STUDIES ON CINCHONAMINE AND OTHER QUINUCLIDINE-CONTAINING INDOLE ALKALOIDS ON CARDIAC ARRHYTHMIAS

by

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INTRODUCTION

Accompanying the longer life span as a result of advances made in medicine, there has been a clearly defined increase in the incidence of cardiac arrhythmias. At the present time in the United States alone, one of every four patients with heart disease will develop atrial fibrillation at some period of the disease. While atrial fibrillation is not a fatal disorder, it does seriously interfere with the performance and enjoyment of the patient, often resulting in "cardiac cripples". Medicine is not responsible solely for saving life, but also strives to prolong the useful life of the people.

Atrial fibrillation, also characterized as an irregular irregularity arrhythmia, is many times associated with a reduction in the heart's performance (34). During atrial fibrillation, cardiac output is significantly decreased (by as much as 60 per cent) (58) and this is restored when the heart beat is converted to sinus rhythm. Associated with the depression of cardiac output is a reduction of blood flow through highly essential arteries; coronary, cerebral and renal for example (52) (100). During atrial fibrillation, the heart's response to exercise is abnormal. The normal response during sinus rhythm to exercise is an increase in heart rate, which, inturn, means an increased cardiac output.

During atrial fibrillation, the heart rate increases following exercise, is excessive and recovery is delayed. Mitral insufficiency or regurgitation is also a consequence of atrial fibrillation (22). Dye-transfer studies show that, during atrial fibrillation, dye "escapes" into the retrograde chamber and that this is a functional event, for there was no organic lesion of any of the cardiac valves. With atrial fibrillation, there is a greater susceptibility to thromboembolic complications. Epidemiological surveys indicate that these may be in the vasculature of the heart itself, in the coronary supply system, or in the vascular system as a whole. If an "ideal", safe drug were to exist, these consequences might all be prevented by drug-induced conversion of all patients with atrial fibrillation to normal sinus rhythm (45). At the present time, many patients remain untreated, their arrhythmias persisting.

Patients with chronic or paroxysmal atrial fibrillation, who receive the standard quinidine therapy, are converted to a normal sinus rhythm in but 60 to 70 per cent of the patients (5) (27); 30 to 40 per cent continue to fibrillate despite maximal quinidine dosage. Of the 60 to 70 per cent who do respond favorably to the quinidine treatment, one-half of these cannot be maintained in sinus rhythm, and they revert to the former state of atrial fibrillation. Thus, the overall per cent success with quinidine would be 30 to 40

per cent. Several clinicians feel that the benefits of quinidine, even for the few patients responding satisfactorily, is not worth the risk; according to Sokolow (96), one of 300 patients under quinidine therapy, died suddenly. The merely unpleasant side effects of quinidine have been studied and evaluated, since its introduction into clinical medicine; to state just a few, the allergic response, drug rash and drug fever, thrombopenia, shock and vascular collapse (12). "Cinchonism" (37), a syndrome common to all of the cinchona alkaloids, is an unusual sensitivity of the nervous system of certain individuals to these alkaloids. Symptoms take the form of tinnitus, vertigo, intense nervous and emotional reactions, and even psychosis. There are also effects of quinidine on the heart itself, such as the increased incidence of conduction block, or the development of ectopic foci (8) (102). Quinidine may depress the heart and suppress the sinus activity so that it may force the heart into escape mechanisms in the form of idioventricular rhythms and ventricular tachycardia. Therefore, large doses of quinidine, or even small doses in susceptible hearts, may precipitate arrhythmias instead of preventing them. Thus, there is a need for a better drug than quinidine.

The pharmacologic "screening" methods for the antifibrillatory drugs employs various techniques, as those on isolated heart preparations or the intact animal, to

determine not only the principle action of the drugs but also the side effects and toxicity. The animal studies are paradoxical in the evaluation of antifibrillatory agents, as regards to predicting their clinical usefulness. The results of the animal studies have been used as the basis for many theories and hypotheses, but the correlations between antiarrhythmic potency in experimental animals and effectiveness in patients with arrhythmias is very disappointing (18). This makes very difficult the evaluation and interpretation of antifibrillatory drug "screening" studies in animals (31). In the preliminary experiments that must be conducted on the animals, prior to giving the drug to humans, the experiments on dogs have been found to be useful in identifying the safe maximum dosages and rate of administration for the human application (95). The electrocardiographic changes that occur with the administration of various antiarrhythmic agents, may also lead to a possible mechanism of action for these drugs. The ability of the drugs to alter the depolarization rate of the heart is shown by the change in the width of the QRS complex and a change in the width of the P wave (25). The QRS wave is the electrical manifestation of the depolarization time of the ventricles and the P width, the depolarization time of the atria. Drug changes indicate a slowing of the rate of depolarization of atria and ventricles, can be seen in the accompanying

electrographic recordings. (See Figure 1). Any changes (broadening) of the QRS interval is interpreted as a direct result of the drug action, for it has been reported (28) that the duration of the QRS complex is not altered by a change in heart rate. Both the Q-T and P-R intervals are known to lengthen with a decrease in heart rate.

Quinidine is the standard drug used clinically for atrial fibrillation and is the basis of comparison for any new agent submitted as an antifibrillatory drug. In designing a new drug, the pharmacologic concept of biochemorphology or chemobiodynamics is involved. Schueler (90) in his treatise on the subject, brings out the fact that in the complex structures of drugs, such as quinidine, certain portions of the molecule are necessary for activity, or for "successful interaction" of the drug molecule and the biological system for a real or hypothetical "union", therefore, a beneficial pharmacologic response. The other portions of the compound may produce the harmful effects due to the "unsuccessful interaction" and actually elicit a blockade or antagonism to the biological system. The quinidine molecule is composed of a methoxy quinoline ring, bridged to a bicyclic structure called the quinuclidine moiety, which also has a vinyl side chain. On the bridge between the quinoline and quinuclidine, there is a secondary alcohol group. The author feels that the quinuclidine portion of the molecule is the portion or

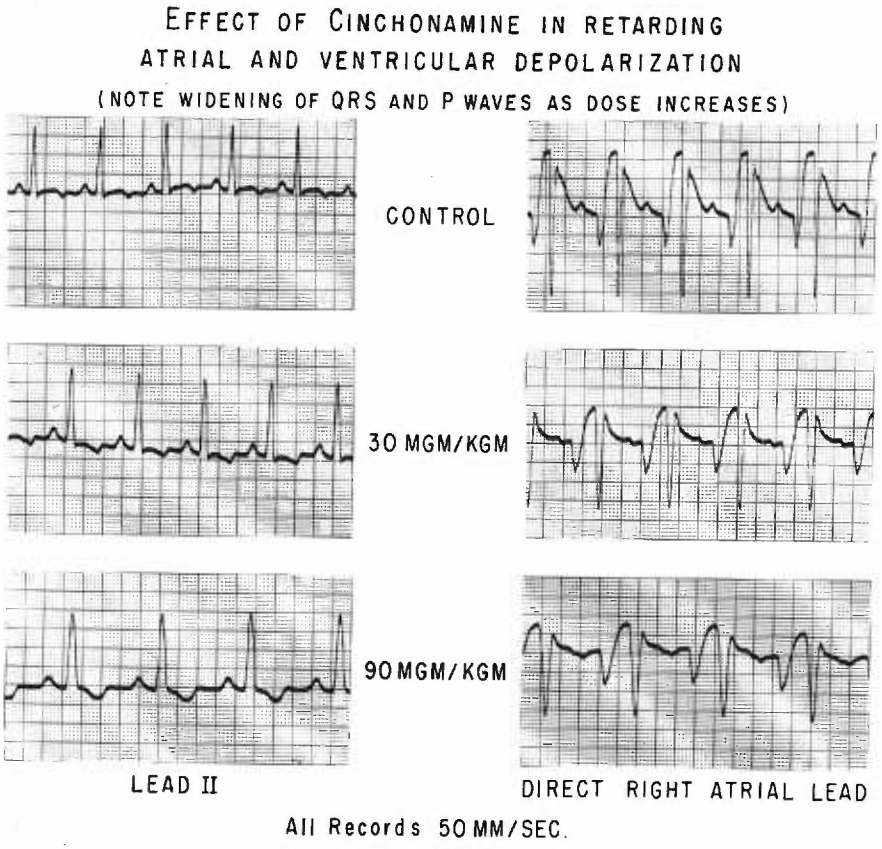

Figure 1. A typical electrocardiogram of an anesthetized dog, illustrating the period prior to drug administration as the control on the top, with the center and bottom recordings obtained during the infusion of cinchonamine sulfate at 1 mg./kg./minute. All records were taken at 50 mm./second.

"active" component, responsible for the antifibrillatory activity. Certain quinoline compounds not containing the quinuclidine ring are devoid of antiarrhythmic activity, where at the present time, quinuclidine ring containing compounds not substituted by other ring systems are not available. Therefore, we considered that the substitution of the quinoline portion by some other heterocycle or alkyl group may increase the activity of the compound and decrease many of the undesireable side effects. In search for such a structure, the compound best fitting the requirements was the alkaloid cinchonamine. (See Figure 2).

Cinchonamine contains the quinuclidine portion with a vinyl side chain and is bridged to an indole nucleus. This structure is interesting, in that it is the first alkaloid containing a quinuclidine ring also to have an indole nucleus, whereas all other cinchona alkaloids contain the quinoline ring system. The indole does not have the methoxy radical, but it does contain an ethanol side chain, primary and not secondary as with quinidine. The various portions of the molecule will be discussed later as to their activity.

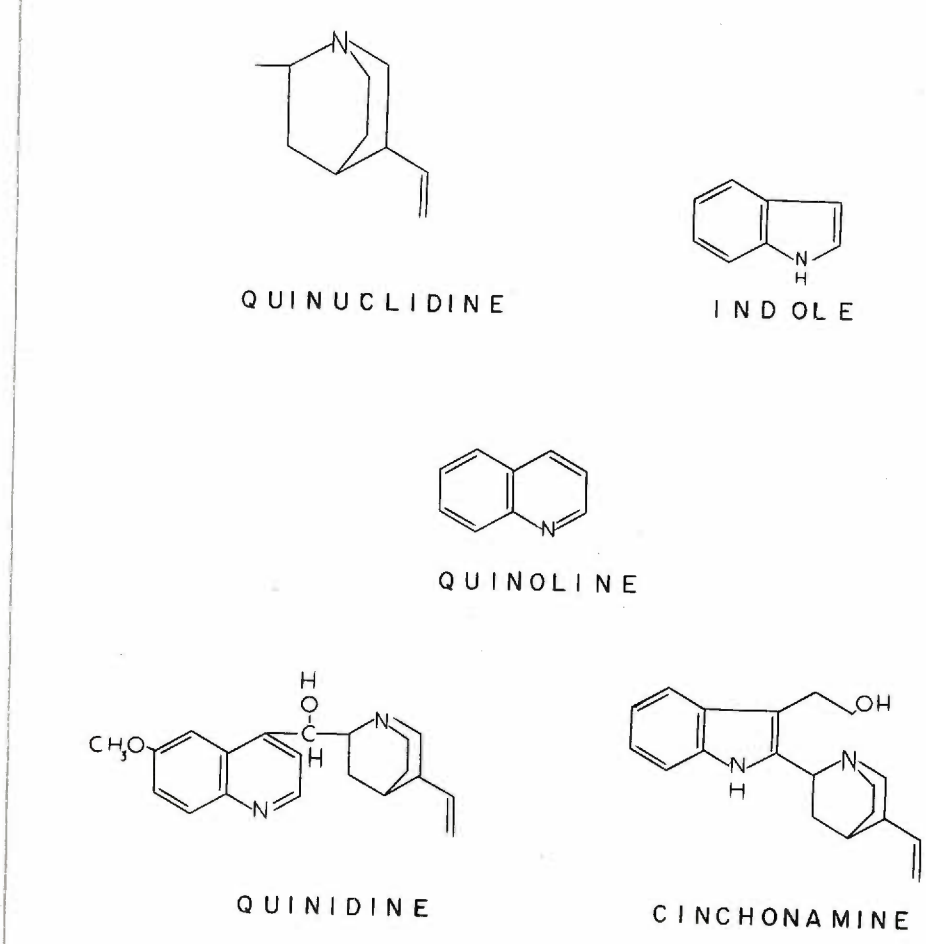

Figure 2. Structural formulae for quinidine and cinchonamine. Note that quinidine is a quinolyl-quinuclidine ring system, while cinchonamine is an indole-quinuclidine structure. Both alkaloids also have an alcohol and vinyl radicals.

THE PURPOSE OF THIS STUDY WAS TO TEST THE HYPOTHESIS THAT DRUGS CONTAINING THE QUINUCLIDINE RING SYSTEM, BUT LACKING THE QUINOLINE PORTION, WILL BE EFFECTIVE IN CONVERTING ATRIAL FIBRILLATION TO REGULAR SINUS RHYTHM.

A DRUG'S ANTIARRHYTHMIC ABILITY (AND POTENCY) IN ANIMAL EXPERIMENTS, DOES NOT SHOW SATISFACTORY CORRELATION WITH THERAPEUTIC EFFECTIVENESS IN PATIENTS. THUS A LIMITED SERIES OF QUINUCLIDINE CONTAINING ALKALOIDS WERE STUDIED IN DOGS WITH NORMAL HEARTS, USING ELECTROCARDIOGRAPHIC CHANGES AS AN INDEX OF CARDIAC ACTIVITY. AN ATTEMPT WAS THEN MADE TO CORRELATE DRUG-INDUCED CHANGES ON THE NORMAL HEART WITH THE BASIS OR BASES FOR TERMINATION OR PREVENTION OF CLINICAL CARDIAC ARRHYTHMIAS. THESE PRELIMINARY STUDIES, IT IS HOPED, WILL PROVIDE INFORMATION IMPROVING OUR UNDERSTANDING OF THE MECHANISM OF ACTION OF QUINIDINE AND IN TURN ON THE GENESIS OF HUMAN CARDIAC ARRHYTHMIAS.

Historical Background:

Drugs With Antiarrhythmic Activity

Quinidine was first identified in 1848 by van Heyninger (97), as one of the alkaloids present in the bark of Cinchona ledgerderia, then used widely in the treatment of malaria. It was first prepared as a pure crystalline entity by Pasteur in 1853 (97). Although exploratory pharmacologic experiments on quinidine are reported in the nineteenth century, no real interest in it was shown until the time of World War I. Wenckebach introduced the l-isomer of quinidine (quinine) into the treatment of atrial fibrillation in 1914 (101). He relates that a Dutch merchant presented himself with a history of two diseases, atrial fibrillation and malaria. When the merchant used quinine to suppress his malarial attacks in Java, he discovered that his episodes of atrial fibrillation also ceased. Wenckebach subsequently used this drug successfully in the treatment of other patients with atrial fibrillation and those with other cardiac arrhythmias. von Frey was the first to employ quinidine, the d-isomer of quinine, in the treatment of atrial fibrillation (75). He also found that other cinchona alkaloids, cinchonine and cinchonidine, were effective in restoring sinus rhythm of patients with cardiac arrhythmias, but concluded that they were less useful than quinidine.

Gold (37), recently has shown the superior effectiveness of quinidine to be due to its more complete and uniform absorption from the intestinal tract, when compared with quinine or the non-methoxyl containing congeners, cinchonine and cinchonidine. All of these cinchona alkaloids are quinolyl-quinuclidine derivatives. It is thus logical to assume that any quinolylquinuclidine-containing cinchona alkaloid, injected intravenously, would exhibit an "equipotent" antiarrhythmic potency on the heart. Such a hypothesis is not subject to simple experimental proof, for the intravenous administration of these alkaloids to patients with atrial fibrillation is quite hazardous.

Non-cinchona drugs have been used effectively in the treatment of chronic atrial fibrillation and other cardiac arrhythmias. These drugs include: allocryptopine (alpha fagarine), quinacrine (Atabrine®), sparteine, procaine amide (Pronestyl®), and lidocaine (Xylocaine®) (11). While allocryptopine and sparteine were effective in chronic atrial fibrillation, they were too toxic for general use, provoking serious ventricular arrhythmias and death in as many as 8-10 per cent of the patients treated (26). The remaining drugs could not terminate chronic atrial fibrillation, though they find practical use in paroxysmal atrial or ventricular arrhythmias.

Thus, it was concluded that a search for a more effective drug for chronic atrial fibrillation most probably would center on alkaloids related to quinidine.

A striking characteristic of Cinchona bark is its red color, due to the presence of anthocyanine pigments. Commercial samples of bark were often graded on the basis of this color. By 1880, the cinchona trees of South America were so depleted that copper colored barks were substituted. It was noticed then that the copper colored barks imported into Europe were not effective in treating malaria. Upon chemical examination, they were found to contain no quinine. Histologic section of these barks revealed that they were not true Cinchona, but a related species named Remijia, after the Brazilian physician who first described it. This new bark, Remijia purdieana, Wedd., contained two entirely new alkaloids, given the trivial names cinchonamine and concusconine (47). Few papers on these alkaloids were published between 1881 and 1950; only their melting points and emperic structures were established. In 1950, Goutarel and associates (39), identified the structure of cinchonamine as being that of an indole nucleus bridged to a quinuclidine ring, with an ethanol side chain on the indole molecule. This structure was interesting in that it is the first alkaloid discovered containing a quinuclidine ring, but without a quinoline nucleus. Still other important alkaloids

such as reserpine and yohimbine, psilocybin, bufotenine and others, although containing the indole ring, do not have the quinuclidine portion. (See Figure 3). The quinuclidine ring, in a disguised or condensed form, has recently been described in ajmaline of Rauwolfia (Apocyanaceae, family), and also some of the other derivatives from the same genus, as for example ajmalidine, rauvomitine, and sandwicine. Other compounds that possess the quinuclidine structure are shown, but very few of these alkaloids were available for study. An isomeric form of the quinuclidine ring is present in the alkaloid ibogaine obtained from the African shrub, Tabernanthe iboga Baillon (acanthaceae). Thus, from Remijia, Rauwolfia, and Tabernanthe plants, it was possible to obtain several indole-quinuclidine alkaloids for comparison with quinidine as potentially useful drugs in the therapy of atrial fibrillation. Only in the last few years has it been possible to synthesize the quinuclidine ring (14) (41); only a few derivatives have so far been prepared and the synthesis is difficult, as starting materials are not readily available. The natural quinuclidine-containing alkaloids were thus chosen as a starting point to learn if the indole-quinuclidine compounds possesses antiarrhythmic properties. Synthetic quinuclidine derivatives for pharmacologic study remain in the future.

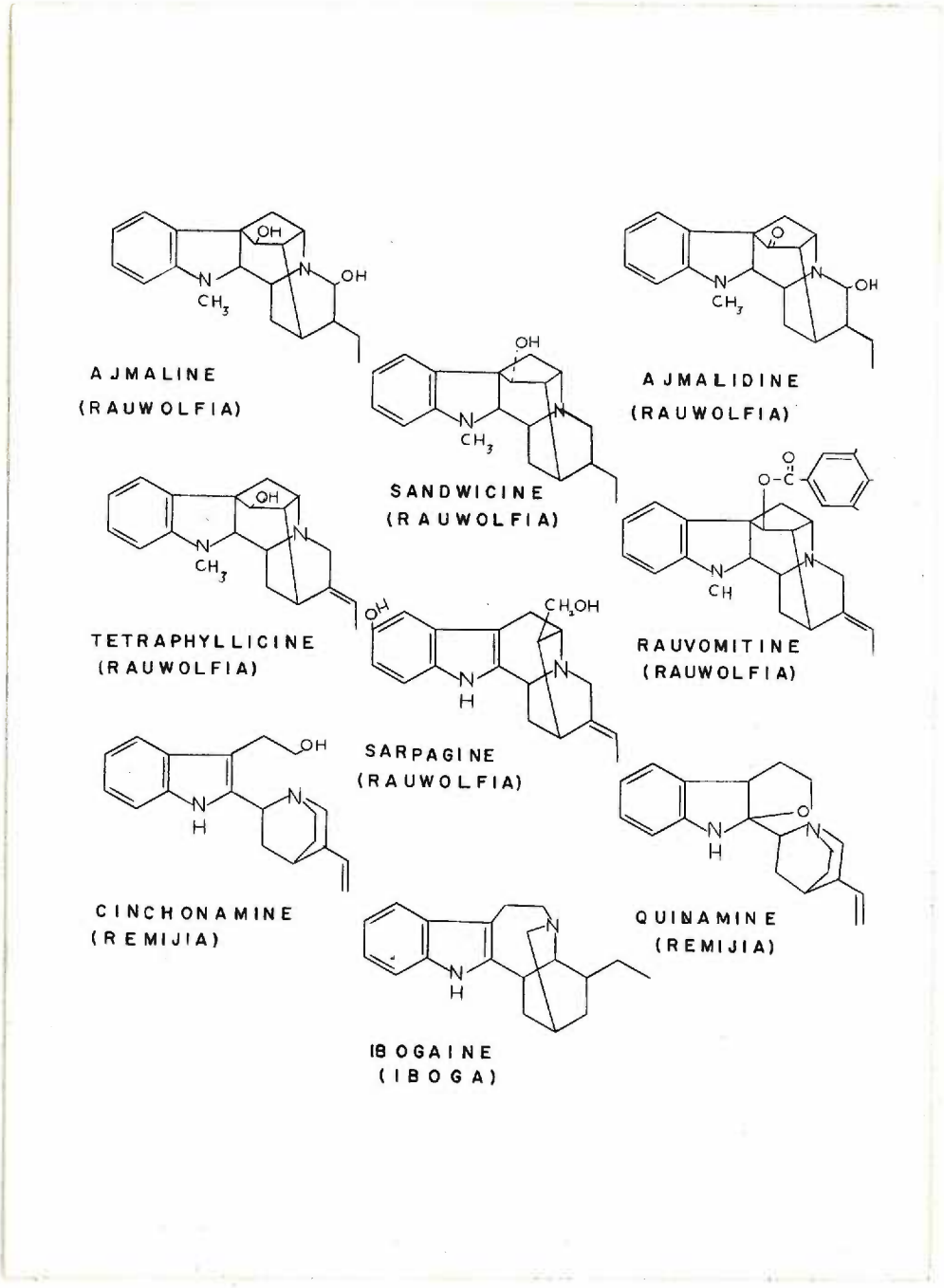

Figure 3. The various alkaloids and their plant sources are shown. The indole-quinuclidine alkaloids fall into two groups: 1. those with a single bond "bridge" between the two heterocyclics and 2. those wherein the connecting bridge forms an additional ring system.

Recently (63), it was proposed that the quinoline ring of quinidine is not essential for its cardiac activity, but rather is responsible for peripheral vasomotor collapse. The ring systems of compounds that we have selected for study allow testing of the theory, that the quinuclidine ring system is essential for the antiarrhythmic activity and that the indole portion may prove to have a lesser amount of the vasomotor side effects.

The replacement of the quinoline ring with an indole may not only be effective as an antifibrillatory agent, but may also have the ability to decrease the frequency or intensity of the other side effects that are present in those agents containing the quinoline nucleus.

Quinidine has many side effects, some accounting for death, others merely troublesome. Among the untoward effects are those on the cardiovascular system, such as are seen in the electrocardiogram; broadening of the QRS and P waves, changes in the heart rate, "escape" mechanisms, in the form of idioventricular rhythms or even ventricular tachycardia

It is to be hoped that the alkaloids containing the quinuclidine nucleus and the indole moiety can be shown to be as therapeutically effective as quinidine, but with fewer toxic actions on the heart. Other undesireable actions of quinidine include non-cardiac actions summarized under the syndrome of "cinchonism" (12). The actions of quinidine on

the central nervous system are many and varied and are characterized by a ringing in the ears, blurred vision, tremor, light-headedness or giddiness, emotional changes, and even psychosis (9). Other side effects that may be occasionally seen are; headache, excitement, confusion, delirium, photophobia, diplopia, apnea, convulsions and shock (13). The actions of quinidine on the gastro-intestinal system include: nausea, vomiting, abdominal pain and diarrhea (95); while on the renal system, the effects take on the form of nephrosis and anuria (57). The allergic responses to quinidine are similar to those found for any drug given for a prolonged period of time, namely; drug fever, rash, urticaria (73) (94), gingival hemorrhaged (98), exfoliative dermatitis, eczematoid reactions (99), sinus thrombosis (86), leucopenia with transient spenomegaly (48), and thrombocytopenic purpura (10) (60). Despite all these side effects, quinidine is the drug of choice for the treatment of most cardiac arrhythmias and has remained the standard of comparison during the 44 years since its introduction. To be accepted however, an effective antiarrhythmic indole quinuclidine alkaloid must be shown to have fewer side effects than quinidine.

The biochemorphologic approach (90) to the study of compounds intended for therapy of cardiac arrhythmias has two objectives; it may lead to the design of an "ideal" drug

or may provide information about the site or mechanism of action of the drug which may shed light on the basic mechanism of the disease itself. In the biochemorphic approach, attention is focused on the chemical structure of drugs. Various portions of the molecular structure may be removed by chemical degradation, or a chemically related series obtained by the addition of various radicals to the basic molecule. All drugs are then examined pharmacologically and chemical structure-biological activity relationships derived. The various cinchona alkaloids and available chemical derivative have been studied in a preliminary fashion for such structure-activity relationships (23) (3). The methoxy group, though not essential, contributes to the increased activity of quinidine, as compared to cinchonine, its congener, apparently by virtue of improved absorption from the intestine. The secondary alcohol radical may be replaced by an alkyl or ether moiety without loss of effectiveness in malaria. The quinoline portion may be replaced by a number of aromatic radicals, such as naphthyl or phenyl without loss of the antimalarial activity of the compound. Certain animal studies would suggest parallel changes for antiarrhythmic action, but these derivatives have not been tried in patients. On the quinuclidine ring is a vinyl side chain, which seems to decrease the antifibrillatory potency, as compared to the saturated form (91). For in the

commercial preparation of quinidine sulfate, there is approximately 20 per cent of the saturated form, or dihydroquinidine, which when tested alone in patients with atrial fibrillation appears to be more potent than the parent derivative, quinidine. This apparent advantage for dihydroquinidine is subject to question, for differences in reported potency may not be statistically significant; double-blind studies have not been made. Dihydroquinidine is used by physicians in Algeria, the rest of the world uses quinidine. It seems justifiable to conclude that either a vinyl or ethyl side chain on the quinuclidine ring retains the antiarrhythmic activity.

Cinchonamine was the simple choice of a compound which fulfilled the above chemical requirements. It does contain the quinuclidine portion and has a primary alcohol on the indole nucleus. The vinyl side chain is present on the quinuclidine portion, so that structurally the two compounds, quinidine and cinchonamine, are quite similar. (See Figure 2).

Clinical Cardiac Arrhythmias

A discussion of atrial fibrillation and other clinical cardiac arrhythmias is necessary for two reasons; first to examine the basic pathophysiologic or pathobiochemic mechanisms establishing the arrhythmias and so to provide a pharmacologic point of attack for drug studies, and secondly

to establish the deleterious effects of the arrhythmia on the patient and so provide indications for the administration of antiarrhythmic drugs. One of the earlies hypotheses concerning the nature of atrial fibrillation was proposed in 1874 by Aubert and Dehn (6) (7). They suggested that the altered rhythm was produced by injury to a "nervous coordination center" in the heart. Other than the sino-atrial node, no such "coordination center" in atria can be demonstrated. Engleman (33) then noticed that various parts of the fibrillating atria were in different phases of contraction at any given instant of time, and in 1895 proposed the existence of multiple isolated areas of impulse, or foci. From this tentative suggestion, has developed the "ectopic foci" theories of the genesis of atrial fibrillation and other cardiac arrhythmias as well. The "circus movement" theory of atrial fibrillation was developed from MacWilliams' hypothesis of a re-entry phenomenon (64). In the conduction of an electrical impulse from sino-atrial node to atrio-ventricular node, the impulse wave was believed to re-enter areas previously stimulated, and restimulating them. Garrey, in 1914 (35), proposed the existence of a circular movement for this re-entry wave in atrial fibrillation. The results of the detailed studies by Sir Thomas Lewis and his co-workers (30 (61), has resulted in an almost universal acceptance of the "circus movement" theory.

Lewis, in 1918, concluded that atrial fibrillation was a "condition in which a single wave curculates continuously the auricular muscle. The path taken both by the central and centrifugal parts of this wave is sinuous and varies in greater or lesser degree from cycle to cycle (58)." This conclusion was proposed as an alternate theory to that of MacWilliams (64), who postulated that the excitation wave of fibrillation pursued a sinuous course through the atria, traveling in a direction determined by the excitability of the muscle fibers in its path, and that the cause of fibrillation was to be found in the condition of the cardiac muscle itself. In recent years Lewis' concepts have been challenged. At the present time no one theory for the mechanism of atrial fibrillation or indeed any cardiac arrhythmias is universally accepted. The theories do find counterparts in animal experiments providing a basis for pharmacologic experiments. That is experimentally produced disease (arrhythmias) are produced in normal dogs to detect antiarrhythmic potency of the drugs to be tested. By placing aconitine or acetyl choline on the atria, arrhythmias are formed presumably because of the formation of an ectopic focus. Rosenbleuth and Garcia-Ramos (87), crushed the intercaval bridge of the heart of dogs with forceps and the arrhythmias which resulted are presumably caused by establishing an inter-atrial circus movement.

The deleterious effects of cardiac arrhythmias are well established, particularly through the recent studies of Prinzmetal and Corday (75). The heart provides blood supplying all tissues and cells of the body with oxygen and nutrients, and provides for the removal of carbon dioxide and waste products of metabolism. An average adult male's heart, weighing approximately 350 grams, will, during the 70 year life span, beat some 3 billion times, pumping and circulating a total of 15 million gallons of blood (11). Complete failure of the heart for as brief a time as 85 seconds results in death of the individual; partial failure results in heart disease, severely limiting the activities of the victim.

Disturbances in the rate or rhythm of the heart, which are followed by a lowered cardiac output, an inefficient coronary blood flow, formation of a functional mitral or tricuspid valve regurgitation and an increase in the frequency of the complication of thrombo-embolic phenomena, are the main problems that need attention in the fibrillating patient (75). Blood flow to other vital areas, due to the deleterious effects of the arrhythmia is also impaired; for example the renal blood flow is decreased, along with that of the cerebral and mesenteric flow (52). Therefore, a termination of the arrhythmia would be accompanied by; an increase in the cardiac output, an important reduction in

the complication of the thrombo-embolic phenomena, an increase in the blood flow to the various important areas of the body, coronary, mesenteric, cerebral etc, the disappearance of mitral and/or tricuspid insufficiency, an efficient response of the patient to exercise and a decrease in the venous pressure.

Atrial fibrillation, perhaps the most common of the arrhythmias, may be considered as the spring-board of other arrhythmias. In the patient with chronic sustained fibrillation, only quinidine and the toxic allocryptopine are effective in restoring a normal sinus rhythm. Quinidine is also effective in arrhythmias other than atrial fibrillation; paroxysmal atrial fibrillation, atrial flutter, paroxysmal atrial tachycardia, premature ventricular systoles and ventricular tachycardia. Other drugs are effective for a specific type of arrhythmia, but only quinidine can be said to be a general antiarrhythmic drug. Therefore, drugs having a close structural relationship to quinidine may also have a general antiarrhythmic action.

Atrial fibrillation, a disturbance in the rate and rhythm of the heart is the most common persistant arrhythmia. Except in hyperthyroidism, it is rarely seen in the absence of organic heart disease. Paroxysmal atrial fibrillation is however, often found in hearts without organic

disease. Atrial fibrillation is commonly associated with mitral stenosis, thyrotoxicosis, arteriosclerosis, congestive heart failure and hypertension. It is rare in aortic disease and in subacute bacterial endocarditis. In the paroxysmal type, the patient may suddenly develope tachycardia, palpitation, precordial pain, dyspnea, vertigo, nausea, pallor or even collapse. These attacks are often associated with hyperthyroidism, undue exertion, acute infection or the use of drugs, alcohol or tobacco. Recurrent or persistent attacks give rise to the chronic form, commonly associated with congestive heart failure. Other than the enlarged atria and sustained increase in the intra-atrial pressures, no characteristic pathologic lesion can be identified as responsible for the onset or continuance of atrial fibrillation.

The orderly sequence of atrial contraction is replaced by a chaotic, inefficient contraction of auricular muscle. The engorged and functionless auricles show rapid, futile, fibrillatory twitchings at a rate of 450 or more per minute. The ventricles, unable to respond to but relatively few of these atrial stimuli, because of functional atrioventricular block, usually have a rate of about 120 and rarely over 140 per minute. Their contractions are totally irregular, both in force and rhythm. This rapid and irregular heart action leads to inadequate

diastolic ventricular filling, with a resultant inability, due to weak contractions of many beats, to open the aortic valve leaflets. A pulse deficit results; the difference in heart rate at the apex and radial pulse varying from 10 to 100 beats per minute (9).

Atrial fibrillation and flutter are similar in nature. Fibrillation, according to the interpretation by Lewis' hypothesis, is caused by the "circus movement" within the auricular musculature, which travels at a variable rate anf follows an inconstant path. Flutter is then believed to be due to the "circus movement" within the auricular muscle, following a constant path at a constant rate. Thus, there has been proposed, because of the close similarity of these arrhythmias, a "unitarian" theory (75). This theory suggests that there is no essential difference among all atrial arrhythmias; premature atrial contractions, atrial tachycardia, atrial fibrillation or atrial flutter. They differ only in the rate of stimulation (circus wave) and the rate of response, thereby they are classified as different arrhythmias. Atrial premature systolic rhythms appear to be almost identical to a normal slow sinus rhythm. Only occasionally is a circus wave activated, resulting in an occasional P wave of abnormal configuration. In atrial tachycardia (paroxysmal), the

rate of discharge from the sinus node exceeds the rate of discharge from the A-V node, and no aberrant circus wave need be proposed, or the wave is rapid and uniform. The ventricles respond to each auricular beat. Then, with atrial flutter, the rate of discharge from the circus wave falls into the range of approximately 300 to 400 beats per minute (62); atrio-ventricular block occurs and a discrepancy of atrial and ventricular contractions occurs. Atrial fibrillation also follows when the rate of discharge from the circus wave exceeds the ability of auricles to respond rhythmically. This threshold for fibrillation is usually between 400 to 600 per minute. A similar "unitarian" theory can be developed using the ectopic focus (foci) mechanism for all atrial arrhythmias. The various types of arrhythmias are caused by variations in rate of discharge and/or sites of ectopic focus (foci). Since there is a unity of cause of these arrhythmias, therefore these arrhythmias will all respond to but a single antiarrhythmic-antifibrillatory drug.

The ventricular arrhythmias might also be considered from the point of view of the "unitarian" theory. That is, that premature ventricular systoles, ventricular tachycardia and ventricular fibrillation originate from the same ectopic foci, therefore a unity in the cause of the

arrhythmia. Ventricular premature contractions are initiated in either ventricle by an ectopic focus (foci), and other than the occasional "prefiring" of the ventricle, the rhythm is that of normal sinus rhythm. Paroxysmal ventricular tachycardia differs from premature ventricular systoles only in the increased rate of impulses from the ectopic focus. Ventricular fibrillation is the same as ventricular tachycardia except for the further increase in rate of firing of the ectopic focus. With this hypothesis, all of the ventricular arrhythmias would respond to but one "antiarrhythmic-antifibrillatory" drug.

There are drugs which can be used to prevent or terminate cardiac arrhythmias, but cannot, or should not be classified as antiarrhythmic drugs because their action on arrhythmias is secondary to their more important pharmacologic action. For example, hydroxyzine pamoate(Vistaril®) is used as an antiarrhythmic to abolish ventricular extrasystoles and paroxysmal ventricular tachycardia, but its main pharmacologic action, like that of phenobarbital, is on the central nervous system; a tranquilizer drug. Presumably it suppresses the discharge from the anterior or posterior hypothalamus. Electrical stimulation of these areas, using a stereotoxic instrument, has been shown to provoke extra systoles (69). Procaine amide (Pronestyl®) may be said to depress the "irritability" of

the cardiac muscle, and for both atrial and ventricular extra-systoles, by virtue of its local anesthetic action. This drug fails when the arrhythmia is not associated with an organic lesion causing hyperirritability. Norepinephrine (Levophed®) is effective in suppressing arrhythmias only when they are caused by an abrupt fall in the blood pressure such as may occur in myocardial infarction, when this compound is really acting as a pressoramine. Since these and other drugs do not have a primary pharmacologic action on the heart, they cannot be considered to be antiarrhythmic drugs.

Studies of Antiarrhythmic Drugs In Experimental Animals

The first attempts to study antiarrhythmic drugs in animals followed immediately on the publication of Sir Thomas Lewis' (61) concepts of the circus movement. He believed that if a drug were to prolong the refractory time period of the heart, then it would so lengthen the path of the circus movement that it would collapse by meeting unreactive tissue and sinus rhythm would resume. Quinidine was shown to prolong the relative refractory period of the beating heart and study of other drugs soon followed. During World War II, Dawes (24) devised a simple technique to measure the effect of drugs on the atrial refractory period. Isolated rabbit atria are placed in a heated bath and

stimulated electrically. As the frequency of the stimulus is increased, a threshold will be reached, where the atria will no longer comply with a 1:1 response to the applied stimulus. The reciprocal of this maximum response rate (frequency) is a measure of the refractory period. Since the stimulus intensity is some arbitrary value above rheobase, the measurements are termed "effective refractory period". Quinidine, local anesthetics, antihistamines and a large variety of other drugs prolong this relative refractory period (25).

The means of producing atrial fibrillation in experimental animals in the laboratory for the testing of antifibrillatory drugs may be divided into three main groups; first the drug or chemically induced form of fibrillation, as in the direct application of the alkaloid aconitine to the surface of the myocardium; secondly there is that form of fibrillation seen as a result of electrical stimulation, and lastly there is the mechanically induced fibrillation of the atria by injury or surgical manipulation. Those experiments resulting in atrial fibrillation from electrical stimulation of the auricle, at a rate of 500 to 1000 per minute do show a characteristic brief atrial fibrillation. At a critical threshold current the arrhythmia appears <u>after</u> the current is shut off, "nachflimmern". After the threshold current has been derived, drugs were then infused and the

stimulation reapplied. If there was a rise in the threshold current required to elicit the post-stimulation arrhythmia, the drug was considered to have potential antifibrillatory activity. The chemical induction of atrial fibrillation by aconitine was first used by Matthews and Cushny (21). It has been used both as topically applied crystals or as a solution in benzene, often on a paper pledget, or can be injected subepicardially in an area near the sino-atrial node (20). The local application of the aconitine seems to be preferred by most investigators using this technique (75). Acetyl choline or acetyl beta methyl choline (Mecholyl®) also has been applied in the same manner. They provoke atrial arrhythmias apparently identical to those of aconitine in nature and cause, but are of shorter duration. The third technique, that of injury, as in the crushing of the auricle, or by teasing the atria with a wooden applicator or by heating the atria with hot lamps, were found to be the least reliable (75). All of these techniques for provoking atrial fibrillation in animals whether electrical, chemical or mechanical, are believed to involve a common cause, the local tissue release of acetyl choline. Atropine is effective in preventing or terminating all of these arrhythmias. Atropine in large doses, however, does not terminate clinical atrial fibrillation. Therefore, the results of drug studies on the experimental arrhythmias in experimental animals may

have no value whatsoever in predicting usefulness of the drug in man.

A variety of techniques have been developed to provoke ventricular arrhythmias in experimental animals. Premature ventricular contractions and other ventricular arrhythmias follow aconitine or acetyl choline applications to the ventricle and result from electrical or mechanical stimulation. Ventricular arrhythmias follow the administration of several drugs administered systematically; for example, digitalis overdosage, Amodiaquin (Camoquin) produces ventricular bigeminy; epinephrine alone provokes all types of atrial and ventricular arrhythmias; epinephrine following cyclopropane inhalation causes ventricular tachycardia and epinephrine following chloroform inhalation results in ventricular fibrillation (68).

There is general dissatisfaction with the results of all of these antiarrhythmic pharmacologic studies, so much so that in turn, it casts grave doubts on all hypotheses for the mechanisms proposed for the genesis of arrhythmias. Luther Terry in a speech at the National Institutes of Health stated that 1284 compounds were selected as having antiarrhythmic potency from animal studies, 58 had low enough toxicity to be given clinical trial. None of the 58 drugs tested in patients with arrhythmias showed useful therapeutic effects.

There are, however, many observations of the pharmacologic actions of quinidine on the heart, which may prove to be useful in the evaluation of the potentially useful antiarrhythmic indole-quinuclidine alkaloids. The hypothetical basis for these studies can be described briefly as a "toxic parallelism". The assumptions are that, quinidine exerts some unknown action which is responsible for preventing or terminating arrhythmias. This action is subtle and not yet recognized in studies on animal hearts in sinus rhythm. With toxic doses of quinidine this subtle action of quinidine on the heart is intensified and can be measured. It must be assumed that these same actions, when elicited at lower therapeutic doses are in fact, responsible for the therapeutic (antiarrhythmic) effect. Further, it is assumed that new drugs showing toxic actions parallel to those of quinidine on the heart, that these new drugs will have a high probability of antiarrhythmic potency when given in lower (non-toxic) doses to patients with arrhythmias.

The direct actions of quinidine on the heart are best measured by the electrocardiogram. They are, in general, dose-intensity related and include: broadening of the QRS and P waves, prolongation of the P-R and Q-T intervals; appearance of U waves; transitory tachycardia, then bradycardia; reversal to an upright deflection of the T wave and with an increased height and area; change in S-T junction

position; decreased height of R and an increase length of S wave (31).

Therefore, the effects of various indole-quinuclidine containing alkaloids were administered by intravenous drip providing a continuously increasing dosage and the electrocardiogram recorded (also continuously). At the present time, only successful clinical trials can show that the "toxic parallelism" concept is useful in the discovery of new antiarrhythmic drugs.

METHODS AND MATERIALS

The evaluation of the indole-quinuclidine alkaloids as antiarrhythmic drugs involves three different types of experimental approach:

A. Chemical: isolation, purification, and identification of alkaloids from plant sources;

B. Pharmacological: observation and measurement of the responses of the dog heart to the indole alkaloids;

C. Clinical: human pharmacologic investigation of the indole alkaloids in patients with cardiac arrhythmias.

In some instances, there will be no mention of an experimental method and/or materials used, but this information will be found in the results section for it seemed preferable to include their description along with the experimental data later on in the text. This will facilitate understanding the results obtained. The experimental procedures actually used are thus outlined here, giving details of those techniques which are not adequately described in the section of results.

A. Chemical Methods.

The isolation of cinchonamine from Remijia purdieana, Wedd., followed the standard methods for the extraction of alkaloids from plant materials. The salts of weak organic acids of nearly all tertiary alkaloids are soluble

in alcohol. Thus, ground Remijia bark was extracted with acid-alcohol. Sufficient acetic acid was added to obtain a pH of 4.5 to 5.5. By this process much tannin, cellulose, lignins and other polymer materials were separated from the desired alkaloids.

The next stage in purification takes advantage of the amphoteric nature of plant alkaloids. The aqueous-alcoholic solution of the alkaloid-organic acid salt is made strongly alkaline; forming alkaloid bases. Ammonium hydroxide is used, for water-soluble sodium phenates which are formed if the alkaloid contains a phenolic hydroxyl group. Using a relatively non-polar solvent, such as ether or chloroform, the alkaloid bases are transferred to the organic solvent phase, leaving behind aqueous-alcohol soluble impurities, which are not amphoteric by nature. Finally the alkaloid bases are transferred from the organic solvents to water by forming salts of strong acids. Briefly, the ether is extracted with dilute sulfuric acid. Much colored matter, anthocyanine dyes and chlorophyll and its degradation products remain in the organic solvent phase and are discarded.

Purification of the alkaloids is a highly individual, empiric process. Fractional crystallization, using a variety of non-polar, semi-polar and polar solvents was tried. The nine transfer Craig counter current distribution

system, involving a two phase system of buffers at varying pH and mixtures of solvents, is very effective in purifying alkaloids and separating them as well. The right combination of components of the counter-current system must be determined by trial and error. Column chromatography, particularly using "activated" alumuna or the newer polymer resins and differential elution of alkaloids, removes impurities and is also a powerful purification tool. Conditions again are established by trial and error.

Recent developments of analytic techniques have enormously facilitated the identification of alkaloids. No longer must we depend alone on melting points of the alkaloids and their salts, although they are still done. The general alkaloid precipitating reagents, Wagner's, Dragendorff's and Millon's, were useful to detect the presence of alkaloids during the fractionation process. The precipitates depend on the reduction of aqueous solubility of the alkaloids upon the addition of iodine or mercury. The ultraviolet absorption spectra, using a Carey recording ultraviolet spectrophotometer, provides some clues as to identity of the alkaloid but is most valuable in establishing purity by comparing the molecular extinction coefficient of a characteristic maximum or peak, with that obtained from known samples or reported in the literature. The infrared absorption spectra, obtained from a potassium iodide pellet

in the Perkin-Elmer 221 G instrument is a most valuable aid in identifying alkaloids. The multiple absorption bands in the infrared region of the alkaloid may be compared with those of a known sample or the published literature. It is also possible to identify particular structural moieties which have a characteristic resonance resulting in an absorption band at a known wave length (or frequency). Neither the nuclear resonance spectrograph nor a polarimeter with sufficient resolution were available to provide their information on fine structure of heterocycles or stereo isomerism.

B. Pharmacological Methods.

The pharmacologic procedures adopted for the anti-arrhythmic studies in animals were of two types. The influence of the various indole alkaloids and quinidine on the electrical properties of the heart were studied from electrocardiographic recordings. Secondly, various ventricular arrhythmias were produced in dogs by various techniques and the indole alkaloids used in an attempt to prevent the arrhythmias being established or to restore sinus rhythm, the ventricular arrhythmia already present.

Electrocardiographic methods: Adult mongrel dogs of either sex were weighed and anesthetized with sodium pentobarbital administered either intraperitoneally or intravenously. The dose of pentobarbital, usually 30 mg.

per kilogram, was supplemented as necessary trying to maintain a constant (light surgical III.) level of anesthesia.

The electrocardiogram was recorded using either the portable viso-cardiette or Sanborn Model 150 four channel recorder. A 50 mm per second paper rate facilitated measurements. A lead II record was obtained in all experiments. In most experiments selected records were taken using augmented limb leads AVR, AVL and AVF and right chest V_3R or left chest V_6 to V_8 leads these electrocardiograms were recorded almost continuously throughout drug administration and intermittently (10 to 15 min. internal) for a 3 to 4 hour post-drug time period.

For analysis, the electrocardiograms were placed in an opaque projector (Rectolineoscope) and enlarged on a screen. The manification was 55 to 60 diameters. Four heart beats from each record were traced on millimeter graph paper using the upper border of the trace made by the electrocardiographic stylus. The interpretation of the electrocardiogram followed the standard clinical practice as established by the New York heart association. To measure the length (or duration knowing the paper speed) of the QRS wave a lead was selected having both a Q and S wave. QRS width was measured from that point where the Q leaves the isoelectric line until the end of the S wave. Since the S.T. junction was not always at the isoelectric

line, the end of the QRS wave, therefore, may not always be on the isoelectric line. The beginning and end of the waves of the electrocardiogram is a mathematic asymptote. In order to facilitate a decision as to origin and termination of waves, tangent lines were drawn through the slope of the wave and also the isoelectric line. In this way the small arc formed between the intercept aids identification at the beginning and end of any wave.

The measurements of Q-T and P-R intervals offered no difficulties, although those leads were chosen depicting the largest waves (i.e., the greatest voltage) and inverted T or isoelectric T waves avoided when possible. It is noted that as in standard practice, the term P-R interval is used, though it is a P-Q interval.

Unlike the width of the QRS and P waves, the duration of the Q-T and P-R intervals varies inversely with heart rate. Thus any drug-induced change in Q-T or P-R interval may be the direct result of drug action or secondary to a change in heart rate. The influence of heart rate on these intervals was eliminated by using "corrected" time intervals. In this procedure, the Q-T and P-R interval duration is computed at the arbitrarily selected heart rate of 60 per minute. This rate was chosen because the reciprocal of heart rate, the R-R interval is 1.0 seconds or 1000 msec.;

a simple mathematical divisor. Bazette's formula (25):

$$QT_c = \frac{Q\text{-}T}{\sqrt{R\text{-}R}}$$

was adopted for the "corrected"* $Q\text{-}T_c$ interval. Fujiwara's relationship between P-R interval and heart rate (25) was transformed algebraically to the following expression:

$$P\text{-}R_c = \frac{1.754\ P\text{-}R}{R\text{-}R^{0.122}}$$

The time intervals of electrical silence or "diastole" for atria and ventricles were derived by difference. Thus, the time required for electrical diastole was subtracted from the total time of electrical systole viz.,

for atria	S_eT_c	=	$P\text{-}R_c$	- P width
for ventricles	S_eT_c	=	$Q\text{-}T_c$	- QRS

The measurements of various waves, or time intervals, of the electrocardiogram, from any one lead, were found to vary widely from dog to dog. Further variation is encountered in those same measurements from one lead to another.

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*The interpretation of these corrected time intervals must be used with caution. The original data was obtained on volunteer human subjects varying the heart rates from 58 to 130 per minute by exercise. The validity of adapting these formulae to anesthetized dogs with heart rates varying from 130 to 200 per minute has not been examined.

To clarify the interpretation of drug-induced changes of these wave and interval durations, the results are expressed interms of per cent change. In this way, each dog served as its own control, the drug effect being some per cent increase or decrease from the pre-drug control record taken as 100 per cent. To cite an example, a control QRS interval of 80 msec. broadened to 160 msec. by drug action, would be expressed as 100 per cent increase.

All drugs were administered by continuous intravenous drip at a constant rate. Although screw-driven syringes, using constant speed motors were tried, the calibrated Murphy-drip technique was most practical. A femoral vein was exposed by cut-down and catheterized with a polyethylene tube of suitable size. The Murphy-drip devise was adjusted or calibrated to deliver 1.0 ml. per minute. The concentration of quinidine gluconate or the indole alkaloids were then varied to obtain the desired dose in mg. per kg. per minute. Sterile normal saline was used as a diluent. A few preliminary experiments were necessary to arrive at an optimal dosage rate. For each drug, an arbitrary rate of administration was chosen and the intravenous perfusion continued until the dog died. A satisfactory or optimum rate of administration was then obtained when death occurred within about 30 to 90 minutes

after starting the injection of the drug. These times were chosen merely to allow time for 2 to 4 dog experiments to be performed in one working day. This technique provided a large series of many dosages with a correspondingly large number of observations (the electrocardiogram). The rate of administration, chosen in this manner also yielded a progressively increasing series of dosages, permitting plotting of a dose-response curve for each drug on every parameter studied. The dose increased, for the rate of administration exceeded the rate of drug loss from liver metabolism or renal excretion.

In some, but not all experiments, respiratory movements and blood pressure were recorded simultaneously with the electrocardiograms. The trachea was exposed, cannulated and connected to a Marey tambour in the traditional fashion. The rubber tambour, however, was connected to a strain guage transducer and a carrier wave Wheatstone bridge pre-amplifier used in the Sanborn multichannel recorder. Blood pressures were recorded from a polyethylene catheter inserted in a femoral artery. A Statham P 23 D strain guage transducer and carrier wave Wheatstone bridge system were used.

Techniques for producing ventricular arrhythmias in dogs.

The details of the techniques used to provoke experimentally-induced ventricular arrhythmias is presented coincidentally with the recording of results of the indole alkaloid experiments in preventing or terminating these arrhythmias. This, it was believed, would ease understanding the results obtained. Moreover, each technique has been modified to adapt to equipment available and modernized more in terms of modern pharmacologic concepts. In one instance, that of amodiaquin-induced ventricular bigeminy, the technique was devised from an observation in the literature. Thus, while an experimental method, it actually was developed during the course of these studies.

C. Clinical Method and Material.

Before any new drug can be submitted to a large number of physicians (clinicians) for clinical trial, to establish its potential place in our therapeutic armamentarium, human pharmacologic studies must be performed. In these human pharmacologic studies, the general plan followed is to administer a single dose of the drug that is believed well below that dose provoking any pharmacologic response. Some quide to select this dose is to be had from the animal experiments done earlier. However, there are still unexplained differences in mg. per kilogram doses between

animals and man. This dose is then increased until a definite pharmacologic response or desired therapeutic change takes place in the human volunteer or patient. The dosage increment adopted for the new drug is usually based on previous clinical experience with similar drugs. In the antiarrhythmic drug field, the dosage increment has been traditionally a factor of the square root of two, or two itself (i.e., doubling). It is obvious that the appearance of any troublesome or potentially dangerous side effect necessitates immediate discontinuence of the drug.

The clinical human pharmacologic studies of ajmaline and cinchonamine in patients parallels the cardiac study of these same drugs in the anesthetized dog. That is, for the electrocardiogram, blood pressure and respiratory activity were recorded at frequent intervals as the dosage level of the drug was increased. This parallelism of study, decreases the risks involved when a drug is to be given for the first time in history to a human being. That is, despite differences in dosage between dog and man, certain quide posts or warnings of danger, are available. It was known, for example from the study in dogs, that following a prolongation of the QRS interval from 50 to 100 per cent, that A-V block would occur. Similarily an excessive rate of administration of the indole alkaloids in the dog caused tachycardia and hypotension. These warning signs of

potential danger were accidentally confirmed in a patient when a defective Murphy-drip tube allowed too fast a rate of administration of the drug.

It was possible to solicit information about the subjective symptoms of the new drug effects in patients.

Some of the experimental details of necessity are included in the description of the clinical results.

RESULTS

A. Extraction of Alkaloids from Remijia purdieana.

The procedure as described by Hesse (47), was used for the isolation of the alkaloids of Remijia purdieana, Wedd. The following is a simplified outline "flow-sheet" for the process: (See Figure 4). A 40 kilogram sample of the dried bark of Remijia purdieana was obtained from Bogata, Columbia. The botanical identification was certified by Sr. botanico, Dr. Rafael Romero Castaneda, Ministerio de Agricultura, Republica de Columbia.* The bark was ground in a Wiley Mill using 1 mm. mesh screen. The total alkaloids of the ground bark were then extracted with 95 per cent alcohol at room temperature by maceration and percolation. Ground Remijia bark was mixed with Monterey white sand, 1 part sand to 10 parts bark, to minimize packing in the percolator. A total of 170 liters of alcohol was used, with sufficient glacial acetic acid added to maintain a pH of 4.5 to 4.9 (ca. 600 ml.). The alcohol was divided into five portions, each allowed to stand with the bark from 24 to 48 hours; the final alcohol extract

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*This material was supplied through Astra Products Farmaceutico Ltd., Bogata, through the courtesy of Dr. Bengt Christiansson AB Astra Sweden, without who's generous assistance, this work could not have been done.

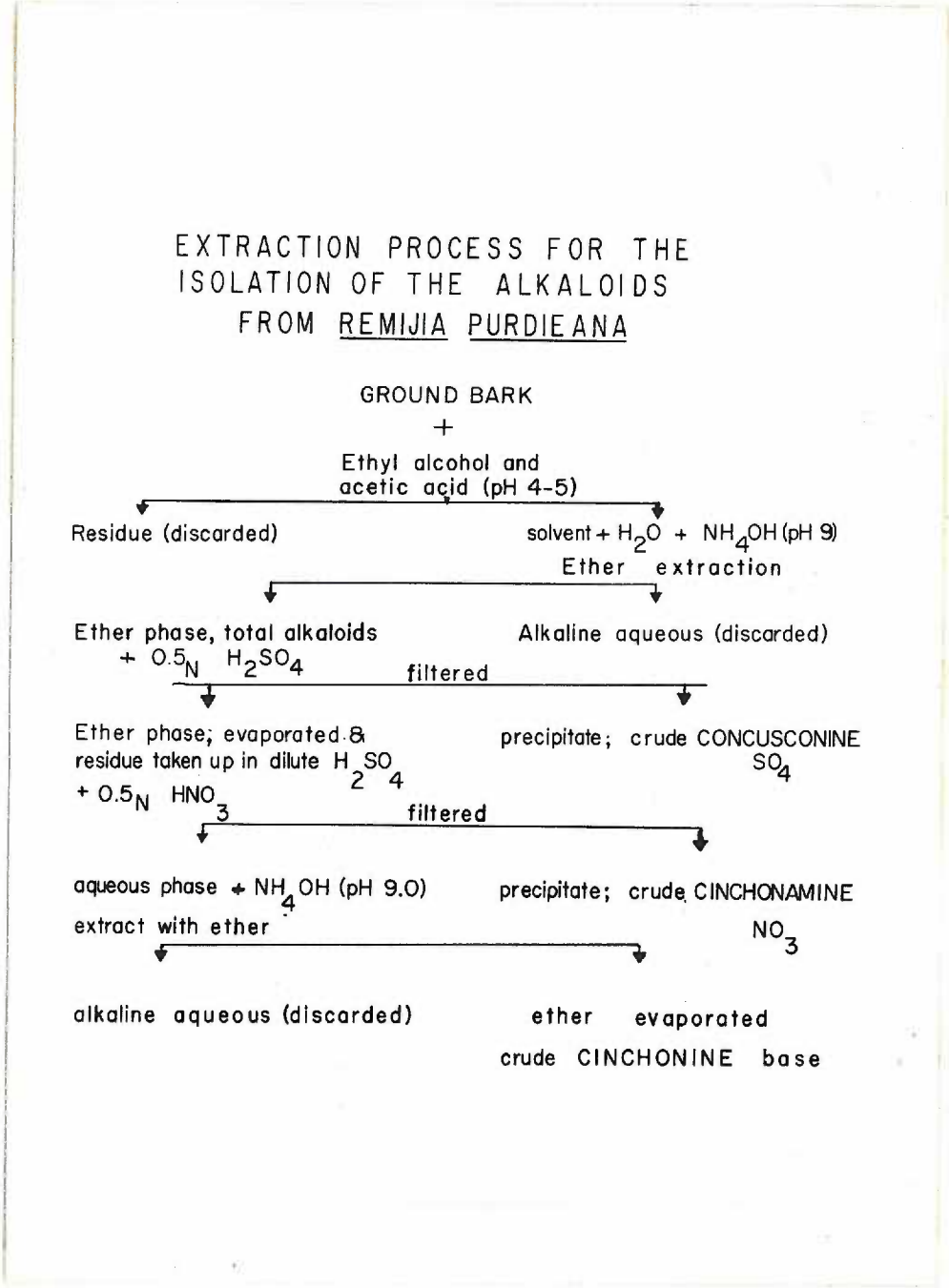

Figure 4. Outline of the extraction process for the isolation of alkaloids of Remijia purdieana, Wedd. Some of the minor steps have been omitted, but the overall scheme shows at which points concusconine, cinchonine and cinchonamine were obtained.

gave a positive Wagner's test at a dilution of from 1 to 8, to 1 to 16 (70). The dark red-black alcohol extract was next concentrated. The alcohol was distilled off *in vacuo* (water aspirator) using a stream of nitrogen gas, and maintaining the bath temperature from 30 to 40° C. The last of the alcohol was removed by fan from evaporating dishes. These precautions were observed because the indole-containing alkaloids are unstable to heat, light and oxygen.

The black tarry liquid remaining, 21.3 liters, was chilled, saturated with nitrogen gas and made alkaline with 2.5 N ammonium hydroxide, to a pH of 9.0. 400 ml. aliquots of this extract were placed in separatory funnels with 200 ml. of ether (purified to remove peroxides) and placed on a rolling extraction apparatus for 20 minutes. A second and third extraction was necessary using 200 and 100 ml. aliquots of ether respectively, to complete the transfer of alkaloid bases to the ether phase.* Emulsions formed but two layers separated out on standing. The intensely green ether extract, 38.7 liters, was washed with ice water.

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*A brown clay-like precipitate formed in the aqueous phase after extraction with ether. This was collected, filtered and dried and an additional alkaloid yield recovered by Soxhlet extraction using ether. This alkaloid crop was added to the main yield.

The green, chlorophyll-containing, ether extract was next extracted with 0.5 N sulfuric acid, transferring the alkaloids to their sulfate salts and thence into the aqueous layer. Most of the chlorophyll and other pigments remain in the ether phase. Sufficient sulfuric acid extractions were done until the Wagner's test was positive at a dilution of 1 to 16, or 1 to 32. A green, cheesy, gelatinous precipitate appeared at the solvent interface and on the sides of the separatory funnel. This precipitate, identified by Hesse (47) as crude concusconine sulfate, was removed and dried for later work up. Yield of crude concusconine was 40 grams.

To the acid aqueous phase, containing the remaining alkaloid sulfates, 0.5 N nitric acid was added forming a copious yellow precipitate, the insoluble nitrate salt of cinchonamine. After the precipitate was removed by filtration, a second and third crop could be obtained by addition of more nitric acid and chilling overnight in the refrigerator. Total yield of crude, air dried cinchonamine nitrate was 205 grams, or a maximum content in the bark of 0.5 per cent.

After removal of insoluble cinchonamine nitrate, the aqueous phase containing the sulfate and/or nitrate salts of still insoluble alkaloids, was worked up. This aqueous phase was made alkaline, to pH 9.0, using 7 per cent

ammonium hydroxide. The alkaloid base(s) was then extracted with ether. The ether extract was then set aside in the fume hood to evaporate in the air. The precipitate which formed after the removal of the ether Hesse identified as cinchonine base. This fraction was washed with ice water and dried in a desiccator; the yield was 35.5 grams.

Purification of the alkaloids from Remijia purdieana. Concusconine: This fascinating alkaloid, named after the Inca city Cusco, high in the Cordielleras, is very resistant to purification. Crude concusconine sulfate was transformed into the salt of a weak acid, hoping that enhanced solubility in water, and therefore a better ability to crystallize, would follow. It was believed possible to avoid mixed salts, interferring with crystallization, which could occur, by forming mono- or di-sulfate salts (assuming the presence of two basic nitrogen nuclei). Concusconine sulfate, on gentle warming, was dissolved with glacial acetic acid and diluted with water. On concentrating the solution by fan, concusconine acetate crystallized in long needles, lightly tan in color. The yield was however, several fold greater than theoretical and this was presumed to be water of crystallization and possibly poly-acetate inclusion, which did not lose all of the water or acetate on prolonged standing in a

vacuum desiccator over phosphorous pentoxide.

Concusconine acetate and concusconine sulfate were then converted to the base by the addition of 7 per cent ammonium hydroxide. The concusconine base was then extracted into peroxide-free ether. After washing the dark colored ether with ice water, the solvent was removed by evaporation. The concusconine base was dissolved in the minimum amount of boiling water. When cooled, the whole solution formed a greenish-brown transparent gel. This was washed with ice water which removed much colored material. When the gel was dried, a light yellow powder of concusconine remained. Thisprocedure, although not satisfactory, was the best method for the purification of concusconine. Hydro-alcoholic mixtures were also tried, but on cooling these also formed gels and appeared to offer no advantage. Concusconine base is readily soluble in benzene and ether, but only slightly soluble in petroleum ether, but would not recrystallize from these solvents. Crude concusconine sulfate (another portion) was treated with a 20 per cent solution of sodium carbonate with trituration and gentle heating. The base was then treated with only stoichiometric amounts of sulfuric acid to form the mono-sulfate salt; the amount of water of crystallization was not determined. The concusconine sulfate thus formed did not recrystallize satisfactorily

from either 80 per cent alcohol or boiling benzene.

Preliminary trials of purification by column chromatography, using Dowex 1 and Dowex 50, with either concusconine or concusconine sulfate were unsuccessful. Hydro-alcoholic solutions were adsorbed on the columns and elution attempted using 1 N sulfuric acid. The alkaloid material appeared to remain on the column. Preliminary trials using paper chromatography also were not rewarding. Using a n-butanol, acetic acid and water system, no spot separation appeared when the paper was sprayed with Wagner's or Dragendorff's reagents. Further attempts, using different adsorbants and solvents, are indicated.

Hesse(47) indicates that the crude concusconine sulfate is composed of several alkaloids. He dissolved the crude concusconine sulfate fraction in boiling 80 per cent alcohol; purified concusconine sulfate crystallized on chilling. To the mother liquor, Hesse added hydrochloric acid, precipitating out chairamine hydrochloride. Next to the mother liquor, potassium thiocyanate was added(potassium rhodanate), and conchairamine precipitated out. By adding to the remaining mother liquor, potassium thiocyanate in excess, chairamidine and conchairamidine precipitated and were separated by fractional crystallization from benzene. When this procedure was repeated, the crude concusconine

sulfate formed a gel-like precipitate, when the 80 per cent alcohol cooled. Neither hydrochloric acid nor the potassium thiocyanate added to the mother liquor formed precipitates.

Howard and Chick (50) in 1909 prepared concusconine but were not able to detect Hesse's chairamine or chairamidine stereoisomeric pairs and noted that concusconine sulfate does not recrystallize easily from alcohol. The physical data from the following table are consistent with the hypothesis that the chairamine and chairamidine alkaloids are in fact, crystalline crops, from alcohol, of impure concusconine sulfate, contaminated with the dextro-rotatory cinchonamine and/or levorotatory cinchonine.

The structure of concusconine has not yet been determined, though much information regarding its nature is available. The early work done by Hesse and others in the nineteenth century identified the cinchona-like barks as red or true China or quinquina barks, Cinchona ledgerderia; copper barks as Cinchona pelletierana or Remijia pelleteriana. Hesse (46) isolated from the latter, both cusconine and aricine and though at first denied it, he later stated that Remijia purdieana and cuprea bark, Remijia pedunculata, contained aricine. It thus becomes a possibility that concusconine and aricine are the same alkaloids or are stereoisomers.

Aricine was first isolated from Peruvian, light colored Rubaciae (Cinchona) barks, whose origin was in the province of Arequipa and shipped from the port of Arica. Aricine was also named cinchovatin and possibly for a time was confused with paricine (46). Aricine assumed greater interest, when, like cinchonamine, it was shown to have an indole nucleus on the basis of color reactions (p-dimethyl amino benzaldehyde) (39).

The structural formula of aricine was established by Goutarel et al (39) in 1954, as being an alkaloid of the ajmalicine group of alkaloids (see Figure 3). Aricine, like concusconine, has a molecular composition consistent with the empirical formula $C_{22-23}H_{26}O_4N_2$. Both alkaloids have two labile methyl (or methoxyl) groups, from the Zeisel determination. Goutarel and co-workers (39) depict the ultraviolet and infra-red absorption spectra for aricine. This permitted comparison with data obtained for a concusconine sample recrystallized three times from hydro-alcohol solvents. In figure 5, it can be seen that aricine and concusconine both have an ultraviolet absorption maximum at ca. 220 mμ. In the infra-red spectrum of concusconine, the same carbonyl (lactone) bands are seen at 5.9 μ and 6.1 μ as are found in the spectrum published for aricine. While additional band similarities in other regions of the infra-red spectrum between concusconine and

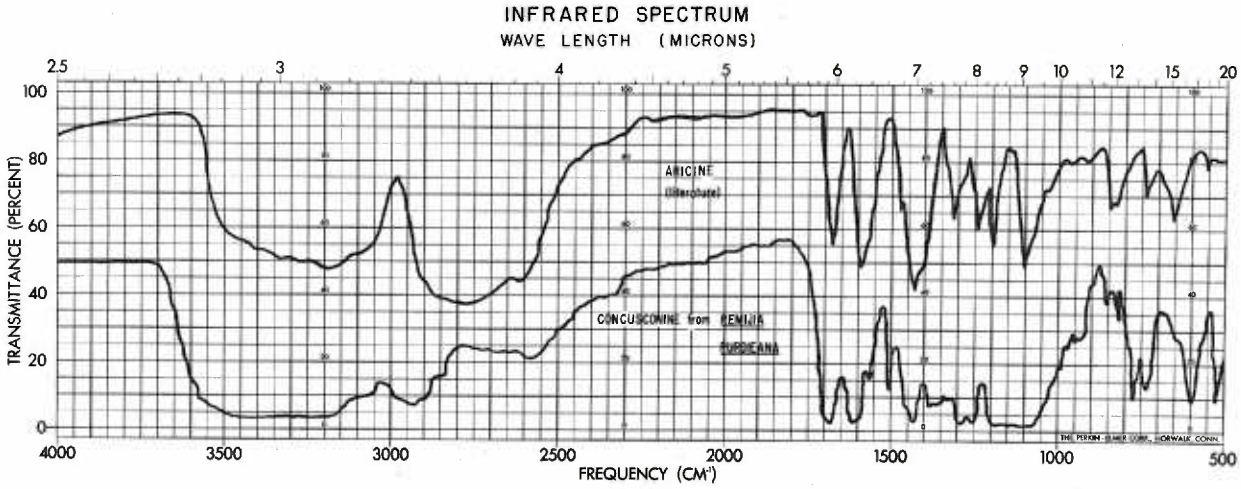

Figure 5. Comparison of the infra-red spectra for aricine and concusconine. The upper curve was obtained from the literature, the lower from that of a sample of concusconine.

aricine can be seen, they are not identical. Thus, it does not seem highly probably that concusconine is the dextro steroisomer of aricine although they may be rather closely related in structure. It is quite pertinent to note that the infra-red spectra for concusconine and cinchonamine are quite dissimilar. In particular the 10.9 mμ vinyl band of cinchonamine is absent in concusconine's spectrum. Thus, since physical properties suggesting concusconine's structure did not seem to imply the presence of a quinuclidine ring system, it was not examined further for anti-arrhythmic properties.

Cinchonamine:

Like that of concusconine, difficulties were encountered in the purification of cinchonamine; a golden fluorescing decomposition product of cinchonamine is formed.

Following the procedure described by Hesse (47), the crude cinchonamine nitrate was washed with ice water and converted to the base by the addition of 7 per cent ammonium hydroxide. The alkaloid base was then washed with ice water and recrystallized from dilute boiling alcohol. (In separate experiments both 20 per cent, volume to volume, and 50 per cent alcohol was used). The precipitate, at first a bright white, soon turned to a canary yellow color as the alcohol cooled; and, on standing, became a bright

orange. The cinchonamine base was sucked dry on a Büchner funnel. To this material, 1 N sulfuric acid was added to a pH of 4.5, to form the mono-sulfate (neutral sulfate) salt. This also was recrystallized from boiling alcohol (50 per cent). Once again the initial crystal crop was white to ivory in color, but on standing, developed an intense yellow-orange color. Ellran (32) previously noted that alcoholic solutions of cinchonamine were decomposed by sunlight, with the addition of oxygen, and recalled that Pasteur found that all cinchona alkaloids were unstable in light and alcohol. It may be suggested that the presence of the nitrate ion may accelerate this oxidation for the cinchonamine. Pure white cinchonamine base was obtained by multiple recrystallization from benzene.* A 20 gram portion of crude cinchonamine nitrate was treated with an excess, 45 ml. of a 20 per cent, of sodium carbonate solution. This was triturated for thorough mixing, and placed in a separatory funnel containing 300 ml. of water and 300 ml. of benzene. This solvent system was flushed out with nitrogen and kept in dim light. Due to the insolubility of cinchonamine nitrate, conversion to the base is slow and shaking of the separatory funnel was continued for 20 minutes. Fresh benzene (saturated with

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*I am indebted to Dr. Gordon A. Alles of the Alles Laboratories, Pasadena, California, for the suggestion and am grateful for other suggestions as well.

nitrogen) was added until the benzene extract showed less than 1 to 16 positive reaction for Wagners' reagent, and 0.5 N nitric acid no longer caused a visible precipitate. Anhydrous sodium sulfate (5 grams per liter) was added to the benzene to remove water, and the hydrated sodium sulfate was filtered off. The volume of benzene was reduced by *in vacuo* distillation, using a stream of nitrogen and a bath temperature not exceeding 40° C. The benzene solution was then divided into two fractions. One portion of the benzene was placed in an evaporating dish and the solvent removed by a jet of nitrogen passing over it. The crystalline cinchonamine base thus obtained was recrystallized from boiling water. The cinchonamine solution was boiled for 20 minutes to remove any trace of benzene (benzene is said to "poison" activated charcoal). The pale brown-yellow solution was boiled an additional 20 minutes with decolorizing charcoal (carbon), and filtered. The pearly-white cinchonamine base crystallized on cooling.

The other benzene portion containing cinchonamine base was used for salt formation. 5 N hydrochloric acid was added dropwise to the solution, and cinchonamine hydrochloride precipitated immediately. The hydrochloric acid addition was continued as long as significant precipitate appeared. The resulting cinchonamine hydrochloride was recrystallized from boiling water, using activated charcoal. For the sulfate salt

of cinchonamine, the benzene was extracted with 0.5 N sulfuric acid; the pH was then adjusted to 4.0, and the volume reduced. The resulting neutral sulfate of cinchonamine was recrystallized from a small amount of water. The melting points of some of the cinchonamine derivatives are listed below;

	Found °C	Literature °C
Cinchonamine base	185-6	184-5 (Hesse) 186 (Goutarel)
Cinchonamine nitrate	195	195 (Hesse)
Cinchonamine hydrochloride	221-223	225-7 (Hesse)
Cinchonamine sulfate	250-251	—

The infra-red absorption spectrum for cinchonamine base, in a potassium iodide pellet, and the ultra violet absorption spectrum of cinchonamine base, in 95 per cent alcohol, agree in every respect with those published by Goutarel et al (38).

Remijalutein:

Considerable interest in the yellow decomposition product of cinchonamine was aroused, because its appearance reduced the yield of the desired cinchonamine. This product has not been previously described, and was assigned the trivial name of "remijalutein", being obtained from the genus Remijia and having a yellow color and yellow fluorescence. Remijalutein is present in the alcoholic extract

of the Remijia purdieana bark, but whether it is preformed in the bark itself, or results as an artifact during extraction, cannot yet be determined. Remijalutein is formed from pure cinchonamine base or the mono-sulfate salt in alcohol solutions. The process is accelerated by exposure of the cinchonamine to an incandescent light and bubbling in oxygen. Cinchonamine in chloroform, exposed to light and air, also forms remijalutein.

Remijalutein was purified by colmn chromatography. Using Dowex 50, a deep yellow material formed from cinchonamine sulfate was adsorbed. Elution with 0.05 to 0.5 N sulfuric acid removed the remijalutein, leaving the cinchonamine still on the column. On another column, using 40 per cent alcohol and 60 per cent water, containing 0.1 per cent ammonium hydroxide, remijalutein appeared in the first few tubes, cinchonamine in the later ones. Using a 30 cm. column, 2 cm. in diameter, Dowex 1 (16 mesh) was added and "activated" by running through 20 per cent alcohol 80 per cent water with 0.1 N sulfuric acid. Elution with the same solvent released the yellow remijalutein sulfate in the first few tubes. The cinchonamine was removed by elution with alcohol-water containing 0.1 N ammonium hydroxide.

The remijalutein was obtained as a dark brown amorphous solid (melting point 106-109°C). Only very small amounts were obtained and a chemical identification is

incomplete. Remijalutein, in dilute acid solution (1 N sulfuric acid), fluoresces a bright golden-yellow color, but does not fluoresce in alkaline solutions, in polar or non-polar solvents. In the ultra violet, remijalutein has the same 270 and 220 mu maxima, but a new one is added toward the longer wave lengths, around 340 mu. The infra-red absorption spectrum for remijalutein also shows similarities to that for cinchonamine, but new bands appear as shown in figure 6.

A small amount, ca. 30 mg. of remijalutein, was dissolved in anhydrous diethylether and added dropwise to lithium aluminum hydride. The yellow color (and fluorescence in acid) disappeared. This experiment was inconclusive, and should be repeated because the isolation of the reaction product was not done.

A quite similar reaction has been described for another indole-isoquinuclidine alkaloid, ibogaine (40). Ibogaine, heated in chloroform in the presence of light and air, forms a yellow fluorescent product, ibolutein. By reduction with lithium aluminum hydride, ibolutein is converted back to ibogaine. Other examples also are known; for example, mavacurin from calabash curare is converted to the fluorescing fluorocurin (66). Thus by analogy with ibogaine, a provisional structure for remijalutein may be proposed, as illustrated in figure 7.

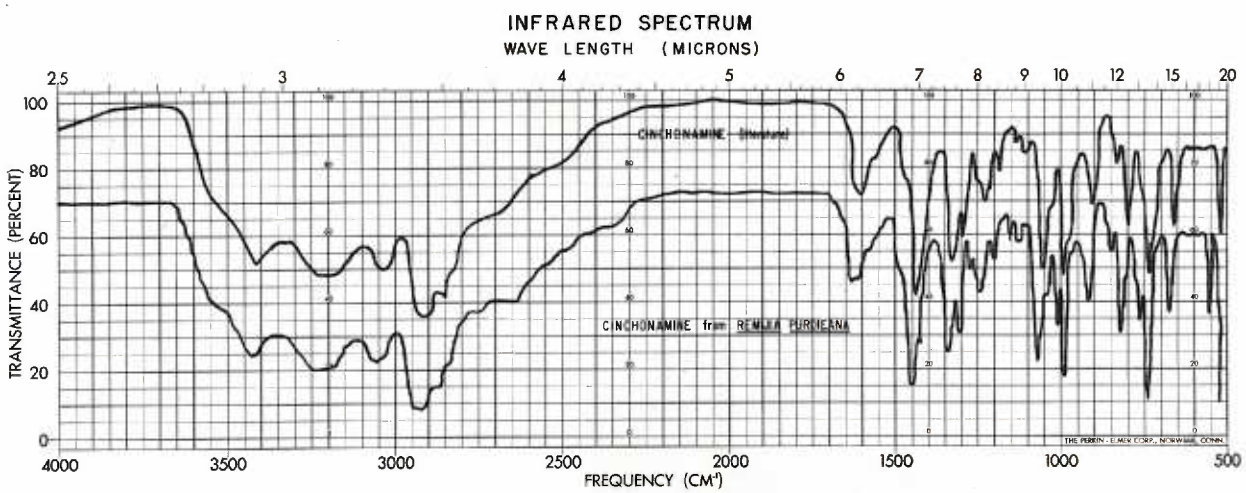
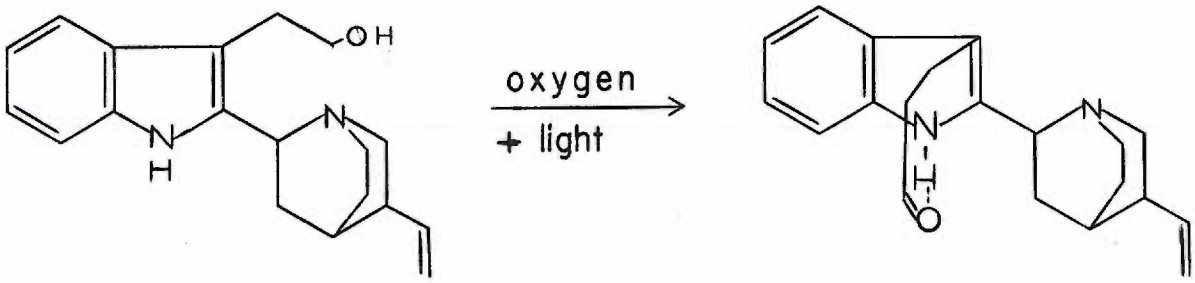


Figure 6. Comparison of the infra-red spectra for cinchonamine. The upper curve was obtained from the literature, and the lower from a sample isolated from Remijia purdieana.

PROPOSED REACTION for the FORMATION of REMIJALUTEIN



CINCHONAMINE

REMIJALUTEIN

Figure 7. A proposed reaction for the formation of remijalutein, and its possible structure, based on the formation of ibolutein from ibogaine.

Cinchonine:

The material remaining after precipitation of the cinchonamine nitrate is, according to Hesse (47), cinchonine. The crude cinchonine base was recrystallized several times from boiling benzene. Beautiful, glistening, white crystals formed. The hydrochloride salt was prepared by adding 1.0 N hydrochloric acid to a benzene solution containing the base and recrystallizing from boiling water. The melting points of the base, 265° C and of the hydrochloride salt, 215° C, agree with the published data for cinchonine (65). The ultraviolet and infra-red absorption spectra also are the same as those published for cinchonine (38). (Figure 8).

The co-existence of the indole-quinuclidine, cinchonamine and the quinolyl-quinuclidine, cinchonamine, in Remijia purdieana and other Rubaciae is unusual. Woodward (104) has proposed a scheme for the biogenesis of the two classes of alkaloids from the same source. Using as starting materials tryptophane, 3,4-dihydroxyphenyl-alanine and formaldehyde, he has proposed that strychnine, cinchonamine, quinine and aricine alkaloids may be formed. (Figure 9).

Based on this hypothesis, Ochiai and associates (71) (72) (42) (53) have synthesized many derivatives of the quinuclidine alkaloids; for example, dihydrocinchonamine

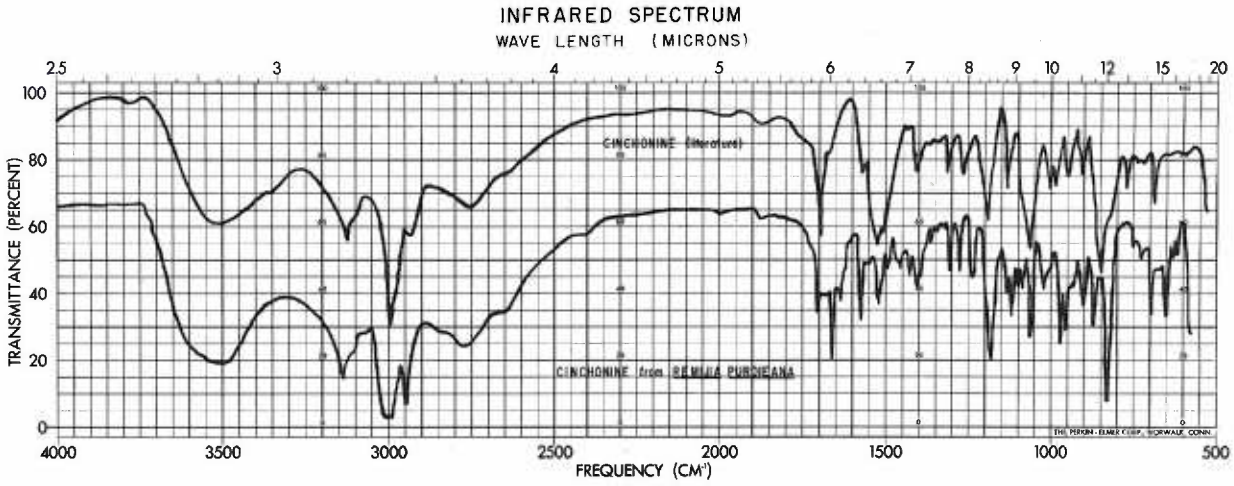


Figure 8. Comparison of the infra-red spectra for cinchonine obtained from commercial sources and the literature, as illustrated in the upper curve, and cinchonine obtained from Remijia purdieana.

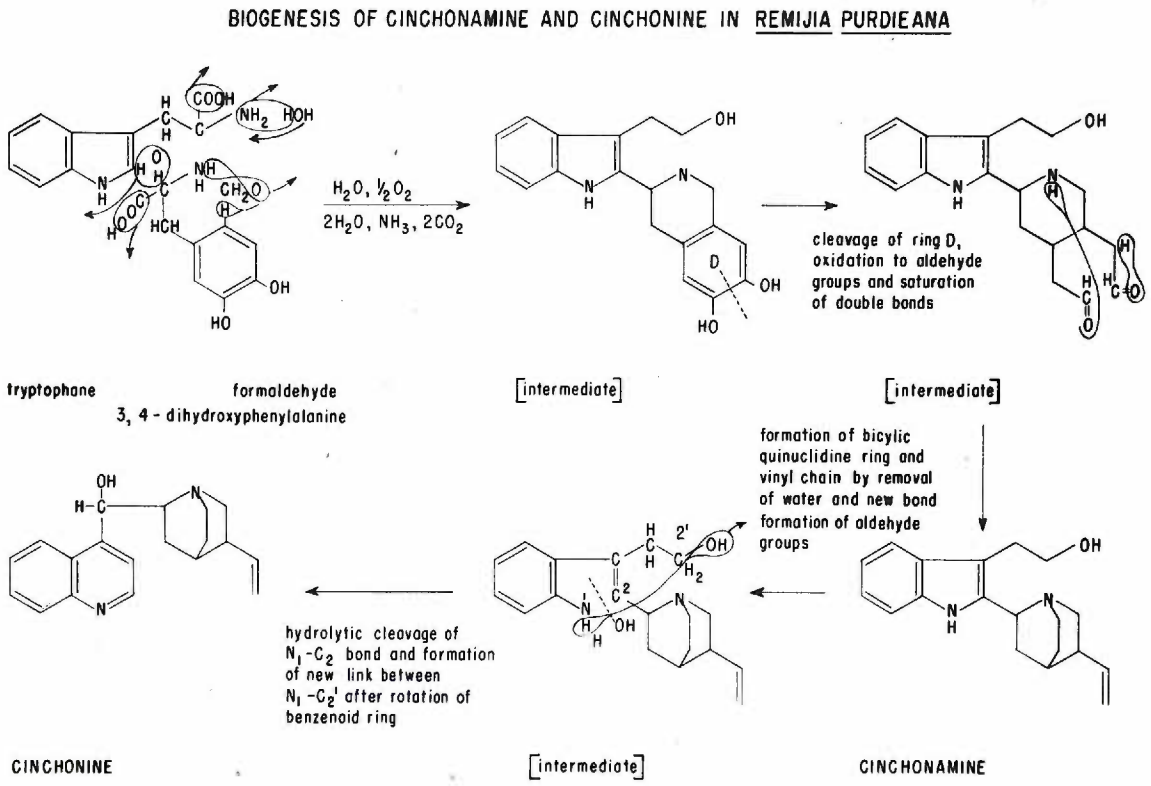


Figure 9. The biogenetic of the two different ring containing compounds, cinchonine and cinchonamine, as proposed by Woodward. The intermediates have not been isolated, but both the reactants and products are found in the plant Remijia purdieana.

has been prepared from cinchonine. (Figure 10). The first stages of this synthesis were confirmed in our laboratory, but limitations of time prevented completion of this synthesis for inclusion in this thesis.

B. Pharmacological Results.

Quinidine.

The effects on the heart obtained by the intravenous infusion of the indole-quinuclidine alkaloids have been observed by recording the electrocardiogram of the intact anesthetized dog. The width of the P and QRS waves; the P-R and Q-T time intervals; and the height (voltage) of P, R, S and T waves were measured. Various leads were employed, selection being based on the widest interval (of control records) and clearest inscription of waves. At least 6 measurements of each observation were made and a mean value derived. At least two dogs were employed for the perfusion of each alkaloid; in some instances 8 replicate experiments were done. A total of 48 dogs were used. To facilitate comparison of different alkaloids with each other and to minimize variation of individual dogs, post drug observations were calculated as per cent changes from the pre-drug control values taken as 100 per cent. Each dog, thus, served as his own control. Since there is a difference in the toxicity and cardiac potency

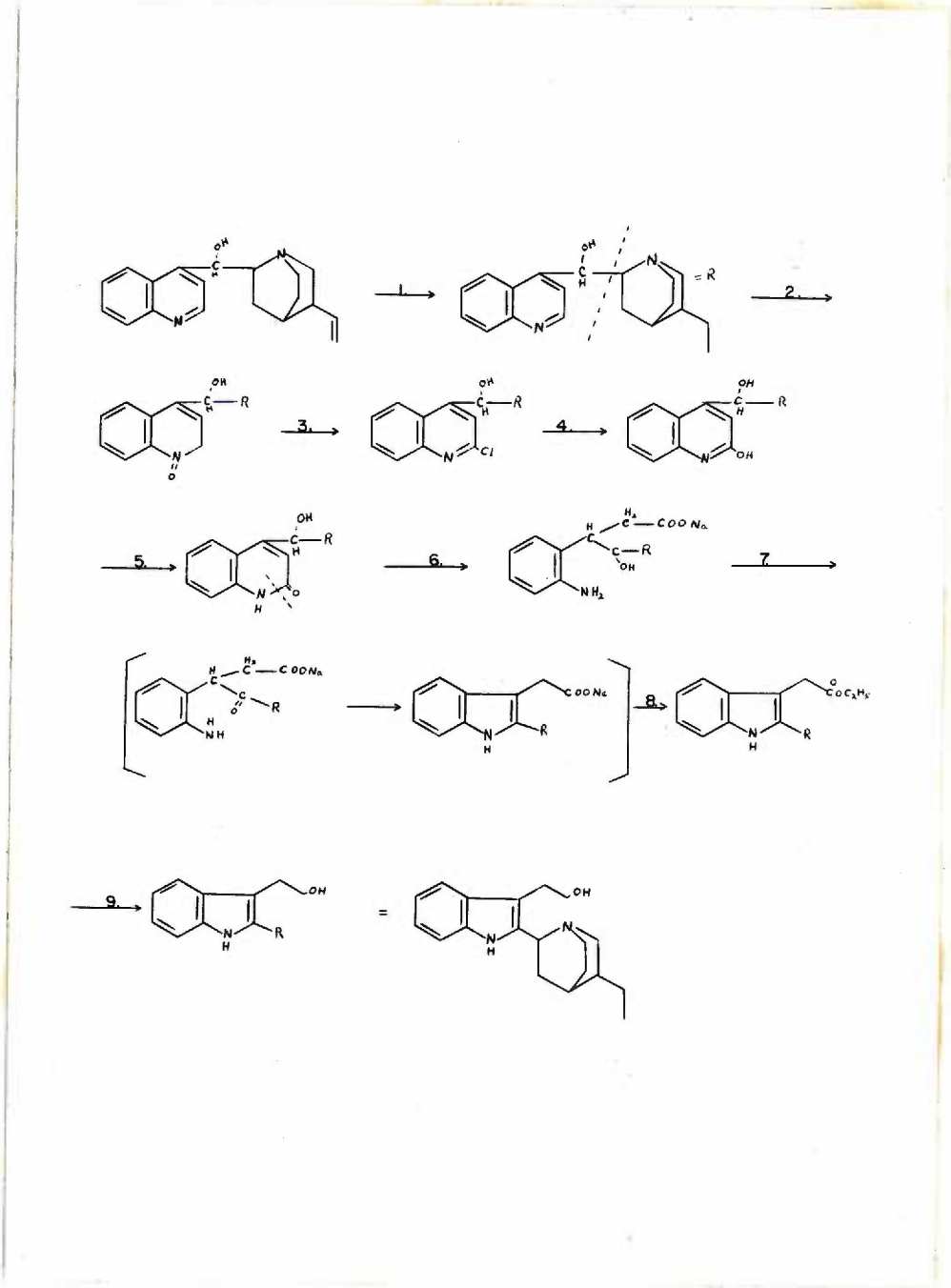


Figure 10. Ochiai's synthesis of dihydrocinchonamine from cinchonine.

of the six drugs, they were administered in different rates. Tetraphyllicine, ibogaine, ajmaline and quinamine were administered at a rate of 0.25 mg. per kilogram per minute. Quinidine and cinchonamine were administered at a rate of 1.0 mg. per kilogram per minute.

The effects of quinidine on the electrical properties of the heart are illustrated in Figure 11. These results are then to be compared with the observations of the indole-quinuclidine alkaloids, quinidine serving as the standard drug.

It is evident that quinidine provokes a widening, of P and QRS waves, that is dose-related. After approximately 90 mg. per kilogram of quinidine, these intervals are twice as wide as pre-drug control values, i.e., 100 per cent prolongation. Concommittant with this effect is an increase in voltage of S wave.

This prolongation of QRS and P wave can be interpreted as being a slowing of conduction rate throughout the ventricles and atria and also reflects a prolonged time of depolarization.

It is also apparent (Figure 11) that quinidine's action is followed by a widening of the P-R and Q-T time intervals. Interpretation of the basic mechanisms behind these observations is complicated by changing heart rates. With increasing doses of quinidine there is first a transitory

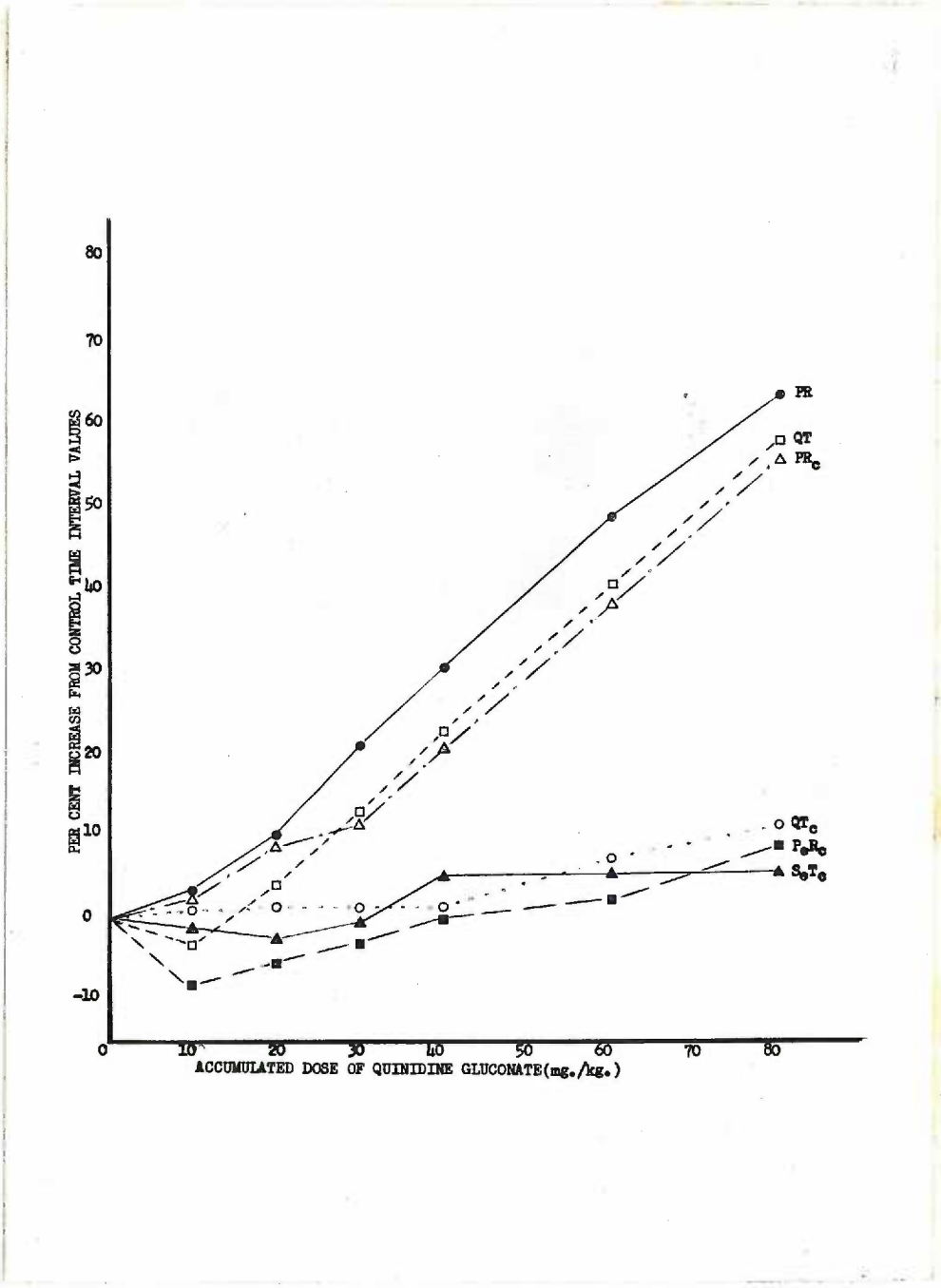


Figure 11. Quinidine gluconate, infused intravenously at a rate of 1 mg./kg./minute, into an anesthetized dog, the electrocardiogram being taken during the infusion. Each point represents the mean of 18 experiments, and each experiment, at the proper time interval three measurements were taken of random heart beats.

increase in heart rate followed by a slowing of heart rate. The influence of heart rate on P-R and Q-T interval durations was removed by computing "corrected" P-R or Q-T intervals, representing the estimated values at a heart rate of 60 per minute. It is now evident that quinidine administration is followed by a broadening of $P\text{-}R_c$ and $Q\text{-}T_c$ intervals. These actions are dose-intensity related, as seen in Figure 11 and Table I.

The $P\text{-}R_c$ and $Q\text{-}T_c$ time intervals have been designed as the total time periods of electrical activity for atria and ventricles. As such, the electrical activity is composed of depolarization and repolarization. If the time of depolarization were to be subtracted from the total electrical activity, the repolarization time is the arithmetic remainder. Thus, QT_c - QRS = S_eT_c (end of S to end of T wave) and dependent on the validity of the QRS width as measuring depolarization, S_eT_c represents ventricular repolarization. In using $P\text{-}R_c$, an assumption is made that initiation of the R wave is directly related to the termination of atrial repolarization (that is the end of the "hidden" T_a wave). Atrial repolarization time would thus be measured as $P\text{-}R_c$ - P width = P_eR_c. It is seen that quinidine has little influence on the S_eT_c interval under the conditions of the experiment. There is a lengthening of P_eR_c interval by quinidine, but interpretation

Protocol for
TABLE I

Dose-response changes for various time intervals of the electrocardiogram. The drug induced change, for each parameter, is expressed as a per cent change from the pre-drug control period, which is taken as 100 per cent.

TABLE I

HEART RATE CHANGES FROM CONTROL VALUES

QUINIDINE		CINCHONAMINE		AJMALINE		QUINAMINE		IBOGAINE		TETRAPHYLLICINE	
dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change
10.0	-16	10	8.0	1.25	-.5	5.0	0.1	2.5	48.0	1.0	0.3
20.0	- 4.3	20	9.2	2.50	2.4	7.5	12.0	6.25	109.0	2.0	47.6
30.0	15.9	30	16.3	5.0	14.7	12.5	33.0	7.5	193.0	3.75	61.3
40.0	23.5	40	22.8	7.5	34.8	17.5	41.5		228.7**	4.25	70.0
50.0	31.5	50	25.7	10.0	50.7					5.00	82.4
60.0	44.7	60	29.9	12.5	70.5		49.1*	** 10 min.			
70.0	64.7	70	34.4	15.0	88.3	* 20 min.		after end of			
80.0	91.4	80	43.3			after end of		drug infusion.			
90.0	94.5	90	51.5			drug infusion.					

CHANGE OF HEIGHTH OF "T" WAVE FROM CONTROL

10.0	2.1	10	3.8	1.25	0	5.0	-18	2.5	12.5	1.0	3.6
20.0	14.6	20	35.2	2.50	20	7.5	37	6.25	75.0	2.0	56.4
30.0	45.4	30	78.3	5.0	76.6	12.5	51	7.50	168	3.75	66.2
40.0	78.3	40	103	7.5	101	17.5	62.3		387**	4.25	80.0
50.0	101	50	143	10.0	134		73.7*	** 10 min.		5.0	141
60.0	139	60	163	12.5	140	* 20 min.		after end of			
70.0	168	70	175	15.0	162	after end of		drug in-			
80.0	209	80	229			drug infusion.		fusion.			
90.0	272	90	297								

TABLE I

QRS WIDTH CHANGES FROM CONTROL VALUES

QUINIDINE		CINCHONAMINE		AJMALINE		QUINAMINE		IBOGAINE		TETRAPHYLLICINE	
dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change	dose mg/kg	% change
10.0	20.2	10.0	6.1	1.25	16.7	5.0	0	2.50	14.7	1.00	12.7
20.0	33.4	20.0	15.5	2.50	26.3	7.5	5.6	6.25	30.1	2.00	85.7
30.0	40.0	30.0	24.5	5.00	45.6	12.5	9.1	7.50	43.2	3.75	127.0
60.0	70.1	60.0	59.0	10.0	83.4	17.5	17.6			4.25	141.3
90.0	104.6	90.0	88.5	A-V block						5.00	192.5

P WAVE DURATION CHANGES FROM CONTROL VALUES

10.0	10.0	10.0	2.9	1.25	7.9	5.0	4.1	2.50	16.8	1.00	16.9
20.0	18.2	20.0	15.3	2.50	24.1	7.5	5.1	6.25	26.6	2.00	85.4
30.0	24.3	30.0	22.9	5.00	41.8	12.5	5.7	7.50	37.0	375	137.7
60.0	57.6	60.0	54.0	10.0	75.3	17.5	28.7			4.25	153.8
90.0	95.0	90.0	83.0	A-V block						5.00	210.0

of the meaning of this result is unclear.

Some confirmation of quinidine's retardation of the ventricular repolarization process appears likely from an inspection of its effects on the T wave. Following quinidine's action, an initially inverted T wave becomes upright. Whether inverted, isoelectric, biphasic or upright, larger doses of quinidine increase both the height of the T wave and also the area inscribed by it. In some, but by no means a majority of the electrocardiograms, large doses of quinidine are followed by the appearance of a U wave.

In several experiments, respiratory activity and arterial blood pressure were recorded. These observations on respiration and circulation are not intended to reflect possible side effect activity of quinidine or the indole alkaloids. It is known that the electrical activity of the heart, the width of the QRS wave for example, may be altered by hypoxia. The hypoxic effect may arise as a depression of pulmonary ventilation or due to tissue hypoxemia, resulting from shock-like decreases in blood pressure. As quinidine is perfused, there is an initial rise in the rate and depth of breathing, followed by a fall in the depth of breathing, as the dose of quinidine became greater. Then as the dose of quinidine increases, there is a progressive fall in blood pressure. It is however, noteworthy that for total doses of quinidine of 60 to 80

mg. per kilogram, neither of these two parameters is significantly altered. With these doses of quinidine, respiratory rates were 16 to 24 per minute, with adequate depth and no cyanosis of oral mucous membranes; blood pressure was 100-120/60.

When quinidine was infused, achieving doses greater than 80 mg. per kilogram, both respiration and blood pressure were progressively depressed; death occurring after approximately 120 mg. per kilogram.

Comparative effects of cinchonamine, quinamine, ajmaline, tetraphyllicine and ibogaine on the heart.

When compared with quinidine, as illustrated in Figure 12 and TABLE I, the effects of the indole alkaloids on the heart fall into three groups. Cinchonamine and quinamine resemble quinidine, both qualitatively and quantitatively, in action. There is evident a dose-related broadening of QRS and P waves; a lengthening of P_eR_c interval and an increase in height and area of the T wave. Inspection of the dose-effect curves indicate a similar intensity (potency) of action for quinidine, cinchonamine and quinamine. The second group of alkaloids includes ajmaline and tetraphyllicine. Their action, like quinidine, is a broadening of QRS and P waves, a lengthening of P_eR_c and an increase in the height of the T wave. The dose-response curves are however, much steeper for ajmaline

Protocol for
FIGURE 12.

Dose-response curves for the indole alkaloids and quinidine gluconate. The rats of infusion were 1 mg./kg./minute for quinidine and cinchonamine and 0.25 mg./kg./minute for the other drugs. Each point represents the mean value of three heart beats.

A. Quinidine and Cinchonamine.

B. Ajmaline and Quinamine.

C. Ibogaine and Tetraphyllicine.

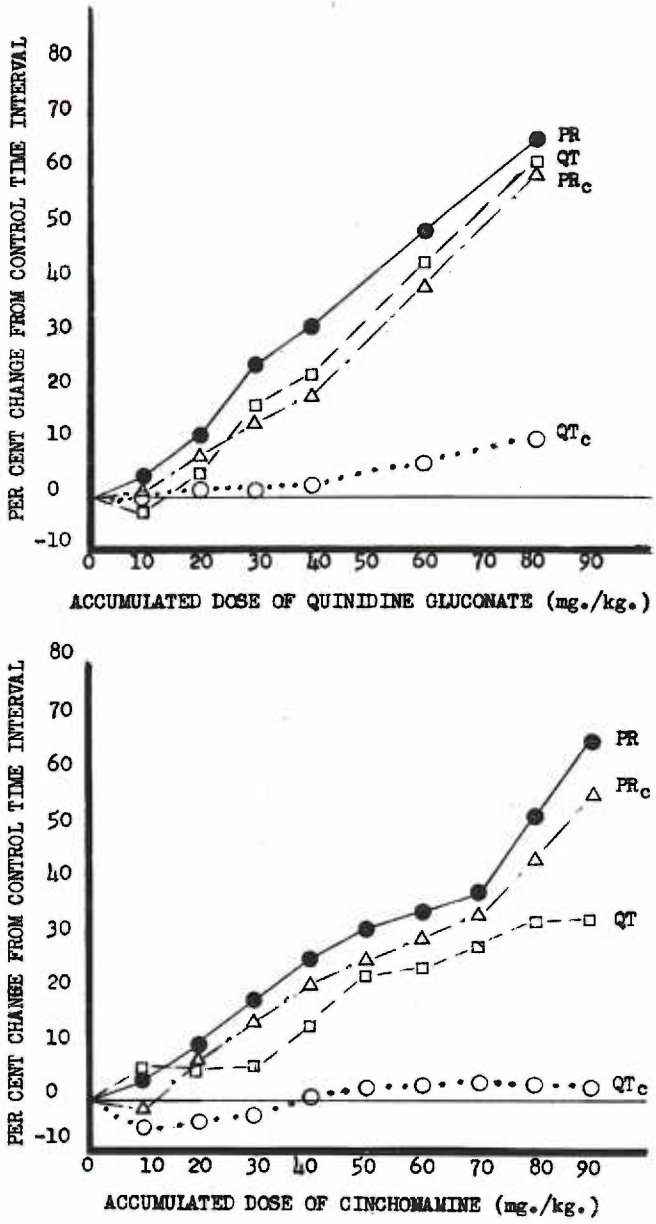


Figure 12.

A. Quinidine and Cinchonamine.

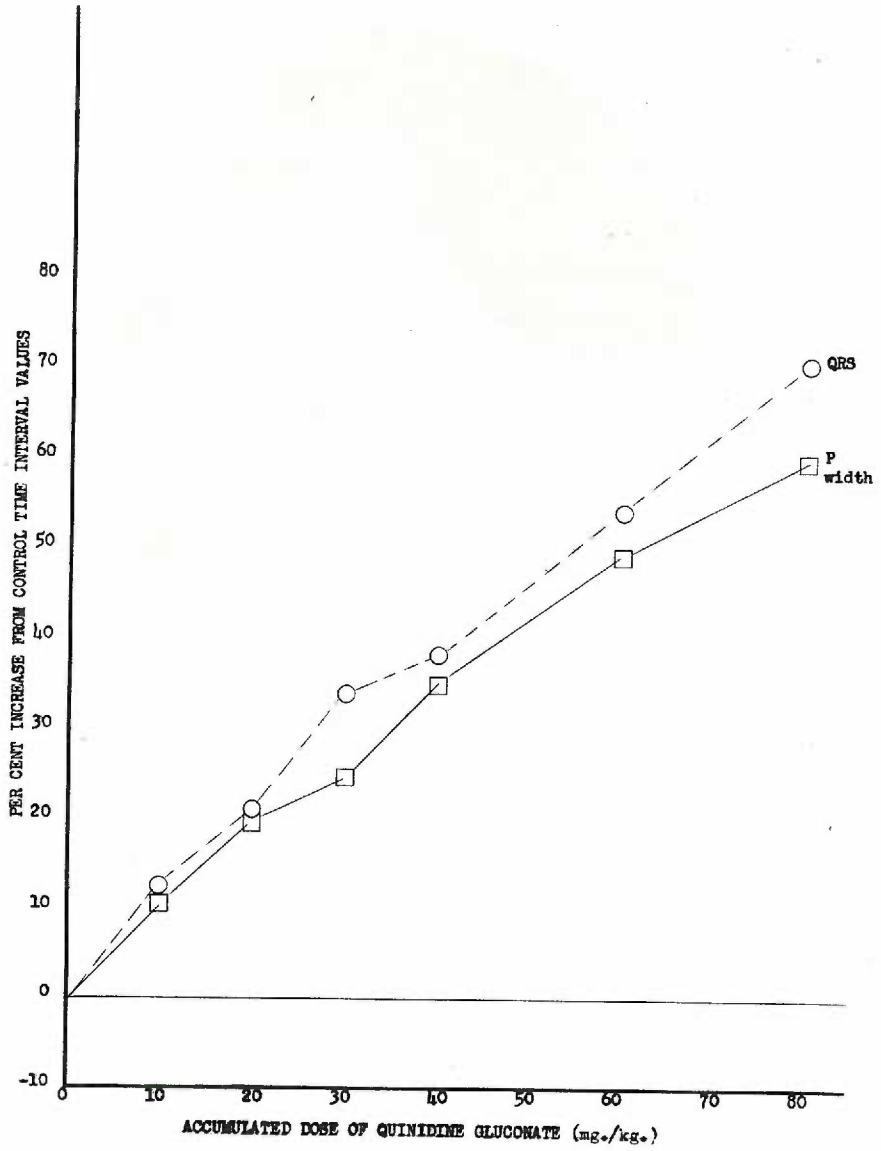


Figure 12 A Quinidine Gluconate.

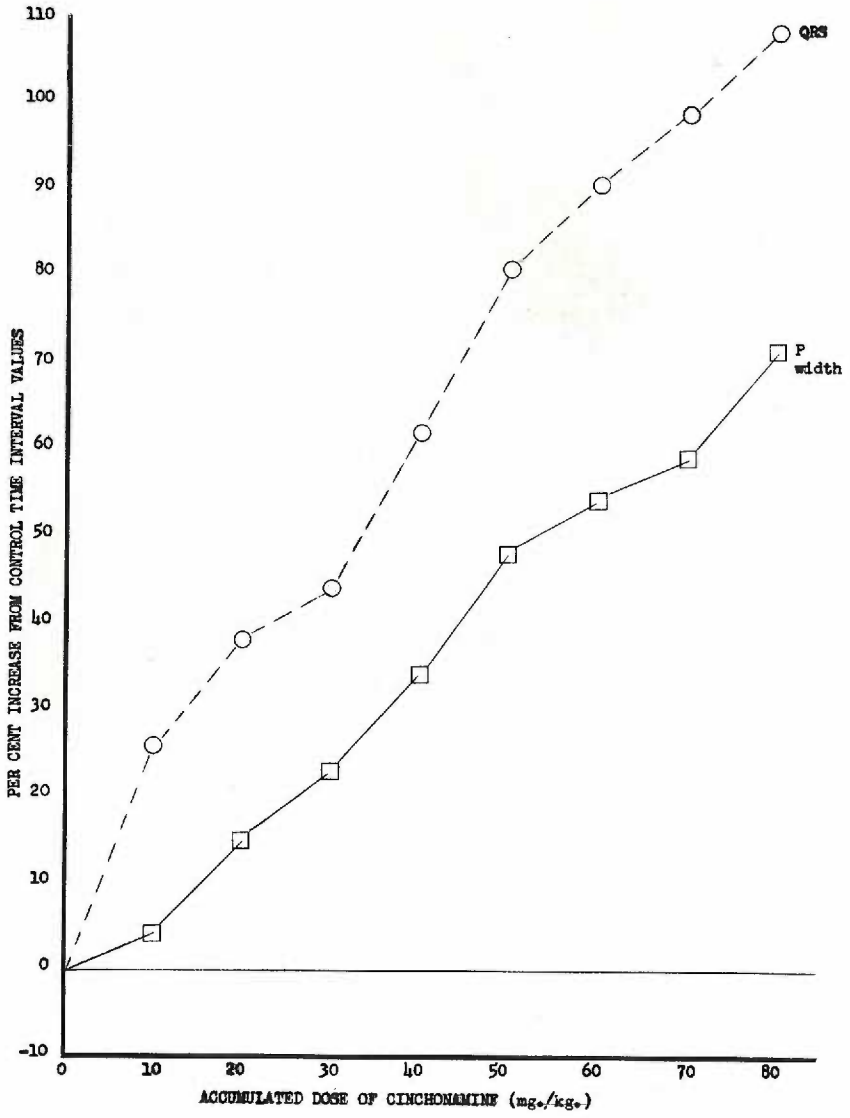
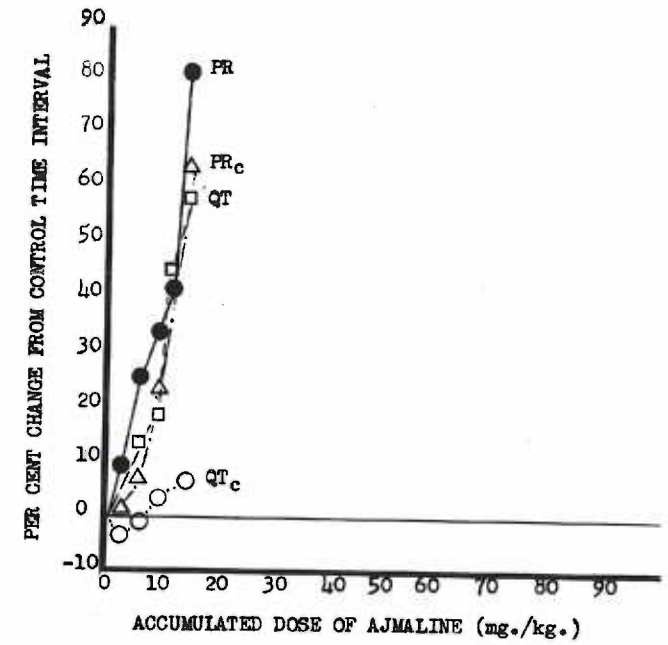


Figure 12 A Cinchonamine.



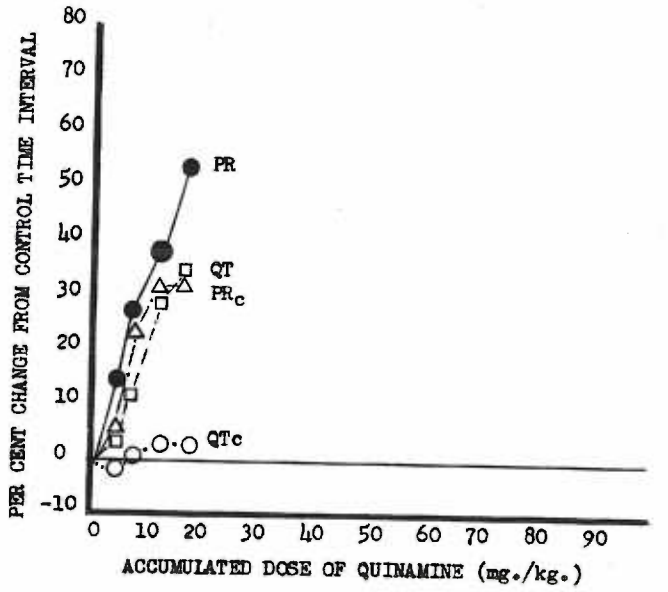


Figure 12.

B. Ajmaline and Quinamine.

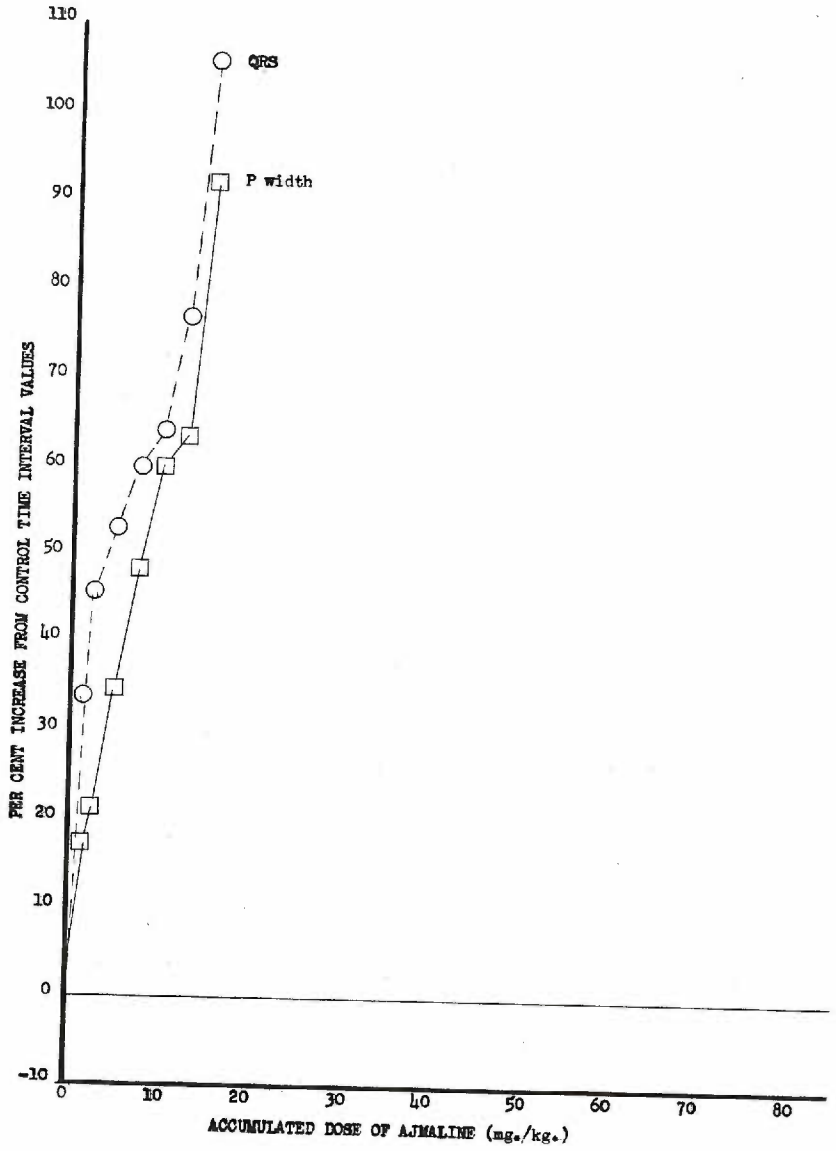


Figure 12 B Ajmaline.

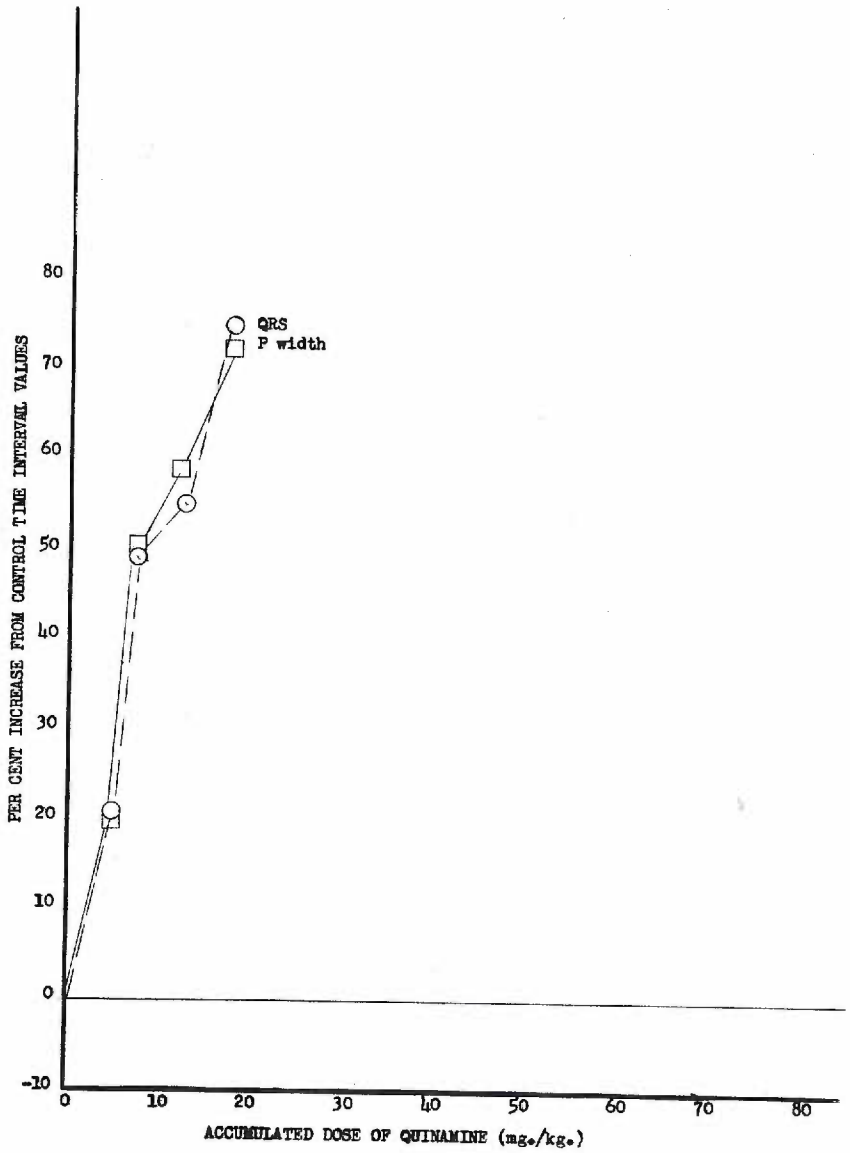


Figure 12 B Quinamine.

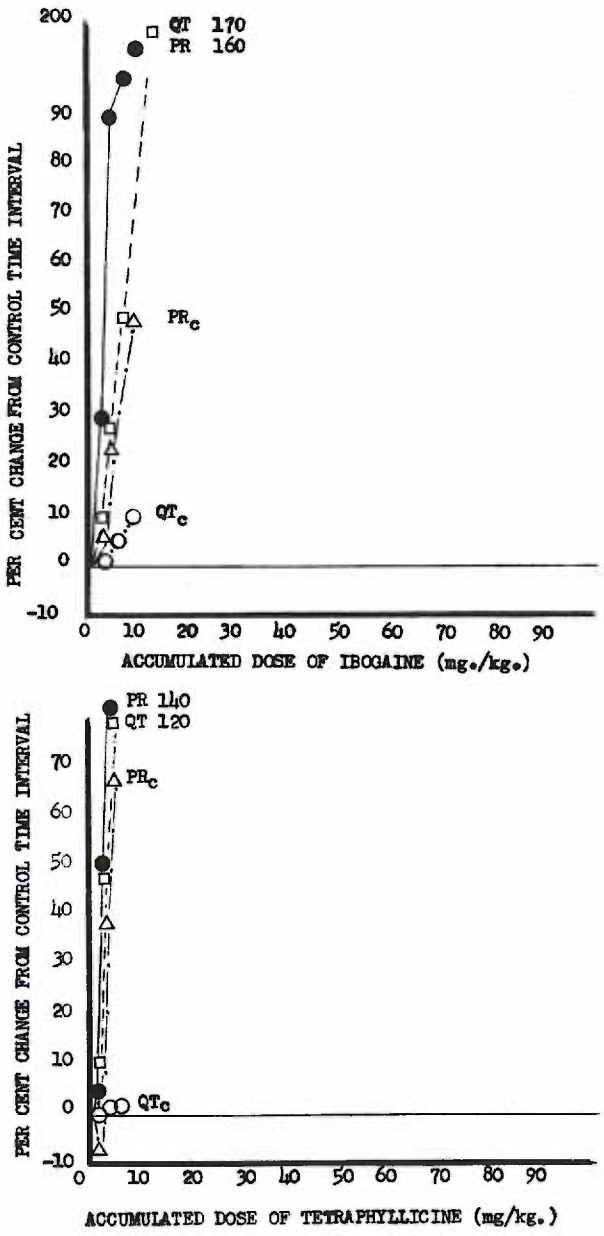


Figure 12.

C. Ibogaine and Tetraphyllicine.

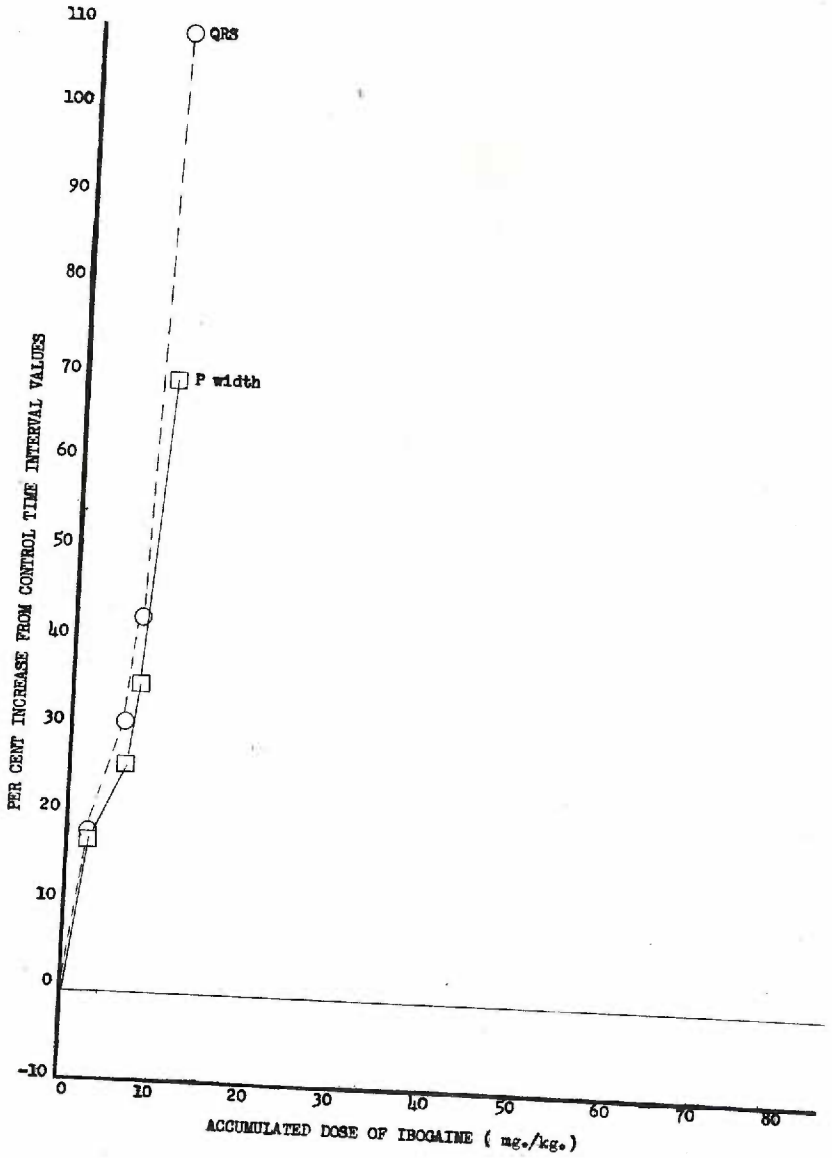


Figure 12 C Ibogaine.

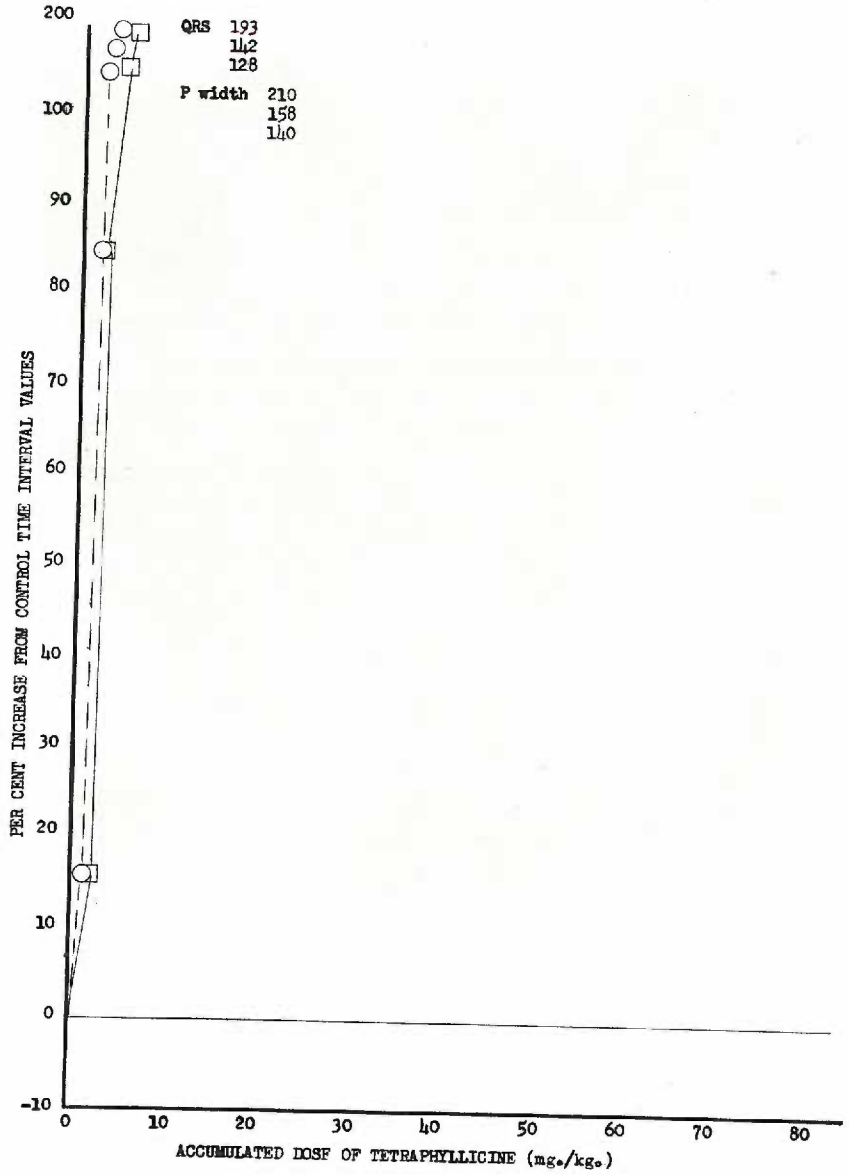


Figure 12 C Tetraphyllicine.

or tetraphyllicine than for quinidine. Direct drug potency comparisons should not be made from constant intravenous perfusion experiments, as the factors of tissue distribution or elimination (by liver and kidney), have not been measured. Nevertheless, ajmaline and tetraphyllicine would appear to exert a more intense action on the electrical actions of the heart than does quinidine; though of the same nature. It is noteworthy that ajmaline at doses of 12.5 mg. per kilogram or greater provokes an atrioventricular block. The P-P interval, though quite regular (ca. 250 msec.), is faster than the R-R interval and totally independent of it. The final group of alkaloids contains but one member, ibogaine. While ibogaine does provoke broadening of the QRS and P waves, as well as the P_eR_e like quinidine, there significant differences. Ibogaine does result in a much greater bradycardia than even nearly lethal doses of quinidine. In addition, there appears to be much greater changes on the P-R interval. These interesting observations on ibogaine must be repeated when more compound is available.

Selected experiments with the indole alkaloids also included observations of respiration and blood pressure. In none of these experiments was there sufficient depression of respiration or blood pressure to alter significantly the electrical responses of the heart.

These effects of the various indole alkaloids and

quinidine on the electrical properties of the heart, suggest several biochemorphic considerations. Thus, the quinuclidine and isoquinuclidine ring systems appear related to a slowing of the rate of atrial and ventricular depolarization, as measured by the width of the P and QRS waves. The quinoline ring system thus is not essential for this property. The connection between the quinuclidine ring and heterocycle need not be a secondary alcohol, as in quinidine, to retain these characteristic effects on the electrical properties of the heart.

Effects of the indole alkaloids on experimentally-induced ventricular arrhythmias in dogs.

The effects of the previously described experiments of the indole alkaloids, on the electrical properties of the dog's heart, were done in lieu of their evaluation in experimentally-induced atrial arrhythmias in animals. This was the result of a poor correlation between drug potency experiments, reported by others, of experimental animal and clinical atrial arrhythmias.

The results of drug studies, using experimentally-induced ventricular arrhythmias in animals, has a more satisfactory clinical correlation. One such drug, procaine amide, was first shown to be effective against cyclopropane-epinephrine ventricular tachycardia of dogs, and subsequently has found a useful place in the therapy of clinical

ventricular arrhythmias. There is, however, no agreement as to which experimentally-induced ventricular arrhythmias in animals is best suited for drug studies with an ultimate goal of clinical usefulness. Thus, it was believed necessary to subject the indole-quinuclidine alkaloids to several experimentally-induced ventricular arrhythmias produced by different techniques. These included ventricular fibrillation provoked by chloroform-epinephrine action; amodiaquin's ventricular bigeminy and the complex ventricular arrhythmias arising from digitalis overdosage or myocardial infarcts which follow coronary artery ligation (Table II).

Melville (68) produced ventricular fibrillation by using a technique of chloroform and epinephrine. Fasting dogs were weighed and anesthetized with 30 to 35 mg./kg. of sodium pentobarbital, administered intraperitoneally. A cuffed-Magill endotracheal tube was inserted into the trachea; the free end was covered with a chloroform saturated 2 x 2 gauze, at the appropriate time. The left femoral vein was catheterized, the tip being placed just below the inferior vena cava; the epinephrine was administered in a 25 to 35 second period of time. The electrocardiogram was recorded continuously throughout the experiment, at a paper speed of 50 mm. per second. (Lead II unless otherwise indicated).

Protocol for
TABLE II

Suppression of experimentally-induced ventricular arrhythmias by quinidine, ajmaline and cinchonamine.

TABLE II

EFFECTS OF VARIOUS INDOLE ALKALOIDS AND QUINIDINE IN SUPPRESSING EXPERIMENTALLY-INDUCED VENTRICULAR ARRHYTHMIAS

QUINIDINE GLUCONATE

METHOD	VENTRICULAR ARRHYTHMIA PRODUCED	DOSE (mg/kg)	"ANTIARRHYTHMIC" EFFECT
left coronary artery ligation. 18 hours.	multifocal ventricular arrhythmia, 50-70 % alternating with sinus rhythm, 30-50 %	2.5	no significant change
		10.0	sinus rhythm
chloroform, 5 minutes.	ventricular fibrillation	1.0	ventricular fibrillation
epinephrine 20 mcg/kg		8.0	multifocal ventricular tachycardia, A-V nodal rhythm
digoxin overdose	A-V nodal rhythm with interspersed PVS, paroxysmal atrial tachycardia with A-V block	1.0	no significant change
		20.0	sinus rhythm
amodiaquin	ventricular bigeminy; QRS 67 & 112 msec alternating; RR 283 msec	2.5	sinus rhythm for 10 minutes
		10.0	sinus rhythm for 4 hours

TABLE II

AJMALINE HYDROCHLORIDE

METHOD	VENTRICULAR ARRHYTHMIA PRODUCED	DOSE (mg/kg)	"ANTIARRHYTHMIC" EFFECT
left coronary artery ligation. 20 hours.	multifocal ventricular arrhythmia, interspersed with bursts of ventricular tachycardia	1.35	sinus rhythm for 10 minutes
		5.0	sinus rhythm for 3 hours
chloroform, 5 minutes. epinephrine 20 mcg/kg	ventricular fibrillation	1.0	ventricular fibrillation
		2.5	no change
		5.0	A-V nodal rhythm, then multifocal ventricular tachycardia
digoxin overdose	alternating A-V nodal rhythm and PVS	5.0	change to ventricular tachycardia; RR 368 msec.
amodiaquin	ventricular bigeminy, QRS 81 & 140 msec alternating; RR 434 msec.	1.0	sinus rhythm; RR 606 msec.

TABLE II

CINCHONAMINE SULFATE

METHOD	VENTRICULAR ARRHYTHMIA PRODUCED	DOSE (mg/kg)	"ANTIARRHYTHMIC" EFFECT
left coronary artery ligation. 24 hours.	multifocal arrhythmia, PVS alternating with A-V nodal rhythm	1.0	regular sinus rhythm, 2 hours.
chloroform, 5 minutes.	ventricular fibrillation	5.0	ventricular fibrillation
epinephrine 20 mcg/kg		10.0	rapid A-V nodal rhythm followed by sinus rhythm
digoxin overdose	not completed for this drug		
amodiaquin	ventricular bigeminy; QRS 68 & 157 msec alternating; RR 535 msec.	2.0	sinus rhythm; RR 560 msec.

After the dog had become stabilized to the barbiturate anesthesia, the electrocardiograph was started. Then chloroform was administered for five minutes. The chloroform administration was not intended to deepen the level of anesthesia; excess chloroform resulted in an electrocardiogram typical of the heart in asphyxia. Epinephrine 1: 10,000 was then injected into the femoral catheter in a dose of 20 micrograms/kg. Irreversible ventricular fibrillation then ensued after a lag period of 30 to 85 seconds. Ventricular fibrillation, under these control conditions resulted in death in 30 out of 30 animals according to Melville and 16 out of 16 animals in this laboratory.

In certain experiments the antiarrhythmic drug was administered at 0.5 to 2.0 mg./kg./minute, just prior to the chloroform-epinephrine challenge. Complete protection was obtained when only a sinus tachycardia ensued. Partial protection was assessed if rapid A-V nodal or ventricular tachycardia appeared for 2 to 3 minutes. Failure to show antiarrhythmic qualities was apparent if, despite the drug administration, ventricular fibrillation followed the chloroform-epinephrine challenge. (See Table II).

Amodiaquin hydrochloride (Camoquin^{R}) was injected at a constant rate in anesthetized dogs until death occurred. The dogs were anesthetized using 30 to 35 mg./kg. sodium

pentobarbital, and an electrocardiogram, lead II, unless otherwise stated, recorded throughout the experiment. In selected experiments, respiratory activity and femoral arterial blood pressures were recorded using strain-gauge transducers. When the rate of administration was between 0.25 and 5 mg./kg./minute, the lethal doses from ventricular tachycardia were 85 and 35 mg./kg. respectively. Respiration and blood pressure were both failing just prior to the lethal dose. In the experimental arrhythmia experiments to follow, the rate of administration was selected at 0.5 mg./kg./minute and at doses of 10 to 30 mg./kg., there was no significant change of blood pressure or respiratory movement.

When 10 to 30 mg./kg. of amodiaquin had been administered, ventricular bigeminy appeared. At first, the arrhythmia might persist only a few minutes. When the amodiaquin administration was continued for five additional minutes (usually 2.5 mg./kg.) the bigeminy persisted for at least 45 minutes and occasionally lasting 7 hours.

Amodiaquin's ventricular bigeminy appeared from an "apparent" fixed focus and was uniform, having a constant rate for each dog. The rate varied from 120 to 220/minute; (R-R 535 to 283 msec). Only one-half of these beats were recorded as blood pressure peaks at the femoral artery. The rhythm consisted of a normally conducted ventricular

contraction preceded by a P wave (QRS 67 to 81 msec. duration), shortly followed by the ventricular ectopic beat and inverted T wave (QRS 112 to 157 msec.) with a pause. From multiple chest leads, it was observed that the ventricular ectopic beat usually had its origin in the left ventricle, however, in some dogs, the origin was in the right ventricle.

With doses of Amodiaquin just subthreshold (just prior to the appearance of the ventricular bigeminy as the intravenous injection dose was accumulating), there were only minor changes in the electrocardiogram. At approximately 4 to 8 mg./kg. of amodiaquin, some decrease in the height of R and an increase in the length of the S wave occurred. The T wave had an apparently earlier "take off" and the S-T segment was sloping. An initially inverted T wave became upright. The ventricular bigeminy was preceded by irregularly occurring premature ventricular systoles from various foci for a few beats. (See Figure 13).

As used as a procedure for establishing experimentally-induced ventricular bigeminy, the amodiaquin was administered at 0.5 mg./kg./minute. The drug was continued for 5 minutes beyond the time of first appearance of bigeminy and then stopped. An antiarrhythmic effect was noted only if the experimental drug could terminate the ventricular bigeminy within 20 minutes.

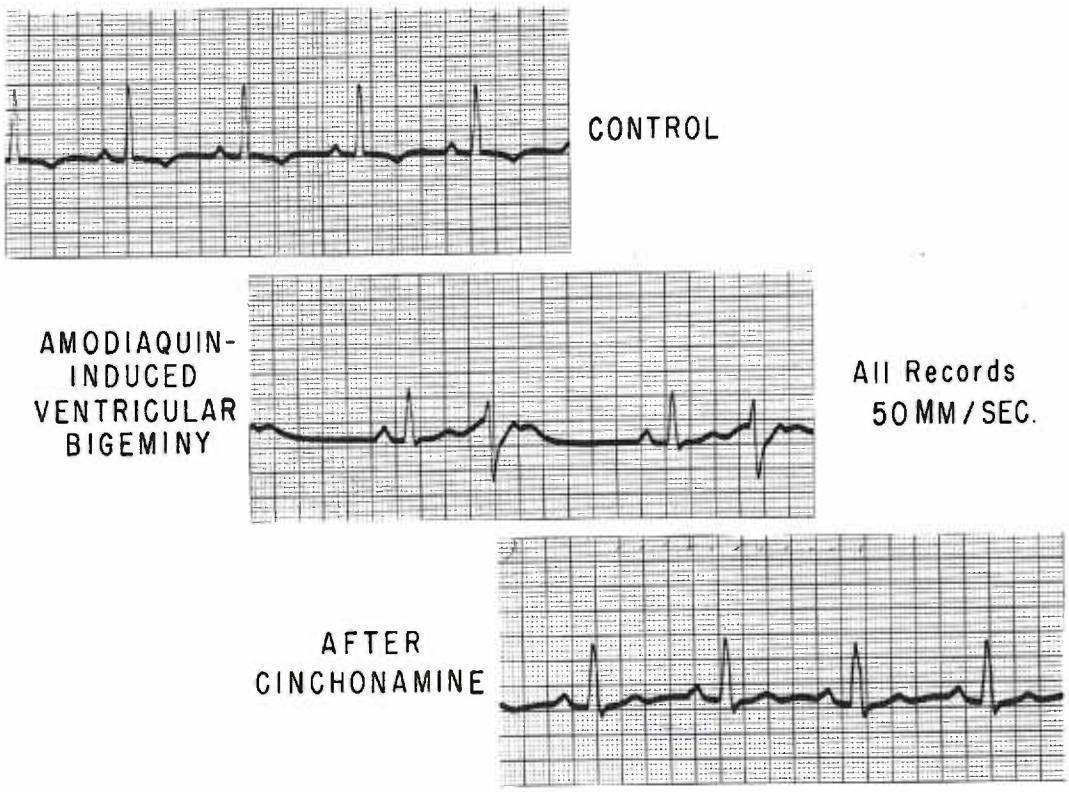


Figure 13. Amodiaquin produced ventricular bigeminy. A control record is shown in the upper record, the middle record after 10 mg./kg. of amodiaquin, and the lower recording illustrates the reversal of bigeminy by 2 mg./kg. of cinchonamine.

Amodiaquin (Camoquin ^{R}) is chemically 4(3'-diethylamino-methyl-4'-hydroxyanilino)-7-chloroquinoline.

The observation was made during wartime investigation of antimalarial drugs, that amodiaquin given intravenously (103), provoked bigeminy and this observation was studied as a possible method for provoking ventricular bigeminy, for the study of antiarrhythmic properties. Amodiaquin's bigeminy is paradoxical, for it, like quinidine, has apparent antiarrhythmic properties of its own (4). (See Table II). These experimental observations for amodiaquin are, therefore, presented in some detail for as a test-method it has not been previously described.

The ligation of the anterior coronary artery was performed just below the bifurcation using the two step procedure as described by Harris. The dogs chest was then closed by layers and suction drainage provided. Ventricular arrhythmias appear immediately after ligation but subside. The arrhythmias then return as necrosis is iniated reaching a maximum in 16-24 hour post-operative period. The anti-arrhythmic drugs were administered during this latter time when ectopic beats occupied 30 to 70 per cent of the total heart beats.

Digoxin* was administered by slow intravenous drip such that approximately 1.0 mg. per kilogram had been given in a one hour's period of time. Each dog (anesthetized with 35 mg. pentobarbital) was "titrated" giving sufficient digoxin to provoke a persistent ventricular arrhythmia. In some instances, paroxysmal atrial tachycardia with A-V block appeared. The antiarrhythmic drug was given over a 10-20 minute time period.

Sufficient supplies of the indole-quinuclidine alkaloids were at hand to test only ajmaline and cinchonamine in these experimentally-induced ventricular arrhythmias. Quinidine gluconate was evaluated in the same way as a standard for comparison. No attempt was made to establish precise antiarrhythmic threshold levels for any drug. However, a high and low estimated dose was used to "bracket" the anti-arrhythmic threshold (each experiment and dosage was duplicated). A total of 22 dogs were used exclusive of those that died from ventricular fibrillation following coronary artery ligation; from chloroform overdosage; and does not include those dogs used to evaluate the amodiaquin technique.

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*Lanoxin injectable obtained through the courtesy of Mr. C. G. Smith, Burroughs-Wellcome Laboratories.

In these experimentally-induced bentricular arrhythmia experiments ajmaline and cinchonamine both demonstrate an antiarrhythmic effect (see Table II). It should be pointed out that with ajmaline and digoxin overdosage ventricular tachycardia was provoked rather than an expected suppression of ventricular ectopic activity.

These preliminary experiments suggest further interest in cinchonamine and to a lesser extent ajmaline as ventricular antiarrhythmic drugs.

Cardiac and non-cardiac Pharmacology of the indole-quinuclidine alkaloids reported by others.

The following summary of the literature described the available pharmacologic knowledge of the indole quinuclidine alkaloids, both cardiac and non-cardiac actions. It is presented, at this time, being thought more germane to results than in historical background and is preliminary to the clinical studies which follow.

Cinchonamine

In the experiments reported in animals, the most clinically significant effect of cinchonamine is that an 0.5 per cent solution is equal to a 0.7 per cent solution of cocaine, in producing local anesthesia in the rabbit's eye (84).

Cinchonamine was noted (85) to have an action antagonistic to lethal doses of digitoxin on the frog heart, and further that lethal doses of cinchonamine caused the heart to be arrested in diastole. Other investigations show that cinchonamine will cause an increase in biliary flow. It does not effect the renal flow nor pancreatic secretions. Other effects that have been observed are, that in the unanesthetized dog, an apparent hallucinogenic state may be ellicited, with death from tetanic convulsions with dilated pupils. At a dosage of cinchonamine of 0.5 to 10 mg./kg., it has been shown that there is a slight fall in the blood pressure of the anesthetized dog. Cinchonamine base introduced into the stomach of a dog will provoke nausea and vomiting, while the sulfate salt of cinchonamine did not have this effect (76).

The approximate lethal dose for cinchonamine varies from animal to animal and some of the results found by See are given below.

Animal	Approximate Lethal dose (mg/kg)
Guinea pig	600
Dog	165
Frog	2500

Arnaud (1), on the other hand, showed a different set of doses for the lethal doses of cinchonamine sulfate.

These doses briefly summarized are:

1. for the frog, 362 mg./kg.; 2. for the rabbit, 61 mg./kg.; 3. for the dog, 23 mg./kg.; 4. and for man, approximately 17 mg./kg.

For a 70 kg. man, this would be a dose of approximately 1.2 grams. It was noticed that in dogs, there was, with the initial administration or the administration of small doses, a tachycardia followed later with larger doses of cinchonamine sulfate, a bradycardia. It was further noted that cinchonamine would block the cardiac effects of electrical stimulation of the vagus (82).

It was further observed that cinchonamine would not arrest a plasmodium infestation of mice, but that it did have a weak antiseptic action on bacteria in vitro and would raise the body temperature to a limited amount (85).

The studies by Raymond-Hamet (81) on the blood pressure effects of cinchonamine were believed to be primarily due to a direct vasoconstriction. As to the effects of cinchonamine on the isolated rat or rabbit uteri, it was proposed (76) that the action ellicited was one of stimulation due to the irritation of the muscle, similar to the action of quinine.

Ellran (32) reported the case of a human volunteer given 200 mg. of cinchonamine hydrochloride, there was a decrease in pulse rate, from 98 to 80 lasting approximately

3 hours and a complaint of slight headache. Cinchonamine was detected in the urine of the volunteer at the 3 hour time period. He further reports the administration of cinchonamine hydrochloride, subcutaneously, to a dog, which resulted in a "hallucinogenic" state, epilepsy-like seizures, convulsions and death.

Ibogaine

Some of the experiments that have been performed using ibogaine include its potentiation of the blood pressure response of 0.6 mcq./kg. of epinephrine, using 4 mg./kg. of ibogaine, but that potentiation of the response of the blood pressure to 1.5 mg. of ephedrine did not occur (78). Ibogaine at 20 mg./kg. augments the blood pressure rise of tyramine. Its action on circulation is largely peripheral, for ibogaine reinforces renal vasoconstriction produced by epinephrine or tyramine as measured with an oncometer (83). Also it has been shown that epinephrine's elevation of femoral artery tone, as measured by restriction of femoral venous flow, is augmented by 0.1 to 1.0 mg./kg. of ibogaine (78).

It has been reported (74) that indigenous people of Gabon have used Iboga for countering the effects of fatigue and sleep. This property was used, with good results, in therapy of patients convalescing from infectious disease

with neurasthenia and congestive heart failure (atonic dilation of the heart) (80).

Ibogaine, 1:100,000, decreases the tone and amplitude of contractions of isolated rabbit intestine, but does not block the spasm caused by acetylcholine. Ibogaine's action on the intestine is reversed by ergotamine (77).

Lambert (59) has shown that 2 to 10 mg./kg. of ibogaine given to an unanesthetized cat will provoke excitement, mydriasis, salivation, partial piloerection, tremors progressing into the rage syndrome, ataxia, clonic extension of the legs and tachypnea lasting from 1 to 2 hours. An arousal picture in the electroencephalogram, similar to a stimulation of the reticular formation has also been noted. This central nervous system action of ibogaine can be blocked with atropine 2 to 3 mg./kg. These effects that have been seen that are similar to epileptic madness, with unconsciousness, drunkenness, excitement, mental confusion and hallucinations which were reportedly on the natives of Gabon on an overdose of ibogaine (89).

Ajmaline

In experiments reported with ajmaline in animals, the most striking pharmacologic effects found were on the heart. With small doses, the cat's heart is slowed, while greater amounts provoked atrioventricular (A-V) block (15).

These effects were not mediated by the vagus, for neither atropine nor severing the vagi could alter them. Ajmaline, following intravenous doses of 10 to 15 mg./kg., doubled the width of the P and QRS waves. The P-R interval was also prolonged and in some experiments, the electrocardiographic pattern suggested right bundle branch block (36), using guinea pigs. Arora and Madan (2) reported ajmaline more effective than quinidine in prolonging the refractory period of isolated rabbit atria.

Of greater clinical interest perhaps, are the reports that ajmaline can prevent or terminate experimentally-induced cardiac arrhythmias. Hartog (44) found ajmaline could prevent ventricular fibrillation which follows electrical stimulation of the frog heart. van Dongen (29) reported that as little as 0.5 mg./kg. of ajmaline prevented electrically-induced fibrillation of atria or ventricles of cats, and also eliminated the arrhythmia which persists after the current is shut off (nachflimmern). He also demonstrated that ajmaline could protect the heart from ventricular arrhythmias which were produced by the injection of barium chloride or epinephrine. Other investigators (2) (88), found that smaller doses of ajmaline than quinidine were needed to arrest or prevent experimentally-induced fibrillation in dogs. The techniques used to establish the atrial

arrhythmias included: crush of the inter-caval tissue (circus movement hypothesis) or the topical application of acetylcholine or aconitine near the sinus node (ectopic focus theory).

Ajmaline's non-cardiac effects would not appear to be a significant complication during clinical trial of the alkaloid. Intravenous doses of 1 to 5 mg./kg. of ajmaline caused a transient fall in blood pressure of the dog, but unlike that resulting from quinidine, and there was no change in peripheral vascular resistance (88).

Daily oral doses of ajmaline (also in dogs), 1 to 3 mg./kg./day, for 5 days, did not result in measurable hypotension (19). Ajmaline applied to an isolated intestinal strip provoked increased peristalsis and tone (17), but contradictory results were obtained in the intact dog (79). Large oral doses of ajmaline, 15 to 30 mg./kg./day for 15 days, produced no detectable changes in cellular elements of blood liver or kidney (88). The LD_{50} following intraperitoneal administration of ajmaline to mice was approximately 250 mg./kg.; respiratory difficulties and convulsions appeared just prior to death (16). Thus, although reserpine and ajmaline are isolated from the same Rauwolfia species, ajmaline does not possess hypotensive or sedative-tranquilizer properties.

Clinical Studies

The final proof of any drug's effectiveness in the treatment of disease must be obtained in patients. In the field of antiarrhythmic drugs, the many failures in attempts to correlate drug potency, in experimentally-induced arrhythmias in animals, with that of clinical arrhythmias, makes the clinical study of paramount importance. The complete therapeutic evaluation of a drug, in patients, is a prolonged effort involving many hundreds of patients, each carefully studied; above all, it must not be merely a testimonial or superficial trial. The results of the clinical studies to follow are, by nature, human pharmacologic investigations.* The primary object of these studies was to detect an antiarrhythmic action; a therapeutic drug evaluation remains to be undertaken.

Preliminary clinical studies were started on two of the indole-quinuclidine alkaloids; ajmaline and cinchonamine. Ajmaline was administered to nine patients on sixteen occasions; one patient receiving the drug six times. At the present time, only two patients have been given cinchonamine.

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*I wish to thank Dr. H. Lenox H. Dick, M.D., Asst. Clinical Professor of Pharmacology, for permission to include the results of these clinical studies in this thesis.

The criteria used for patient selection for the pharmacologic evaluation of ajmaline and cinchonamine were twofold: (a) the atrial fibrillation had persisted for more than 6 months, (b) other circumstances permitted experimental study on "private" patients: terminal carcinoma, cerebral thrombotic episodes ("healed") with mental deficit; or "sensitivity" to quinidine resulting in such syncope and vomiting following a test dose of the drug which precluded its further use. Because of the limitations of case selection, the mean age of the 6 men and 5 women was 71.6 years (range 54 to 84 years). The duration of the atrial fibrillation in the patients actually used in the study was from 9 months to 6 years. In several of the patients premature ventricular systoles usually from a single "ectopic" focus accompanied the atrial arrhythmia. This fact permitted evaluation of the ability of ajmaline and cinchonamine to suppress ventricular arrhythmias. In no instance was the atrial (or ventricular) arrhythmia in a clinical tachycardia classification requiring emergency treatment. The origin of the atrial fibrillation was on an atherosclerotic basis; none had a primary history of rheumatic heart disease and valvulitis was present to but a minimal degree, if present at all. Congestive heart failure, when present, had been adequately compensated by digitalis given at a prior time.

The ajmaline was administered as the sterile hydrochloride salt by intravenous drip. The cinchonamine was administered by mouth. For the ajmaline studies, the electrocardiogram was recorded (50 mm./sec.) almost continuously and blood pressure by sphygmomanometer recorded every 15 minutes. With cinchonamine the electrocardiogram and blood pressure were recorded every 2 hours for a twelve hour time period. The general clinical status of these patients was observed closely during the period of time of drug administration and for an additional time of at least eight hours. Since it was not possible to administer maintenance dosages of ajmaline or cinchonamine because of inadequate supplies of the drug, the patient's arrhythmia returned if conversion had occurred. When this took place, the patients were then given the standard quinidine therapy. It thus became possible to compare ajmaline with quinidine or cinchonamine with quinidine in the same patient.

The ajmaline hydrochloride was diluted with sterile 5 per cent dextrose in water or saline forming 0.02 to 0.1 per cent solutions. Each patient received no more than one liter of gluid during a six hour period of time. The ajmaline was administered for 10 minutes at a constant drip rate, then for the next 5 minutes the non-drug containing fluid was given. The rate of administration was computed over the total fifteen minute period; the total time of

administration involved was one and one-half to three hours.

In a preliminary study, one patient (L. W.) was given ajmaline intravenously on six occasions with one or two weeks interval between injections (see Table III). In this patient, the dosage of ajmaline was increased by increments of 12.5 or 25 mg. until a total of 100 mg. had been given. There were no statistically significant changes in blood pressure, heart rate or respiration. During one of the 10 minute drug injection time periods, the rate of administration of ajmaline was increased to 0.25 mg. per kg. per minute as in the experiments with dogs. With this, the heart rate rose from 68.5 per minute to 84, accompanied by a decrease of the blood pressure from 180/90 to 160/70. Also the frequency of premature ventricular systoles, 17.6 per cent of the heart beats at that time, increased to 35.3 per cent of the total heart beats. Accordingly, on the basis of this experience, ajmaline administration was restricted to 0.1 mg. per kg. per minute or less.

During the course of this preliminary trial, it was observed that with 50 mg. doses of ajmaline the occasional premature ventricular systole disappeared, the atrial arrhythmia persisted. When 100 mg. of ajmaline had been given the atrial fibrillation rhythm was replaced by complete A-V block: that is, atrial (P wave) activity was restored but there was no normal atrioventricular conduction.

Protocol for
TABLE III

The effects of increasing doses of ajmaline on the electrocardiogram and blood pressure of a patient (L.W.) with chronic atrial fibrillation.

TABLE III

EFFECTS OF INCREASING DOSES OF AJMALINE ON THE ELECTROCARDIOGRAM OF A PATIENT (L.W.) WITH ATRIAL FIBRILLATION

AJMALINE HYDROCHLORIDE			ELECTROCARDIOGRAM						BLOOD PRESSURE	
total dose		rate of administration	heart rate (per minute)		QRS duration (in seconds)		Q-T interval (in seconds)		(S/D mm Hg.)	
mg.	mg/kg	mg/kg/minute	initial	final	initial	final	initial	final	initial	final
12.5	0.27	0.0027	79.1	64.5	0.112	0.112	0.306	0.311	190/80	192/80
25	0.53	0.0067	70	66.5	0.100	0.098	0.303	0.307	180/80	170/70
37.5	0.80	0.0084	82.5	82.5	0.092	0.092	0.299	0.318	170/76	170/80
50	1.07	0.017	76.2	70.6	0.100	0.106	0.311	0.327	160/80	170/85
75	1.60	0.018	66	69.2	0.088	0.096	0.320	0.360	150/70	142/74
100	2.1	0.03	53.6	55.6	0.070	0.086	0.327	0.370	144/70	138/68
mean values			71.3	68.1	0.0936	0.0983	0.311	0.332	166/76	163/76

These results in patients paralleled quite closely the results obtained under similar circumstances in dogs.

After these preliminary results, ajmaline was administered to eight additional patients on one or more occasions. Six patients had premature ventricular systoles accompanying the atrial fibrillation; the frequency of the premature beats occupied 6.1 to 17.6 per cent of heart beats. Doses of 50 mg. of ajmaline or greater completely suppressed this ectopic ventricular acitivity (Table IV). With a dose of ajmaline between 25 and 50 mg. the frequency of the extra systoles was diminished by one-half to two-thirds. This appeared to be the minimum effective dosage, for there was no change in frequency of premature ventricular contractions following 12.5 mg. of ajmaline. The ventricular extra systole activity returned as the ajmaline was metabolized and excreted. With a 75 mg. dose of ajmaline, suppression of the extra systoles was complete for three hours; by the fifth post-drug hour they had returned but appeared only one-quarter as often as in the pre-drug period. Hamm, Deiwick, Renschler and Zack in a preliminary note (43) also report 50 mg. of ajmaline effective in suppressing ventricular ectopic activity in patients with recent myocardial infarcts.

Three patients received ajmaline alone at doses of 100, 150 or 225 mg. ajmaline (Table IV). In each A-V block

Protocol for
TABLE IV

The clinical effects of ajmaline on the cardiac rhythm of patients with chronic atrial fibrillation.

TABLE IV

EFFECT OF AJMALINE ON CARDIAC RHYTHM OF PATIENTS WITH ATRIAL FIBRILLATION

patient	dose ajmaline (mg.)	dose procaine-amide (mg)	PVS* % total beats before	after	QRS duration (in seconds) initial	final	per cent increase	rhythm	time intervals (in seconds)
L.W.	100	-	6.1	0	0.070	0.086	23	A-V block	PP 0.220, RR 0.862
R.L.	150	-	8.6	0	0.080	0.091	14	A-V block	PP 0.328, RR 0.830
L.M.	150	150	0	0	0.080	0.120	37	"sinus"	PR 0.160, RR 0.560 QT 0.264
K.H.	150	125	0	0	0.076	0.096	26	"sinus"	PR 0.198, RR 1.120 QT 0.355
M.B.	150	250	7	0	0.094	0.106	13	"sinus"	PR 0.123, RR 0.622 QT 0.332
C.S.	225	-	19	0	0.121	0.168	39	A-V block	PP 0.355, RR 0.756
O.H.	250	200	0	0	0.086	0.123	43	"sinus"	PR 0.187, RR 1.200 QT 0.341

*In two other patients D.D., and J.H. having extra systoles with a frequency of 17.6 per cent and 6.9 per cent respectively, there was also complete suppression by 75 mg of ajmaline.

replaced the atrial fibrillation; the P-P interval was 0.220 to 0.255 sec. and regular, while the R-R interval was 0.796 to 0.862 sec. and variable. Thus, although the R-R interval was not precisely regular, the rhythm had more the character of a sinus arrhythmia than that of the irregular-irregularity of the atrial fibrillation. The P waves appeared to have normal contour, although of low voltage and usually there was complete dissociation of atria and ventricles with no discernible P-R interval. In one patient, the electrocardiogram had the appearance of sinus rhythm, but because the P-R interval was prolonged to 0.234 sec., this was interpreted as being constant A-V block with coincident phasing.

In the "toxic parallelism" experiments of dogs it was observed that ajmaline's A-V block could be prevented or reversed by procaine amide. Thus, ajmaline (150-250 mg.) was combined with small doses of procaine amide (150-250 mg.) in four patients. The small doses of procaine amide were employed with the intent of reducing ajmalines A-V block. Procaine amide, even at doses of 750 mg. or greater, given to some of these same patients failed to convert their chronic atrial fibrillation to sinus rhythm and this same observation is found verified in the literature (27) (75). Nevertheless, ajmaline and procaine amide may potentiate each others antiarrhythmic action.

As seen in Table IV ajmaline and procaine amide administration was followed by conversion of atrial fibrillattion to a sino-atrial rhythm. This conversion was immediate, not delayed as with quinidine. Apparent sinus conducted beats might appear in bursts of two to eight or might persist, as regular sinus rhythm, for twenty minutes. The change in rhythm was always of a temporary nature; complete A-V block returning to be replaced, in turn, by an atrial fibrillation, indistinguishable from the pre-drug records. The procaine amide had been given in one instance prior to the ajmaline; and in three, following it with the same results.

Side effects of ajmaline: There was no significant ($P = 0.05$ of less) broadening of the width of the QRS wave in patients until a dose of 50 mg. of ajmaline had been given. At 50 mg. the broadening was barely detectable, 10 per cent greater than pre-drug values or less. With a dose of 100 to 150 mg., the increase in width was 13 to 26.3 per cent. At larger doses, 225 and 250 mg., the per cent broadening had risen to 39 to 43 per cent. This appeared to establish a maximum single dose based on the warning accepted for quinidine; not to exceed a dose causing 50 per cent or greater prolongation of the QRS wave.

There were no other significant side effect symptoms or signs observed during ajmaline's administration.

The results of this clinical study were interpreted as supporting the hypothesis that an indole-quinuclidine alkaloid did possess antiarrhythmic potency in patients with chronic atrial fibrillation. The appearance, however, of A-V block would preclude any practical interest in ajmaline for therapy, at least at this time.

Cinchonamine: Two patients were given cinchonamine by mouth, one as cinchonamine sulfate the other as cinchonamine base.

Mr. M. W. (197893) aged 66 was admitted to the hospital on 7-20-61 for conversion of atrial fibrillation. The fibrillation was on the basis of atherosclerotic heart disease complicated by congestive heart failure, low grade mitral insufficiency and "healed" cerebral thrombi. The duration of the atrial fibrillation was known for more than one year. He was digitalized with digitoxin 10 days prior to administering the cinchonamine; there were no residual signs of edema or cardiomegaly.

Cinchonamine sulfate was administered in 200 mg. capsules, one at 10 A.M. and two at noon. Within one and three quarters hours the irregular heart rate rose from 80 to 105-115 per minute without significant change of blood pressure (166-170/88-100). The patient noticed slight "heart burn" and dizziness. At 3:30 P.M. the electrocardiogram revealed regular sinus rhythm. (Figure 14). The next

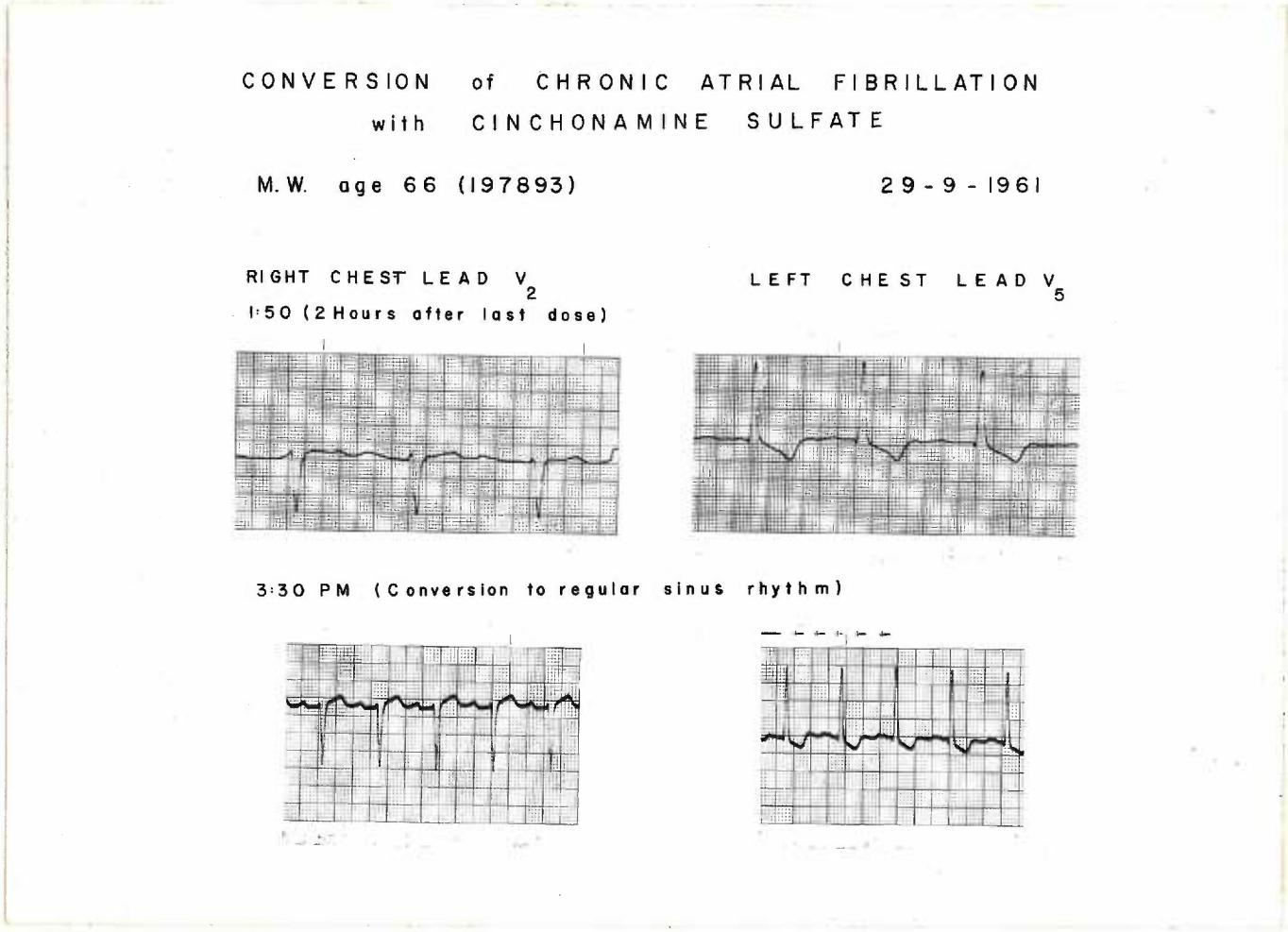


Figure 14. The clinical trial of cinchonamine sulfate in a patient (M.W.), with chronic atrial fibrillation, with conversion to sinus rhythm.

(Upper record 50 mm./second; lower record 25 mm./sec.)

day this patient was started on a regime of quinidine sulfate 0.2 gm. t.i.d. as a maintenance dosage. Sinus rhythm persisted for thirteen days. Four days later the patient was readmitted to the hospital and given quinidine. He was given quinidine sulfate 0.2, 0.4, 0.6, 0.8 and 0.8 gm. by mouth at 2 hour intervals. The atrial fibrillation persisted and additional quinidine was not given because of a protracted vomiting and diarrhea episode which followed the last dose.

Mrs. M. S. (139158) aged 76 had atrial fibrillation for at least 14 months on a hypertensive cardiovascular basis. The atrial fibrillation was accompanied by premature ventricular systoles of apparent single focus origin in the right ventricle; the frequency of these ectopic ventricular beats computed from frequent electrocardiograms taken over a 6 week time period varied from 10.6 to 13.9 per cent of the total heart beats.

Due to the instability of cinchonamine sulfate capsules which had turned red-brown on exposure to light and air and shortness of time this patient was given capsules of cinchonamine alkaloid, 200 mg. The cinchonamine was given at 9 A.M., 12 and 3 P.M. at 200, 200 and 400 mg. dosage. Severe nausea and vomiting was experienced following the last dose, controlled by 1 M injection of 25 mg. diphenhydramine. Due to this occurrence and a late laboratory report of a blood urea nitrogen of 43 mg. per cent no further cinchonamine

was attempted.

Following the first (200 mg.) dose of cinchonamine the premature ventricular systoles disappeared completely. Following the second dose of cinchonamine prominent f waves appeared in several electrocardiograms giving almost the appearance of 2:1 to 4:1 flutter. It is pertinent to recall that Gold and his associates (37) used the appearance of f waves as being an indication of just subthreshold anti-arrhythmic activity of a drug.

These preliminary results with cinchonamine tend to support the hypothesis that indole-quinuclidine alkaloids are effective in converting chronic atrial fibrillation to sinus rhythm and are also at lower dosage, capable of suppressing ectopic ventricular activity. Further studies are indicated.

DISCUSSION

In the experience of Sokolow and others (95) (57) (94), it was found that in the treatment of patients with the drug quinidine, one of 300 died, focusing attention on the need for a new and more ideal antiarrhythmic drug. Quinidine's discovery as a drug was the direct result of the coincidence of two diseases in the same patient; the parasitic infection, malaria and the cardiac arrhythmia, atrial fibrillation. No such accidental discovery can be predicted for the developement of a new useful antiarrhythmic drug. The logical scientific approach in this area, using pharmacologic screening techniques in animals prior to clinical studies, has not met with success. Using this approach, procaine amide and hydroxyzine pamoate have experienced limited usefulness in certain cardiac arrhythmias, but are impotent in the most common clinical arrhythmia, chronic sustained atrial fibrillation. Thus, any attempt to correlate chemical structure and effectiveness in atrial fibrillation is on a tentative and insecure basis. Such correlative relationships are discussed then as merely suggestive avenues of future research.

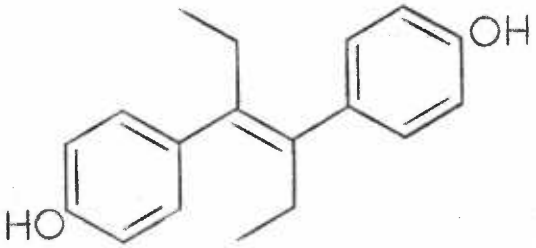
Two heterocyclic rings are evident in the chemical structure of quinidine viz., quinoline and quinuclidine, as shown in figure 2. One fragment, quinoline, has been absorbed in considerable amounts into workers in the dye

industry, and no beneficial effects on atrial fibrillation reported. Contrariwise, drugs of any type, i.e., dibucaine, containing quinoline, have the reputation of high toxicity.

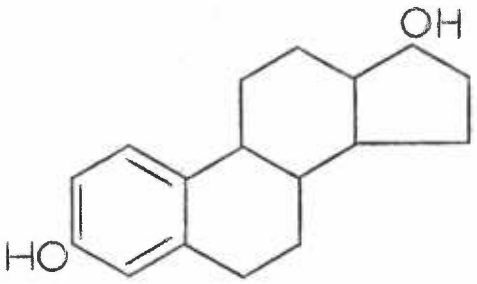
Although simple quinuclidine compounds have recently been synthetized, none has been given to patients with atrial fibrillation. Evidence suggesting that the quinuclidine ring is important for useful antiarrhythmic activity rests on the successful clinical trial of ajmaline and cinchonamine reported in this study. Both compounds contain two ring heterocycles, indole and quinuclidine. From these quite meager observations, the synthesis of the following compound is desirable.

This structure contains the quinuclidine ring and an ethyl (or vinyl) side chain believed essential. It is also a structure with two basic centers, the stronger quinuclidine nitrogen and the weaker base, the primary amine. The primary (or secondary) alcohol moiety is also suggested from its presence in all "clinically effective" antiarrhythmic drugs. The nitrogen nuclei and alcohol are believed to be

centers for covalent or hydrogen bonds, bending with the still unknown antiarrhythmic receptor site in cardiac muscle. The over-all size of this molecule is proposed as necessary to blank the reactive receptor site. This somewhat vague concept has precedent in the estrogenic drugs, stilbestrol and estradiol.



Diethylstilbestrol



Estradiol

The ethylene double bond is necessary to limit rotation of groups about a carbon-carbon single bond. The 'trans' form of stilbene tending to occupy a similar area of space as the more or less planar steroid structure.

Following along this biochemorphic approach, many important questions are raised. To state only one: Is the bicyclic quinuclidine ring really necessary for antiarrhythmic potency, or could there be, by the rupture of one ring, an active piperidine series established? When the the requirements for chemical structure necessary for antiarrhythmic activity have been established, it should then be possible to identify reactive sites in the receptor site

or active enzymes regulating normal cardiac rhythm or vice versa, that pathobiochemical or pathophysiologic mechanism establishing cardiac arrhythmias.

It is pertinent to inquire into the mechanisms underlying the broadening of the QRS and P waves observed for both the quinidine and indole-quinuclidine groups of alkaloids. Two questions are implied in this inquiry: 1. Is the broadening of these QRS and P waves related to the therapeutic effect of quinidine; and 2. Can the broadening of the QRS and P waves be useful to evaluate with confidence new drugs as potentially useful antiarrhythmic drugs?

The classical clinical interpretation of a broadened QRS wave in the electrocardiogram is a retardation of myocardial conduction. This finds use in the diagnosis of intraventricular block or of ventricular hypertrophy or strain. Similarly, a widened P wave might indicate auricular hypertrophy. It is also well understood that the inscription of these waves is the electrical correlate of ventricular and atrial contraction. The contraction process is associated with the phenomenon of myocardial depolarization. Thus, a broadening of the QRS wave (or P wave) reflects both a slowing of the rate of the individual cell depolarization process as well as a retardation of the intercellular conduction process. Now, as in the experiment

on the normal dog heart, there is no enlargement of atrial or ventricular mass and there is no evidence of the block, then a broadened wave reflects a slowing of the rate of the depolarization process.

The hypothesis that the broadening of the P and QRS waves represents quinidine's slowing of the rate of depolarization has been confirmed in other studies. Capillary micro-electrodes were driven into single myocardial muscle fibers of experimental animals, and the mono-phasic action potential recorded on an oscilloscope. This technique minimized complications of interpretation. While specialized conduction tissue (S-A or A-V nodes and the His-Purkinje system) is excluded, intercellular retardation may still occur. Using this technique, Johnson (55) (56) found that quinidine retards the rate of depolarization of atrium or ventricle without significantly altering the resting membrane potential. The biochemical basis for this has been described; as the membrane potential collapses, inscribing a depolarization action potential, there is a loss of intracellular potassium and a gain of sodium ions. Using radioisotopes with isolated rabbit atria, Holland and Klein (49) found that quinidine slows the rate of cellular escape of potassium (efflux) into the interstitial space, as well as, slowing the rate of cellular entrance of sodium. The interpretation that quinidine, by broadening the QRS and P waves

retards myocardial depolarization, thus appears justified.

Caution however, should be exercised in extending this observation to explain the therapeutic effect of quinidine in atrial fibrillation. The widening of the QRS and P waves by quinidine and the indole alkaloids is transitory and completely reversible. When dogs were given quinidine until the QRS wave was 25 per cent greater than pre-injection values, and then the drug was stopped, there resulted a return to the control QRS width in 180 minutes. Broadening of the wave by 50 per cent required 240 minutes for a return to normal values. Similar values were obtained for the P wave. When patients are given quinidine by mouth, with an adequate time interval between doses, there is no change in the width of the QRS wave. Only when quinidine is given to patients intravenously, or by "pushing" large doses by mouth, does QRS broadening occur. The review of the electrocardiograms of hospitalized patients appears convincing; the QRS wave during sinus rhythm following quinidine conversion, while the patient is on a maintenance dosage of the drug, is not any wider than that measured during the period of atrial fibrillation or antecedent sinus rhythm, more often than not, they appear to have narrowed. Broadening of the P or QRS waves thus does not appear to exert a direct influence on quinidine's conversion of arrhythmias to sinus rhythm.

Measurements of a drug's ability to prolong the QRS and P wave in experimental animals may still prove a useful pharmacologic screening technique. It has been reported previously (25) that drugs which are effective in terminating chronic sustained atrial fibrillation, quinidine and allocryptopine do broaden these intervals. Drugs which do not lengthen these waves were clinically ineffective; quinacrine, atropine, procaine amide, diphenhydramine and methantheline. Further studies are however needed.

SUMMARY

The experiments performed in this study were intended to test the hypothesis that in the drug quinidine, the quinuclidine ring structure is essential, but that the quinoline ring system is unnecessary for antiarrhythmic activity. The effectiveness of cinchonamine and ajmaline, both indole-quinuclidine alkaloids, which successfully terminated premature ventricular systoles and sustained atrial fibrillation in patients, effectively supports the hypothesis. Further studies are indicated for both ajmaline and cinchonamine as potentially useful therapeutic agents; the former in suppressing premature ventricular systoles and other ventricular arrhythmias, and the latter for atrial fibrillation.

This study was also intended to develope a new pharmacologic screening technique, which would demonstrate a better correlation than now exists, between antiarrhythmic drug potency in animals and therapeutic effectiveness in patients with arrhythmias. To serve this purpose, quinidine was administered in gradually increasing dosages to anesthetized dogs, with continuous monitoring of the electrocardiogram. Both quinidine and the chemically related indole alkaloids; ajmaline, cinchonamine, ibogaine, quinamine and tetraphyllicine, exhibit similar effects on the electrical properties of the dog heart. These include: broadening of the P wave

and QRS wave, prolongation of P-R_c and Q-T_c intervals and an increase in height (voltage) and area of the T wave. Additional studies are necessary to correlate the clinical predictability of these experiments. Such an experimental design has however, proven of value in the human pharmacologic studies of cinchonamine and ajmaline; as warning "guide posts" of potentially dangerous effects on the heart.

Incidental to the above conclusions, the following findings have been made:

The alkaloid cinchonamine has been isolated from Remijia purdieana, Wedd., and additional knowledge on its chemical properties uncovered. Cinchonamine in the presence of light, air (oxygen) and certain solvents is converted to "remijalutein", and a tentative structure for the latter assigned.

The minor alkaloid concusconine has been isolated also, and evidence suggesting its structure as ajmaline-like is presented. The antimalarial drug amodiaquin provokes ventricular bigeminy, and this observation has been converted into a "screening" technique for the evaluation in animals of drugs with potentially useful action in suppressing bigeminal rhythm.

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