the tissue accumulation of antiarrhythmogenic doses of phenytoin.

Since it has been postulated that ouabain-induced dysrhythmia via a centrogenic mechanism, the centrogenic effect of phenytoin in preventing these dysrhythmias was studied in an attempt to correlate tissue levels and pharmacological effects. Plasma, CSF, and tissue distribution of antiarrhythmogenic doses of phenytoin were studied in the presence of
ouabain-induced sustained ventricular tachydysrhythmia. Also pharmacokinetic and hemodynamic parameters were studied in the presence and the absence of ouabain-induced dysrhythmia. Meanwhile, plasma, CSF, and tissue concentrations of ouabain and phenytoin were studied individually and during concomitant administration. The present investigation indicates: (1) a loading dose of 12 mg/kg, and constant infusion of 30 µg/kg/min of phenytoin, were shown to abolish ouabain-induced dysrhythmias; (2) for phenytoin, steady-state plasma levels of 12.4±0.1 µg/ml and steady-state CSF levels of 2.3±0.5 µg/ml resulted from the dose of phenytoin in (1); (3) this antiarrhythmic dose of phenytoin has a transient depressant effect on cardiac hemodynamic parameters (dp/dt, LVP, BP, and heart rate); (4) in no instance were blood or tissue levels of either phenytoin or ouabain dependent upon the other drug; (5) Evidence suggests that the area postrema may be important in development of digitalis-induced or enhanced dysrhythmia apart from its direct myocardial effect. That both ouabain and phenytoin were recovered from this region in these experiments is consistent with claims that the arrhythmogenic effect of ouabain and the antiarrhythmic effect of phenytoin may be mediated through accumulation in this organ. Because amounts of ouabain accumulated in the
area postrema were unchanged by phenytoin, it can be assumed that if phenytoin exerts its antiarrhythmic effect via this region, it does so by some pharmacologic or physiologic antagonism and not simply by lowering concentrations of ouabain.
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