AN EXPERIMENTAL APPROACH TO THE GENESIS OF AURICULAR FIBRILLATION AND THE ACTION OF ANTIFIBRILLATORY DRUGS

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A THESIS

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INTRODUCTION

Auricular fibrillation, or "*delirium cordis*" as it was once described, was first identified by Hering in 1903. (1) "*Pulsus irregularis perpetuus*" had been known to clinicians for many decades but its significance remained obscure until Hering described it as a cardiac arrhythmia with characteristic features. These features included irregular ventricular contractions and an absence of auricular contractions as shown by the phlebogram. Although his first observations were made on the experimental animal, he states, "This arrhythmia is of exactly the same character as that which we find in *pulsus irregularis perpetuus*." He called this arrhythmia "*Flimmern der Vorhofe*" (auricular fibrillation).

Although its clinical existence was suspected by Hering in 1903 and Cushny in 1906, (2) it was not until 1909 that Rothberger and Winterberg (3) published the first satisfactory evidence that auricular fibrillation occurs in human patients. Their publication antedated by only a few weeks the independent demonstration by Lewis(4) that auricular fibrillation is a common clinical disorder.

Auricular fibrillation ranks third in frequency of occurrence as a disturbance of cardiac rhythm; premature beats and paroxysmal auricular tachycardia ranking first and second. Some sources report that auricular fibrillation

is more common than paroxysmal tachycardia. (5) The reason for this discrepancy can be explained in that auricular fibrillation is a striking disorder, frequently permanent and easily recorded graphically. Paroxysmal tachycardia is usually a transient condition, often scarcely heeded, and difficult to record due to its short duration.

An analysis of 3,000 cardiac patients by White in 1928 (5) showed that 376 or thirteen per cent had auricular fibrillation. Of these, eighty-two per cent were permanent and eighteen per cent were paroxysmal in nature. Ninety-two per cent of all cases of auricular fibrillation showed evidence of organic heart disease, while eight per cent had no demonstrable signs of cardiac pathology.

There are five well-recognized organic causes of auricular fibrillation. The most common of these is mitral stenosis with the most frequent cause of mitral stenosis being rheumatic fever. White (5) found that forty-six per cent of all auricular fibrillators with organic heart disease were in this category. Coronary disease, with its complications of anoxia and infarction, accounted for twenty-one per cent. Hypertension alone, and complicated by other conditions, e.g. cardiac failure, accounted for nineteen per cent. Thyrotoxicosis was the basis of the fibrillation in fourteen per cent of the cases.

Auricular fibrillation in those patients without organic heart disease has certain characteristic features.

It is found in the older age group; symptoms of congestive heart failure are absent; the heart rate is usually normal; cardiac enlargement is absent; and the prognosis is favorable as these patients yield readily to antifibrillatory drugs such as quinidine.

The sexes are unequally afflicted with auricular fibrillation as two males show this arrhythmia for every female. An explanation may lie in the fact that men are generally subject to greater cardiac strain than women and this correlates with the fact that organic heart disease is more common in males than in females.

The clinical importance of auricular fibrillation is not based on the fact that it is a very common disorder. Many syndromes are common, yet so innocuous as to require little or no medical attention. While some individuals look upon auricular fibrillation as an inconsequential disorder requiring no therapy, others view the condition with an inimical eye and take every opportunity to assault it with the most formidable therapeutic agents in their armamentarium.

If fibrillation occurs in the form of transient paroxysms in a person without organic heart disease, it may disturb the patient but little more. Untreated paroxysms usually last a few hours, with extreme limits of a few minutes to several days, and very rarely a few weeks. Paroxysms may occur only once or twice and be absent for many

years, or they may occur frequently at intervals of months or weeks and yet not cause more than passing discomfort if the myocardium is not weakened as in failure or infarction. It is important, however, that these paroxysms be distinguished from attacks of paroxysmal auricular tachycardia.

Even permanent fibrillation may cause little or no disability if there is no associated organic heart disease and if the cardiac rate is not excessive. There are patients with a history of paroxysmal or permanent auricular fibrillation who have lived with this condition for as long as thirty years.

Unfortunately, auricular fibrillation does not always present itself as an innocuous entity but may appear as a serious complication of heart disease. Indeed, it may precipitate cardiac failure and death. Fibrillation is always a burden, even as is tachycardia, to any heart.

The cardiac reserve of the normal heart is usually sufficient to compensate for this added burden, but the heart with organic disease may not have this reserve available and failure results if fibrillation is superimposed.

In general, we may regard auricular fibrillation, premature beats and sinus tachycardias, as functional disorders or symptoms that are the result of underlying organic disease or of an imbalance of normal physiological mechanisms. An analogy can be made with sinus tachycardia, which may be due to organic disease such as myocardial infarction

or it may be the result of hypothalamic overflow as in situational states. We should not attempt to treat the tachycardia per se but rather treat the infarction or alleviate the psychic stress. In some cases, however, it may be to the patient's best interests to control the heart rate until the etiological cause can be discovered and treated.

It has been suggested that the irregularity of rhythm is the serious factor in auricular fibrillation and if we are able to slow the rate even though the irregular rate persists, adequate cardiac output may be maintained. But hearts with a regular rate have a greater cardiac output than those with an irregular rate. (5) This fact alone often makes restoration of normal rhythm lifesaving in those individuals who are on the borderline of decompensation. Let us re-emphasize at this point that these individuals are fibrillating because of the underlying failure and are not primarily in failure because of fibrillation.

While it is true that prolonged fibrillation in individuals with normal hearts may conceivably weaken the myocardium over a period of years, the usual case is not failure due to the fibrillation alone but to underlying organic disease such as mitral stenosis plus the added burden of the tachycardia and irregularity. Thus the early recognition and persistent treatment of auricular fibrillation would seem to be of great importance in prolonging life and reducing disability in cardiac cripples.

There is one condition, thyrotoxicosis, in which auricular fibrillation, either in the paroxysmal or permanent form, is likely to be the earliest sign of cardiac strain. If the thyrotoxicosis is not corrected, the myocardial stress and auricular fibrillation may cause heart failure and death. The use of antifibrillatory drugs alone is not sufficient, because the ventricular rate is difficult to control until the thyrotoxicosis is corrected. In all cases immediate antithyroid therapy should be instituted. It is important to consider the possible existence of thyrotoxicosis in any patient with auricular fibrillation that does not show sufficient cardiac pathology to account for the arrhythmia.

An important complication of auricular fibrillation, besides cardiac failure, is embolism into cerebral, renal, splenic or peripheral arteries from intra cardiac thrombi. While it is recognized that embolism as a complication of auricular fibrillation is infrequent, it does present a serious problem in those cases where it does occur.

Various investigators differ in their reports of successful restoration of normal rhythm in patients with auricular fibrillation. The incidence of successful therapy varies from seven to ninety-four per cent, with the average being sixty per cent. The usual procedure is to give a test dose of 0.2 gram quinidine sulfate to the patient to see if he is unduly sensitive to the drug. If no toxic

symptoms such as tinnitus, deafness, urticaria, nausea, vomiting, diarrhea, or tachycardia develop, then administration of the drug in larger doses may proceed. The drug is usually administered in five doses every two hours for the first day. If conversion to normal rhythm does not occur, the dose is increased by 0.2 gram and the same time schedule is repeated the next day. This procedure continues until signs of toxicity or conversion to normal rhythm occurs. Doses of 6 grams a day have been given with no toxic effects. (5) If normal rhythm appears, the patient should be maintained on approximately 0.2 gram twice a day for two or three weeks. In some instances therapy can be discontinued at once without recurrence of the arrhythmia. If auricular fibrillation recurs it should be treated in the same manner as a new case, however if it recurs often and normal rhythm does not persist for any appreciable length of time, then it may be best to discontinue therapy with quinidine and try to decrease the cardiac rate with digitalis.

Therapy of fibrillation is not without its complications. Emboli from intracardiac thrombi which are broken off when the auricular muscle resumes its normal contractions may result in death or hemiplegia. Sudden death without embolism has been seen in several cases during quinidine therapy and may be the result of the toxic effect of the drug on the pacemakers of the heart. (5)

In a prevalent clinical condition such as

auricular fibrillation it would be logical to assume that over a period of years, there would be advances in techniques of therapy and the introduction of newer and more effective therapeutic agents. Actually, therapy of auricular fibrillation has changed very little since the discovery in 1914 that quinine had a specific effect on the disorder. A patient with malaria and who incidentally had auricular fibrillation reported to his physician, Professor R. F. Wenckebach, that the quinine he was taking for malaria also cured his fibrillation. Four years later, Frey published the results of a study which showed that quinidine, the dextroisomer of quinine, had more effect in abolishing auricular fibrillation than quinine. (6) Since that time, with the exception of the introduction of intramuscular and intravenous forms of quinidine (7), there has been no new drug developed that has replaced quinidine in treating auricular fibrillation.

In 1940 procaine hydrochloride was successfully employed to prevent the ventricular arrhythmias that occur during surgical operations when cyclopropane must be used. (8) Procaine is very rapidly hydrolyzed by blood esterases and it must be administered by continuous intravenous drip. Dr. E. L. McCawley noted that diphenhydramine (Benadryl) is closely related chemically to procaine. Procaine is a dialkylaminoalkyl ester while Benadryl is a closely related dialkylaminoalkyl ether. The prediction that Benadryl

probably would have a longer duration of action due to the stable ether linkage was borne out in experimental studies and limited clinical trial (McCawley and White [9]). Dr. H. L. H. Dick suggested, since ventricle and auricle are composed of the same type of tissue governed by the same properties of contractibility and conduction of stimulus, that Benadryl be given a trial in the therapy of auricular fibrillation.

Many agents have been subjected to trial in therapy of auricular fibrillation; quinacrine (Atabrine), sparteine, alpha fagarine and procaine amide (Pronestyl) have received recent attention. Although much effort has been expended in the synthesis, discovery, rediscovery, and often exploitation of new antifibrillatory drugs, it must be admitted that little of consequence has resulted from these energies to date.

In the past, new antifibrillatory drugs were introduced only as they were accidentally discovered, as in the case of quinine and quinidine, or by a clinical trial and error study of drugs similar to quinidine in biochemorphology. At the present time the available screening tests include a few animal techniques of doubtful validity and a poor record of performance in selecting useful drugs for therapy. The reluctance of the patient and the physician to receive or administer virtually unknown and untested chemicals deters rapid progress toward an ideal antifibrillatory drug. Each year, numerous articles appear in the various

publications announcing new antifibrillatory drugs or denouncing drugs previously introduced. This chaos will continue until we develop reliable screening tests based upon the physiological principles involved in auricular fibrillation.

THE PURPOSE OF THIS THESIS IS TO STUDY THE MECHANISMS INVOLVED IN THE GENESIS OF AURICULAR FIBRILLATION AND THE ACTION OF ANTIFIBRILLATORY DRUGS AND TO EVALUATE CERTAIN DRUGS FOR ANTIFIBRILLATORY PROPERTIES. A REVIEW AND PRESENTATION OF THE RESULTS OF THESE EXPERIMENTS IS PRECIPITATED BY TEMPORAL OBLIGATIONS, NOT THE SOLUTION OF A VERY COMPLEX PROBLEM.

Organization of Thesis: The first section of this thesis will be limited to a discussion of the mechanism and physiology of auricular fibrillation. It will include a definition of the properties of cardiac muscle and the influence of such factors as anoxia and digitalis on auricular fibrillation; experimental auricular fibrillation in the intact animal; and the mechanism of action of the antifibrillatory drugs.

The various methods of testing antifibrillatory drugs and an introduction of several new techniques for this purpose are discussed in the second section. Several drugs including quinidine, antihistaminics and Banthine are evaluated using these procedures.

PART ONE

MECHANISMS INVOLVED IN AURICULAR FIBRILLATION

AND

ANTIFIBRILLATORY DRUGS

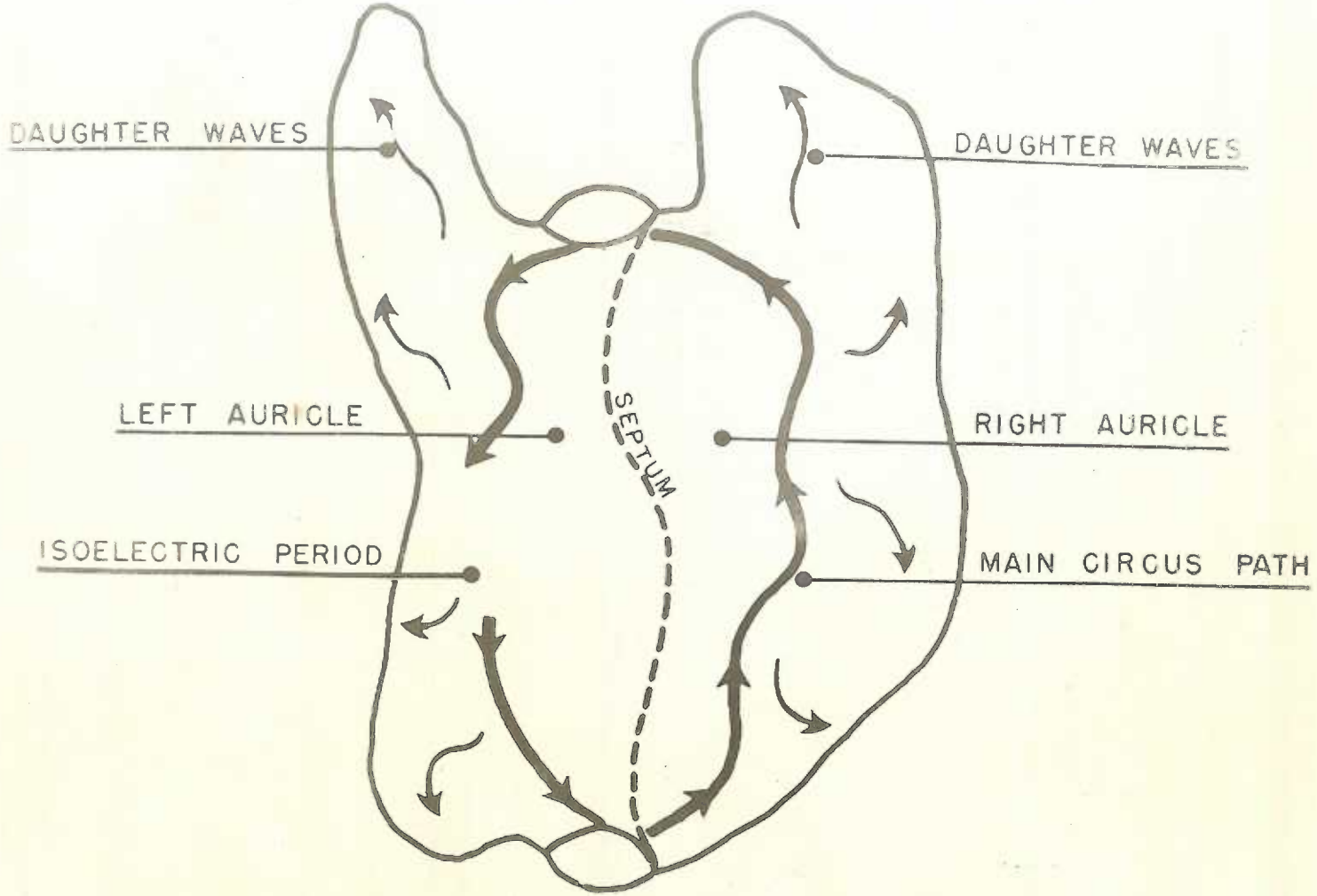
MECHANISMS INVOLVED IN THE GENESIS OF AURICULAR FIBRILLATION AND THE ACTION OF ANTIFIBRILLATORY DRUGS

INTRODUCTION

The first explanation for the mechanisms involved in the genesis of auricular fibrillation was suggested by MacWilliam in 1887. (10) He postulated a circulating excitation wave, perpetuated by re-entry of daughter waves into its site of origin. His re-entry theory was modified by Garrey in 1914 who suggested that the mechanism was a circus movement of excitation around the venae cavae.(11) This conception, called the "circus movement" theory is based on fundamental observations of a circus movement of muscular contraction waves in experimental animals. (12) (13) (14) The detailed studies of Lewis and his co-workers during the period 1918 - 1922 resulted in almost universal acceptance of this theory.

The genesis of auricular fibrillation is explained by Lewis in the following manner. (15) A central wave of excitation, initiated from the sinoauricular node, travels through the atrial myocardium in a circumferential path around the venae cavae. The central wave gives rise to daughter waves which spread to the periphery. (fig.1) The pathway of the central and daughter waves is determined by the excitability of the muscle fibers which lie in its path. Porter in 1894 (16) made the observation that functional

Figure 1. Hypothetical Circus Movement in the Auricle Showing the Daughter Waves



HYPOTHETICAL CIRCUS MOVEMENT

intramuscular blocks (absolute refractory period) in the atrial myocardium could deflect the course of the excitatory impulse into a devious and redundant pathway. If the central excitatory wave has a sufficiently long pathway of atrial tissue to travel in, it may re-enter the site (S-A node area) of the initiating tissue and finding that refractoriness has worn away, continue in its original pathway.

Once an area of myocardium has been stimulated by the excitation wave, it cannot respond again until it has recovered from its refractory period which resulted from the previous contraction. The duration of the resulting iso-electric gap between effective excitatory waves is dependent on the sum of the absolute and "effective" relative refractory period of the atrial myocardium at the time. If the iso-electric gap be shortened, the excitatory wave meets less non-responsive tissue and can travel a shorter circus resulting in a more rapid fibrillary rate. If, by way of contrast, the iso-electric gap or refractory period be lengthened by the action of drugs, the fibrillary rate should be decreased or abolished.

Englemann in 1893,[17] struck by the fact that various parts of the fibrillating auricles were in different phases of contraction at a given instant, postulated that the existence of multiple ectopic foci discharging simultaneously at different rates was responsible for the incoordinated muscular activity characteristic of the

fibrillation. Englemann's theory of "polytopic impulse formation" gained much support because it provided a rational basis for the observation that premature systoles frequently precede the onset of fibrillation.

This theory, and particularly in its simplified form of a single ectopic focus, is being currently revived. Prinzmetal and his coworkers (18) (19) have seriously challenged the circus movement concept of auricular fibrillation and favor an ectopic focus explanation. The major proof of circus movement developed by the Lewis group was the sequential appearance of the excitation wave passing over electrodes implanted at various places in the right auricle, with improved oscilloscopic recording and exposure of the left auricle as well, the data accumulated recently does not support a circus path of excitation but rather indicates a random spread of electrical activity. High speed cinematographic recording also indicates that the contracting waves of atrial myocardium do not follow a circus path but rather exhibit random activity.

Prinzmetal and his group thus describe auricular fibrillation as a chaotic heterorhythmic disturbance involving a sea of irregular contractions that number from 600 to 40,000 per minute. There is a super-imposed regular wave-like contraction that sweeps across the auricle in undulating fashion at 400-600 cycles per minute. For purposes of description these two orders of activity have been

termed "M" or microscopic and "L" or macroscopic activity. Both occur simultaneously throughout all contractile portions of the auricular musculature.

"M" activity is diagnostic of auricular fibrillation, as it is never seen in any of the other auricular arrhythmias. "L" waves arise at a relatively regular rate of 400-600 per minute and arise at various sites in the auricle. In experimentally produced auricular tachycardia and flutter, these contractile waves arise from a single ectopic focus. The distance covered by these "L" waves is variable; some are scarcely visible while others may involve most of the auricle. There is the suggestion offered that macroscopic waves may merely represent a fusion of the microscopic contractions.

Today the battle line is drawn between these two schools of thought. On the side of the circus movement theory is acceptance by medical texts for over thirty years and the fact that it is the only explanation known by the majority of physicians today. The ectopic foci theory is new, and untested. The results and observations have not yet been duplicated by others. It is not too widely known nor is it completely understood by all who have read or listened to the lectures on this work. At the present time I do not believe that the problem has been solved by either group but the ectopic foci theory, with certain modifications, which will be discussed later, more nearly gives us

the key to the problem.

Cardiac muscle has certain fundamental properties. It is these properties and the modification of these properties by such factors as drugs, anoxia, autonomic innervation, temperature, and cardiac disease that provide us with an explanation for the genesis of auricular fibrillation and the action of antifibrillatory drugs. This section is devoted to the discussion of these properties and their response to various extrinsic and intrinsic factors. Where it is feasible, experiments have been devised to illustrate or emphasize these points. Auricular fibrillation has been produced in experimental animals and isolated tissues to demonstrate the genesis of auricular fibrillation, and arrested broken to demonstrate the mode of action of antifibrillatory drugs.

Methods. In order to avoid repetition, the details of the methods used in these experiments will be discussed in Part Two under the heading, "Methods of Evaluating Antifibrillatory Drugs", as many of these same experiments are used to demonstrate antifibrillatory activity.

THE ROLE OF CERTAIN CHARACTERISTICS OF CARDIAC MUSCLE IN THE GENESIS OF AURICULAR FIBRILLATION

Cardiac muscle has certain properties such as rhythmicity and irritability that must become altered in any disturbance of normal cardiac rhythm. A discussion of these properties and their alteration in auricular fibrillation is necessary at this time to provide us with an understanding of the fundamental mechanisms involved in the genesis of auricular fibrillation.

Refractory Period: The term refractory period signifies a state of unresponsiveness or lack of irritability in muscle tissue following its stimulation. The absolute refractory period is that period of time when the tissue will not respond regardless of the intensity of the stimulus. Relative refractory period is the period of time when the tissue will respond but requires a stronger stimulus than originally. It is the refractory period that is the limiting factor in the stimulation and contraction of all muscle tissue including the myocardium.

The existence of auricular fibrillation depends on the presence of muscle tissue that can respond to rapid stimulation, be it from ectopic foci or daughter waves from the circus movement of the central excitation wave. If the normal refractory period is shortened, then myocardial tissue recovers its sensitivity sooner and is able

to respond to stimuli at a faster rate. On the other hand, if muscle tissue which is being stimulated at a fast rate has its refractory period increased, then the response rate of that muscle will be slowed.

Lewis, in his original paper in 1921, reported a shortening of the absolute refractory period preceding auricular fibrillation. (15) However, this observation was retracted in 1926 when he demonstrated that while the relative refractory period in dogs was lengthened by quinidine, there was no change in the absolute refractory period associated with the development of auricular fibrillation. (20) Since that time, it has been demonstrated that quinidine also lengthens the relative refractory period in man. (21)

It has been suggested that relative refractory period be given the more descriptive name, "effective refractory period." This may be desirable in view of the present confusion in regard to the limits of the meaning of refractory period. (22) In any event, it may be stated that the absolute refractory period plays no role in the mechanism of auricular fibrillation as it does not change one way or the other. In general, most of the evidence to date indicates that a shortened relative refractory period predisposes to fibrillation and a lengthened one inhibits fibrillation. (23)

What is the cause of the shortened relative

refractory period in auricular fibrillation? At the present time, we are unable to answer this question with any degree of certainty. However, most investigators agree that the shortened relative refractory period seen in auricular fibrillation is in some manner related to increased vagal tone. It has been shown that cholinergic drugs will decrease the relative refractory period, while vagal blocking agents or anti-cholinergic drugs will increase it. (24, 25, 26)

In a series of experiments using the Dawes' technique with the isolated rabbit's auricle, we have reaffirmed these results and in addition, demonstrated that antihistaminic drugs such as Benadryl, also increase the relative refractory period and block the action of cholinergic drugs on the heart. The results of these experiments are shown below in Table 1. (The details of the experimental methods will be discussed in a later section.)

Table 1. The Effect of Cholinergic and Anti-Cholinergic Drugs on the Relative Refractory Period of the Isolated Rabbit Auricle.

Drug	Concentration	Change in Relative (1) Refractory Period
Cholinergic		
Acetylcholine	1 x 10 - 6	30 per cent decrease
Anti-Cholinergic		
atropine	1 x 10 - 6	50 per cent increase
quinidine	1 x 10 - 6	10 per cent increase
Benadryl	1 x 10 - 6	35 per cent increase

(1) Represents the average of three auricles.

While we realize that the whole problem of auricular fibrillation is still in the theoretical stage, we believe that if we are to make any progress in this field, we must make certain affirmations and defend them. It is with this thought in mind that we make the following statements regarding auricular fibrillation and relative refractory period: We will assume at this time that the relative refractory period of auricular muscle is decreased in clinical and experimental auricular fibrillation and that it is due to an increase in vagal tone or cholinergic drugs such as acetylcholine. We will also assume that an anti-fibrillatory drug through its action on the myocardium reverses this effect of acetylcholine and increases the relative refractory period.

It is the decrease in relative refractory period and other concomitant changes in the auricle which acetylcholine produces that makes possible the initiation of auricular fibrillation. It is the increase in relative refractory period and other concomitant changes in the auricle which makes possible the therapy of auricular fibrillation.

Conduction and Conduction Deficits: The conduction system of the heart is subject to many changes in the auricular arrhythmias. The resulting defects may be in the auricle, in the bundle branches or in the intraventricular conduction system. This discussion will be limited to a consideration of the intra-auricular and A-V nodal blocks.

When experimental auricular fibrillation is induced in dogs, increased auricular conduction time and A-V blocks precede the actual fibrillation of the auricles in almost every instance.[27, 28, 29, 30] These same conduction defects have been shown to exist in the development of clinical auricular fibrillation.[30,22]

The appearance of conduction defects preceding auricular fibrillation is probably an indication of increased vagal tone.[30] Whether or not it has any further significance in the genesis of auricular fibrillation is not known. We have suggested that the close correlation between conduction defects and auricular fibrillation might form the basis of a new method of testing antifibrillatory activity.

It might be expected that the minimal amount of a vagomimetic drug (acetylcholine), required to produce a 2:1 A-V block in the intact dog, would be increased after the administration of an antifibrillatory drug. This property of increasing the "minimal block dose" of acetylcholine[29] should parallel antifibrillatory activity and will be discussed further in a later section of this paper.

Rhythmicity: The inherent rhythmicity of the heart is its most unique characteristic. Many theorys are advanced as to the origin of this activity but as yet the mechanism is unknown.[31]

In auricular fibrillation, the rhythmicity of

the heart is disturbed in two respects. First there is complete inhibition of the pacemaker (S-A node) and second, there is the initiation of abnormal stimuli which give rise to the fibrillatory activity of the auricle.[15, 19] In the electrocardiogram this is demonstrated by the lack of normal P waves and the appearance of the characteristic F waves of auricular fibrillation.

We will assume in this paper that increased vagal tone is the factor responsible for the decrease in relative refractory period, the increase in auricular conduction time and the suppression of normal S-A nodal activity in auricular fibrillation.

The origin of the abnormal stimuli in the fibrillatory auricle is not as easily explained. However, it has been shown that oscillating potentials of sub-threshold intensity exist in auricular tissue. In isolated muscle strips, these potentials gradually reach a threshold level and propagate excitation waves.[32,33] Normally these excitation waves do not appear as the threshold of the auricle is so high that the S-A node is the only focus capable of producing potentials that reach threshold intensity. However if we suppress S-A nodal activity and at the same time decrease the stimulatory threshold of the auricle, these potentials now become effective stimuli and propagate excitation waves.

Indirectly Prinzmetal, Brill, et al,[18,19] have

demonstrated that such a chain of events probably takes place in auricular fibrillation. Normal P Waves are usually 0.1 to 0.3 m.v. intensity. Therefore, it may be assumed that the stimulatory threshold of the normal auricle is approximately 0.1 to 0.3 m.v. In fibrillating auricles these workers observed minute complexes usually less than 0.1 m. v. and large complexes whose intensities ranged from 0.2 to 1.0 m.v. In order for these small complexes to account for contraction waves, we must assume that the stimulatory threshold of the auricle was decreased to less than 0.1 m.v.

If such sub-threshold potentials exist in auricular tissue and they become effective stimuli when the threshold of the auricle is decreased, we have a logical explanation for the origin of ectopic auricular foci. It should be emphasized at this point, that ectopic foci form the basis for probably the most acceptable theory on the genesis of auricular fibrillation. (10, 18, 19)

There are two requisites necessary for repetitive, rhythmic impulse formation from an ectopic focus. The first is the existence of a continually active polarized region with a periodically depolarizing membrane. Secondly, it must be separated from the tissue to be activated by a threshold. As the center becomes polarized, it will discharge itself only when the voltage reaches a critical or threshold level. A practical example of this is demonstrated by the relaxation-oscillator circuit. (See Figure 2) The

condenser is charged slowly by the battery through the resistance until a critical voltage across the gas tube is reached, at which time the tube suddenly becomes a conductor and discharges the condenser. The cycle is then repeated, giving the wave form illustrated in Figure 3.

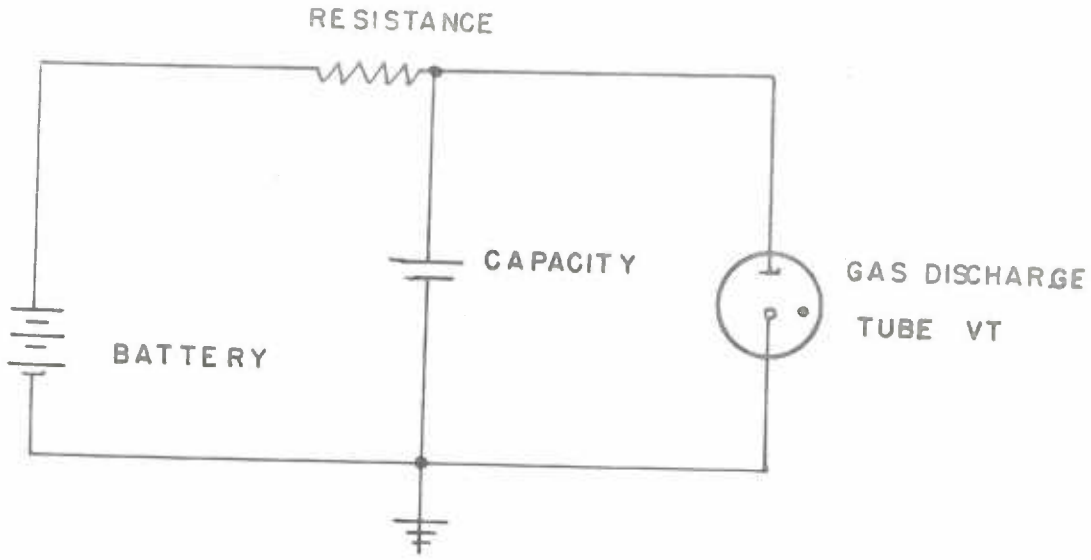
It may readily be seen from this diagram that the speed of impulse discharge from an excitatory focus is dependent upon the threshold and the rate at which polarization takes place. Given an impulse with a constant rate of polarization, we could speed the rate at which impulses were transmitted to the surrounding tissue by simply decreasing the threshold.

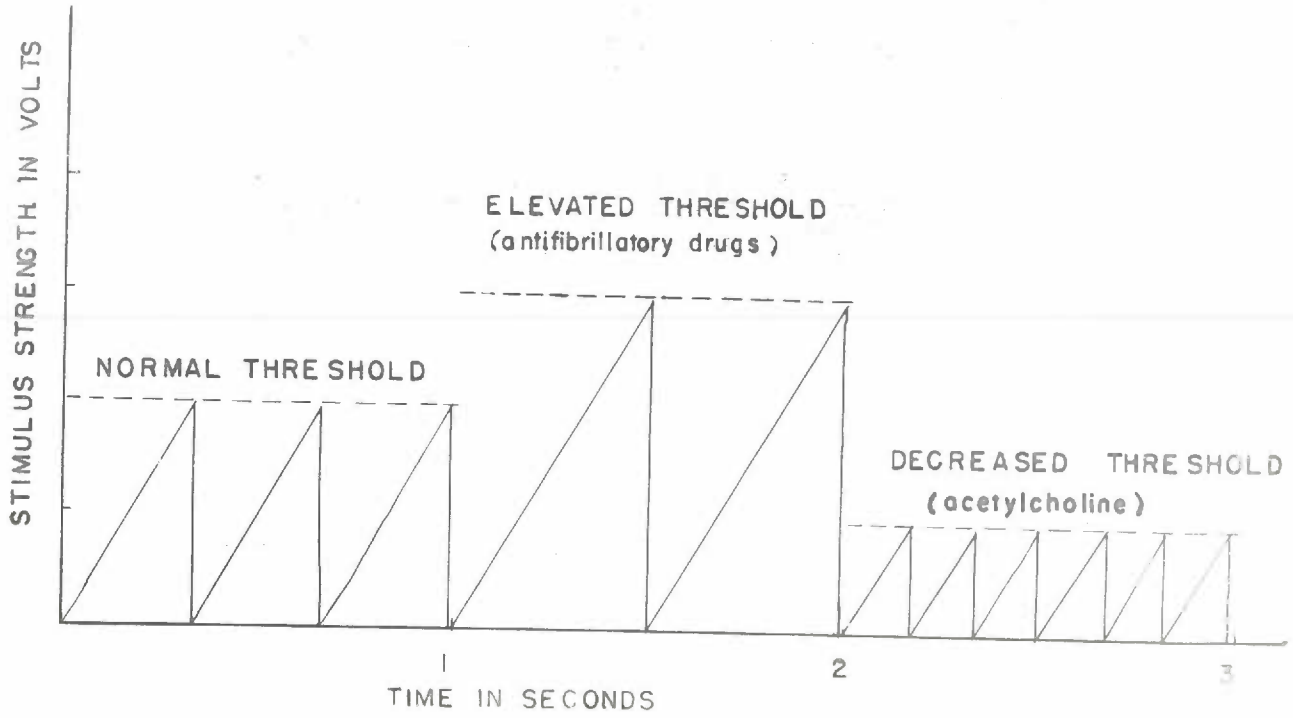
It is entirely possible that existence of ectopic foci which are the source of rapidly formed stimuli, depends upon this type of mechanism. If we wish to slow the rate of impulse formation arising from a focus, then we may do so by increasing the threshold. As the antifibrillatory drugs are capable of increasing the stimulatory threshold of auricular muscle, this may explain their mode of action in converting auricular fibrillation (rapidly firing foci) to normal sinus rhythm (slowly firing focus).

Irritability, Sensitivity and Stimulatory Threshold: By definition, the term irritability when applied to cardiac muscle, means the ability of the muscle to respond to a threshold stimulus. The degree of receptiveness of the musculature to stimulation is known as its

Figure 2. Relaxation - Oscillograph Circuit.

Figure 3. The Relationship Between Threshold and Rate of Discharge in a Relaxation - Oscillograph Circuit or an Ectopic Focus


RESISTANCE
CAPACITY
GAS DISCHARGE
TUBE VT
BATTERY


STIMULUS STRENGTH IN VOLTS
ELEVATED THRESHOLD
(antifibrillatory drugs)
NORMAL THRESHOLD
DECREASED THRESHOLD
(acetylcholine)
1
2
3
TIME IN SECONDS

sensitivity. The stimulatory threshold indicates the level to which electrical potentials must rise before they stimulate the surrounding tissue.

Auricular fibrillation as well as normal sinus rhythm depends upon the presence of irritable tissue for its existence. If there is no irritable auricular tissue, then regardless of the intensity of the stimulus from the ectopic focus or the pacemaker, there will be no contraction waves propagated across the auricular muscle. Without these contraction waves, there can be neither normal or abnormal auricular systoles. The role of irritability of cardiac muscle in the genesis of auricular fibrillation is, therefore, limited as irritability must be present if the heart is to beat at all!

On the other hand, sensitivity of the myocardium is subject to change in disturbances of auricular rhythm. It has been demonstrated (15) that in auricular fibrillation the auricle is in a state of increased sensitivity. In other words, the intensity of potentials required to stimulate the surrounding muscle is less than normal. We believe that vagal stimulation or vagomimetic drugs are responsible for this change in tissue sensitivity in auricular fibrillation.

Stimulatory threshold is inversely proportional to tissue sensitivity. It determines whether the discharge from an ectopic foci will be effective stimulus, and it

determines the rate at which these stimuli are discharged, (See Figure 3). The rate at which an ectopic focus fires determines whether the auricular arrhythmia will be a tachycardia, flutter or fibrillation. (39) We believe that the stimulatory threshold is decreased in auricular fibrillation and that it is a result of increased vagal tone or vagomimetic drugs. We also feel that the main action of antifibrillatory drugs is to elevate the stimulatory threshold of the auricle.

These tenets are based upon the results obtained from a series of experiments on isolated rabbits' auricles and the dog's heart. The exact details concerning the methods used in these experiments will be discussed in the second part of this paper, however, some explanation is necessary at this point for the sake of clarity.

Isolated rabbits' auricles were suspended in Tyrode's solution. They were attached by one end to a writing lever and to fine wire electrodes by the other. The electrodes were connected to a square wave stimulator in which the voltage and rate of stimulation could be controlled. The minimal voltage required to drive the auricle at a given rate was then determined. These voltage determinations were made for rates of 112, 118, 126, 144, 165, 198, 234, 270 and 312. After the normal stimulatory threshold was determined, fibrillatory drugs such as acetylcholine or antifibrillatory drugs such as quinidine were

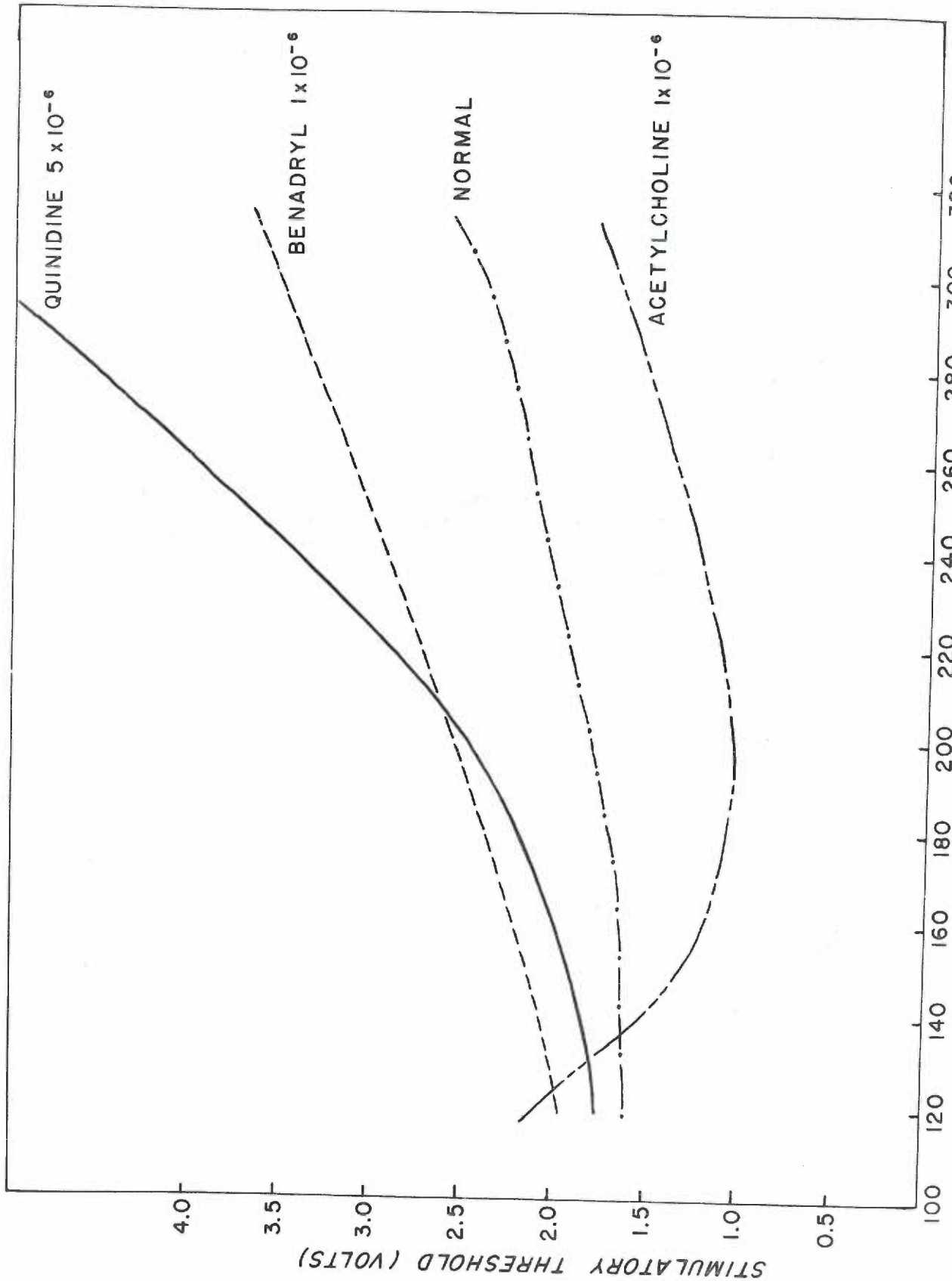
added to the bath. As soon as the drugs had been in the bath long enough to act upon the auricle, usually 15-20 minutes, the stimulatory threshold was again determined. After the maximum effect of the drugs was obtained the solutions were replaced with fresh Tyrode's and the normal stimulatory threshold was again obtained.

The value for each drug was obtained by averaging the results obtained from three auricles. These are shown graphically in Figure 4.

It was observed that antifibrillatory drugs increased the stimulatory threshold of the isolated rabbits' auricle. For example, quinidine increased the stimulatory threshold at all rates of stimulation but had its greatest effect at the higher stimulatory rates. This may well explain why quinidine has little effect on the heart as long as the rate is normal, but has a marked effect in conditions where tachycardia is found. It also explains why it is most effective against rapidly firing foci as in auricular fibrillation and relatively ineffective except in toxic doses against the slow firing focus (S-A node) in the normal heart.

Atropine has a similar action on the stimulatory threshold. It is significant to note that Lewis has reported some cases of auricular fibrillation that responded to atropine therapy. (15) We feel that atropine in large enough doses might be a very satisfactory antifibrillatory

Figure 4. The Normal Stimulatory Threshold of the Isolated Rabbit Auricle and its Alteration by Quinidine, Benadryl, and Acetylcholine.


QUINIDINE 5 x 10⁻⁶
BENADRYL 1 x 10⁻⁶
NORMAL
ACETYLCHOLINE 1 x 10⁻⁶
STIMULATORY THRESHOLD (VOLTS)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
100
120
140
160
180
200
220
240
260
280
300
320
RATE OF STIMULATION

drug if it were not for the annoying side effects of dry mouth, blurred vision, etc.

We feel that the action of atropine, quinidine and other antifibrillatory drugs is to depress the sensitivity and increase the stimulatory threshold by a direct action upon the auricular musculature. This means that the rate of stimulus discharge from the S-A node as well as from ectopic foci will be decreased. (See Table 2 which shows the spontaneous auricular rate of the isolated rabbit auricle before and after the addition of antifibrillatory drugs to the bath.)

When a fibrillatory drug such as acetylcholine is added to the bath, the stimulatory threshold is increased very little, or remains unchanged, as long as the auricle is not driven at speeds over 140 per minute, but in the range 140 to 300 the threshold is decreased with the greatest change being with the higher stimulatory rates! This probably explains why vagal stimulation does not play an important role in the firing of ectopic foci as long as the rate is in the range of normal. But if the foci are firing at rates above 140 per minute then vagal stimulation, which is reflexly brought into play, decreases the stimulatory threshold of the surrounding auricular muscle, allows the rate of firing to be increased, which in turn increases the effectiveness of vagal stimulation in decreasing the stimulatory threshold. It is not difficult to understand how such a

Table 2. The Effect of Antifibrillatory Drugs Upon the Spontaneous Auricular Rate of the Isolated Rabbit Auricle.

Drug	Concentration	Spontaneous Auricular Rate (1)	
		Normal	After Drug
quinidine	1 x 10 - 6	120	105
quinidine	1 x 10 - 5	96	60
atropine	2 x 10 - 6	148	124
Benadryl	1 x 10 - 6	102	96
Benadryl	1 x 10 - 4	87	66
Pronestyl	1 x 10 - 5	87	72
Ambodryl	5 x 10 - 5	90	84

(1) represents an average of three auricles.

The effect of other antifibrillatory drugs upon the stimulatory threshold of the auricle has been determined and the results will be found in the second part of this paper.

vicious cycle can perpetuate itself. We feel that this action of vagal stimulation or vagomimetic drugs plays a major role in the genesis of auricular fibrillation.

While most of the experiments have been done on isolated tissue, the same results can be obtained in the "open chest dog." In reviewing some data tabulated by Lewis et al in 1921, (15) we came across an interesting observation that apparently passed un-noticed by those workers at the time. In a series of experiments in which they drove the dog's auricle with an electrical stimulator, the stimulatory threshold at various rates was determined. In atropinized dogs, the stimulatory threshold increased as the rate increased but in non-atropinized dogs, the stimulatory threshold decreased as the rate was increased. As will be seen from Figure 4, an analogous situation exists in the isolated rabbit auricle. The stimulatory threshold of the control auricle is analogous to the dog whose vagal influence is neutralized by atropine. In both instances the stimulatory threshold increases as the rate of stimulation is increased. However, in the auricle bathed with acetylcholine which can be compared to the dog whose vagus is not neutralized by atropine, the stimulatory threshold actually decreases as the rate of stimulation is increased. This would mean that increased vagal tone or a vagus not blocked by antivagal drugs (antifibrillatory drugs) would actually bring about changes in the auricle favorable to the

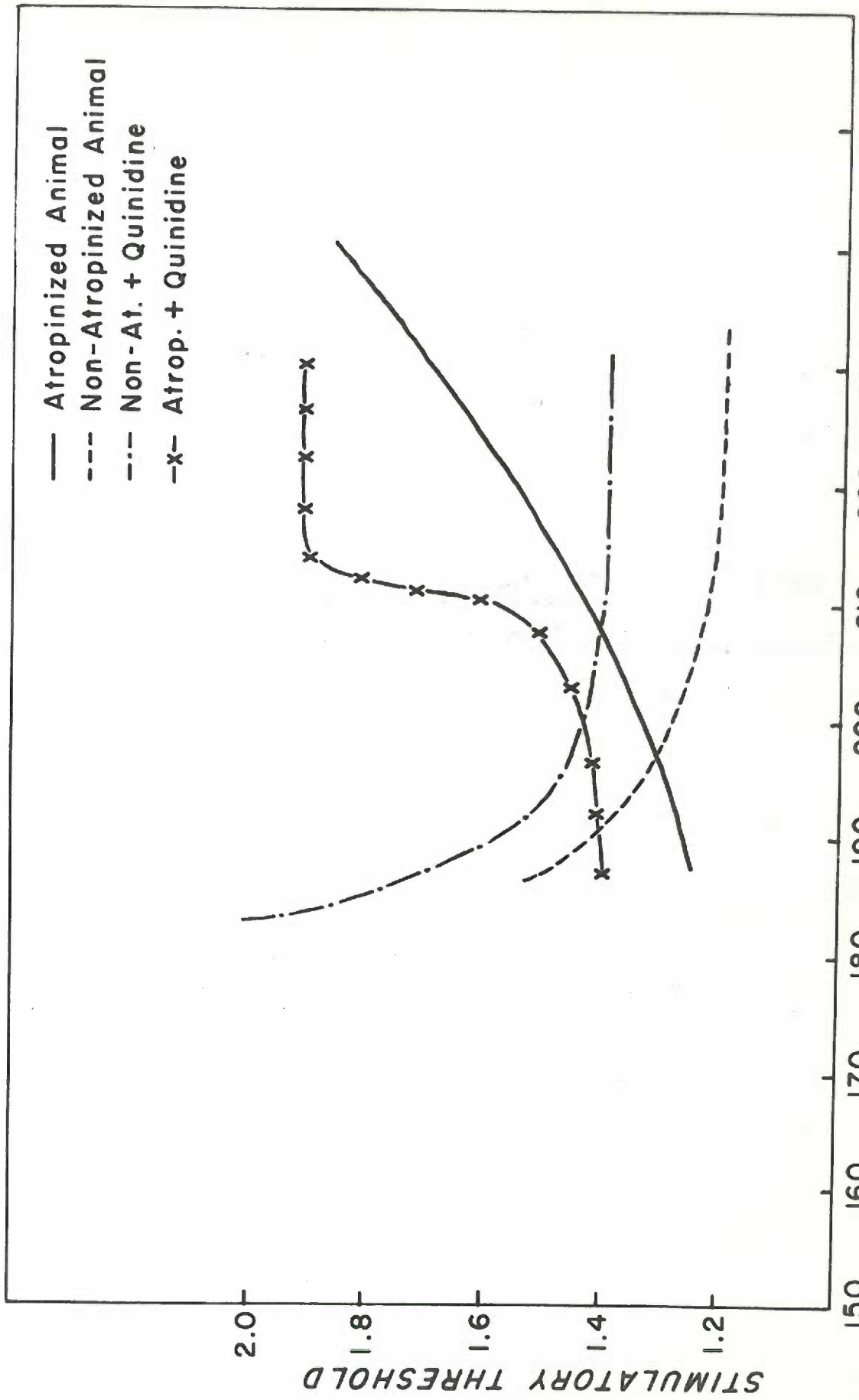
production of auricular fibrillation.

We decided to check the results obtained by Lewis and Figure 5 represents the data obtained from an open-chest experiment using a dog anesthetized with pentobarbital. Small copper wire electrodes were clamped to the tip of the right auricle. The stimulator was a square wave type with which the rate and voltage could be independently controlled. The effects of the uninhibited vagus, atropinization and intravenous quinidine gluconate were determined.

The above experiment correlates closely with experiments on the isolated auricle, but more importantly, it emphasizes the role of the vagus, and acetylcholine in the mechanism of auricular fibrillation. Vagal stimulation or the injection of acetylcholine are important factors in experimental and clinical auricular fibrillation in that they perpetuate and increase the rate of ectopic foci discharge by their ability to decrease the stimulatory threshold of auricular muscle. Digitoxin also has this same effect and probably explains why patients with auricular flutter will often convert to auricular fibrillation when they are digitalized.

This raises the question of the validity of digitalis therapy in the treatment of auricular flutter and fibrillation. It is true that the ventricular rate is slowed when a flutter patient is digitalized but only because he is now in fibrillation or his vagus is stimulated

Figure 5. The Auricular Stimulatory Threshold in an Open-Chest Dog Preparation and Its Alteration by Atropine and Quinidine.

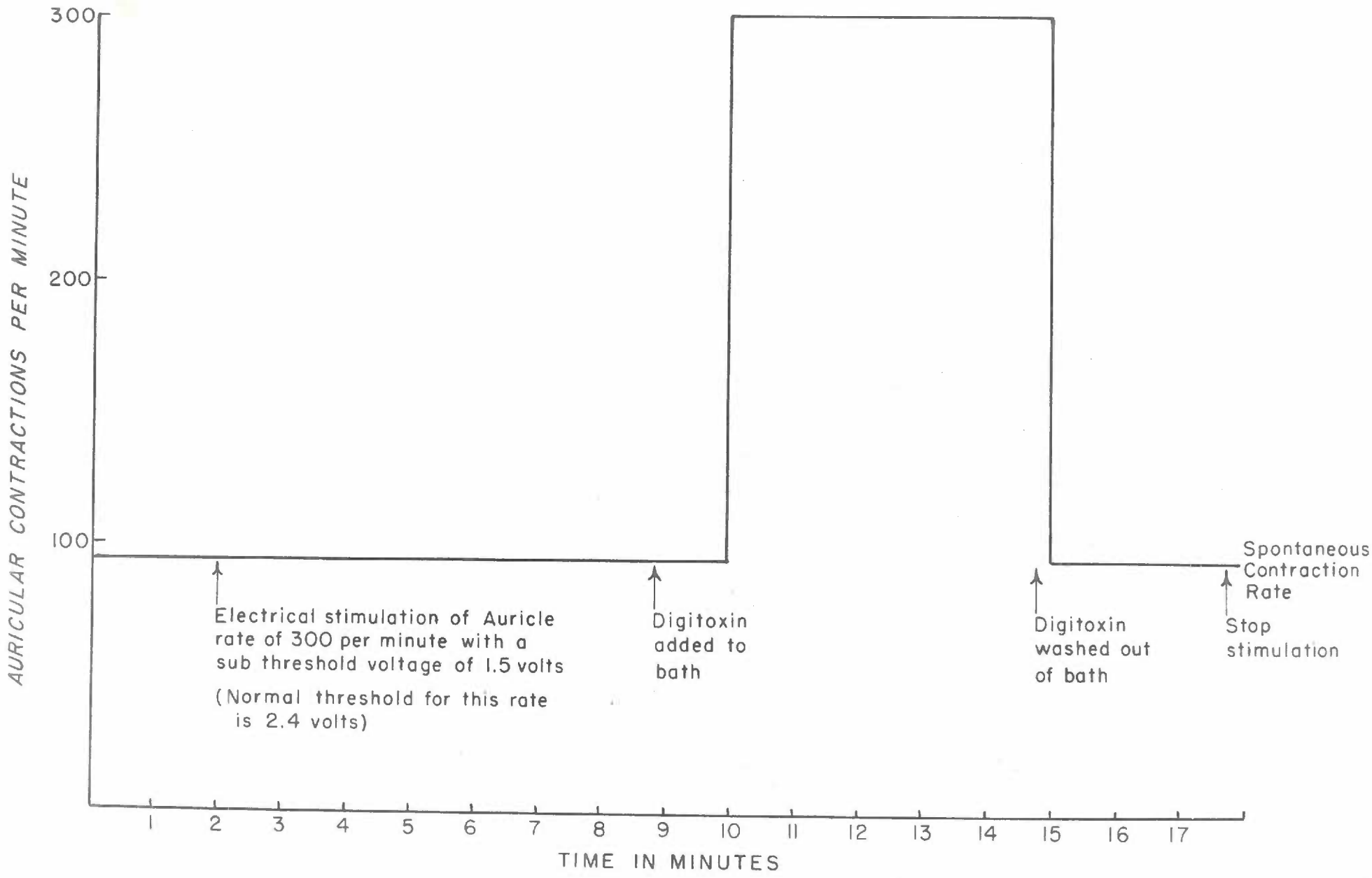

Atropinized Animal
Non-Atropinized Animal
Non-At. + Quinidine
Atrop. + Quinidine
STIMULATORY THRESHOLD
2.0
1.8
1.6
1.4
1.2
150
160
170
180
190
200
210
220
230
240
250
RATE OF STIMULATION

and the S-A node depressed. Many of these patients will revert to a normal rhythm when the digitalis is stopped. This may be explained by the increase in the stimulatory threshold that takes place when digitalis is removed from the isolated auricle bath or from the blood of the fibrillating patient! This can be more clearly demonstrated by Figure 6 which shows the effects of digitalis on the stimulatory threshold of the isolated rabbit auricle.

It was observed that when the auricle was placed in Tyrode's solution it contracted at a spontaneous rate of 92 beats per minute. Then if the auricle was stimulated at 300 beats per minute with a sub-threshold stimulus, in this case 1.5 volts were used with an auricle that had a normal threshold of 2.4 volts, no change in rate was noted. Shortly after digitoxin was added to the solution the threshold of the auricle decreased to the extent that the electrical stimulus now became effective and the rate of auricular contraction was the same as the electrical pacemaker. When the digitoxin was washed out of the bath, the rate of auricular contraction dropped to its original spontaneous rate. When the electrical stimulus was cut off, no change in rate resulted.

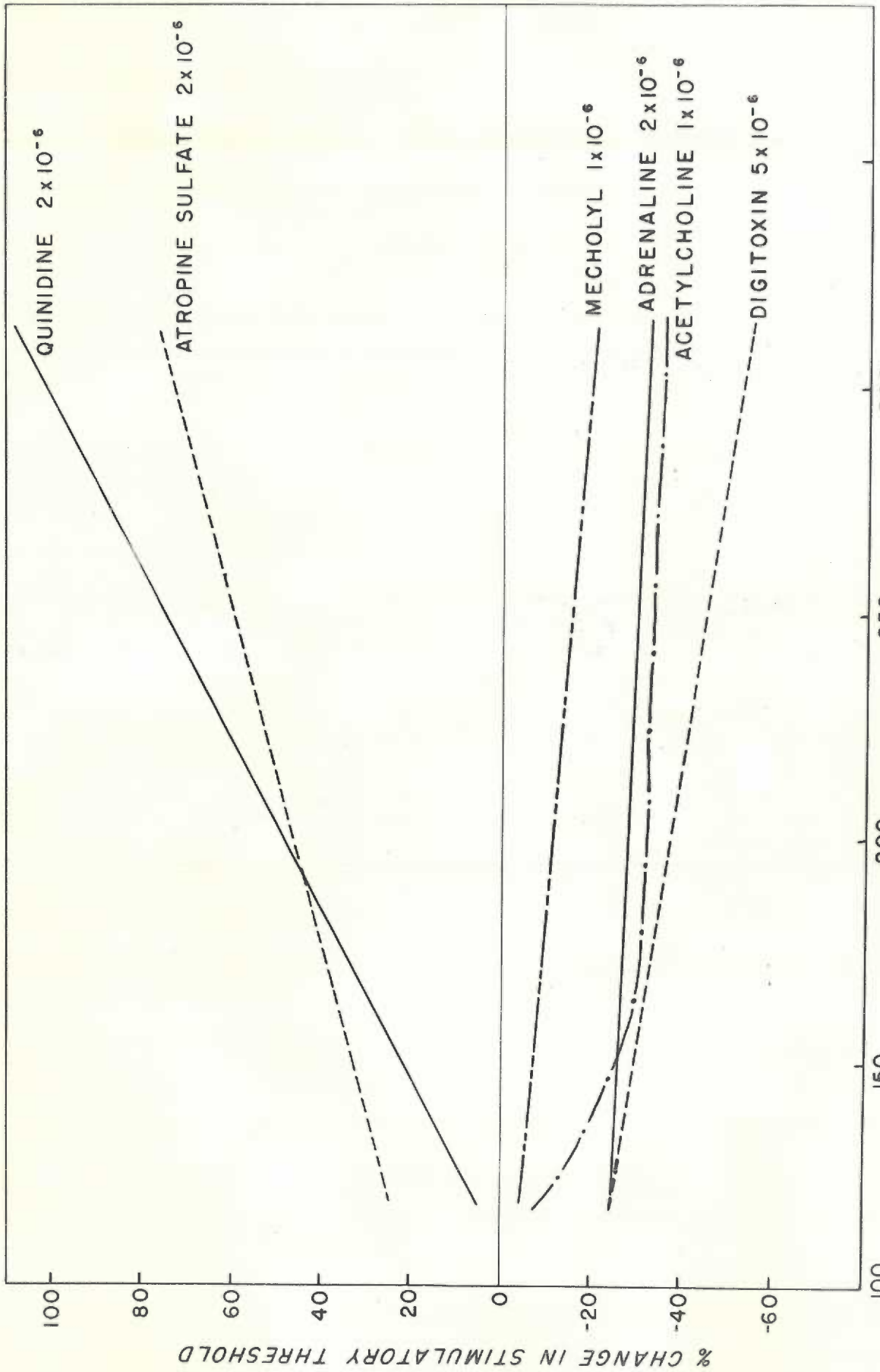
The importance of this experiment can only be appreciated if one realized the relationship of ectopic stimuli formation to the stimulatory threshold of the surrounding auricular tissue (see Figure 3). In a condition

Figure 6. The Effect of Digitoxin on the Stimulatory Threshold of the Isolated Rabbit Auricle.


AURICULAR CONTRACTIONS PER MINUTE
300
200
100
Electrical stimulation of Auricle rate of 300 per minute with a sub threshold voltage of 1.5 volts
(Normal threshold for this rate is 2.4 volts)
Digitoxin added to bath
Digitoxin washed out of bath
Spontaneous Contraction Rate
Stop stimulation
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
TIME IN MINUTES

such as auricular flutter or fibrillation where ectopic foci are responsible for the disorder of rate and rhythm, the effect of digitalis, vagal stimulation, acetylcholine, or epinephrine is to lower the stimulatory threshold and allow more rapid discharge of the ectopic foci. When such drugs as quinidine, atropine or Benadryl are used then the stimulatory threshold is increased (see Figure 7) and the rate of discharge from the ectopic foci is decreased. In most cases the threshold is increased to the extent that the only focus of sufficient potential to be an effective stimulus is the pacemaker in the sinoauricular node and normal rhythm takes the place of the previous arrhythmia.

Figure 7. The Effects of Quinidine, Atropine, Mecholyl, Adrenaline, Acetylcholine and Digitoxin on the Stimulatory Threshold of the Isolated Rabbit Auricle.


QUINIDINE 2x10⁻⁶
ATROPINE SULFATE 2x10⁻⁶
MECHOLYL 1x10⁻⁶
ADRENALINE 2x10⁻⁶
ACETYLCHOLINE 1x10⁻⁶
DIGITOXIN 5x10⁻⁶
% CHANGE IN STIMULATORY THRESHOLD
100
80
60
40
20
0
-20
-40
-60
100
150
200
250
300
350
RATE OF STIMULATION

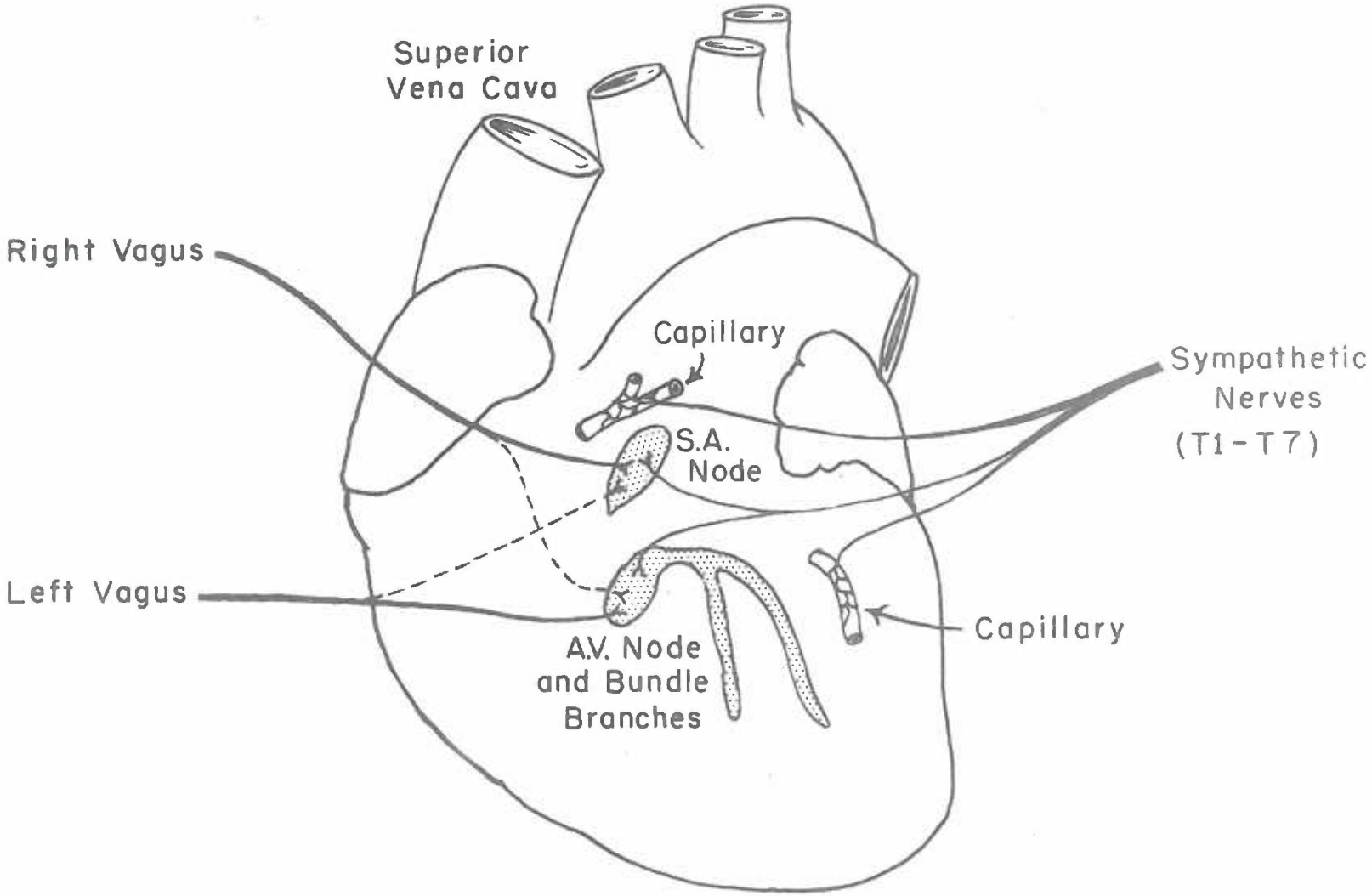
THE ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN THE GENESIS OF AURICULAR FIBRILLATION

Functionally and anatomically the autonomic innervation of the heart is separated into sympathetic and parasympathetic influences (see Figure 8). While the property of rhythmicity is inherent in cardiac muscle, it is the proper inter-play of these influences that regulate the rhythm of the heart. It must be assumed therefore, that in any disturbance of cardiac rhythm the importance of these influences cannot be overlooked.

PARASYMPATHETIC INFLUENCES

It has long been recognized that auricular fibrillation, clinical and experimental, may be precipitated by an increase in the tone of the vagus or by the use of vagomimetic drugs. Andrus and Carter in 1930 reported a shortening of the refractory period and a reduction of the fibrillatory threshold of the dog's auricle with stimulation of the vagus. (34) Actually Lewis in 1921 showed the same effects (15) but was not cognizant of it at the time. It has been demonstrated that the application of Mecholyl directly to the surface of the auricle of anesthetized open-chest dog preparation will produce auricular fibrillation. (35, 36) Nahum and Hoff have shown that intravenous Mecholyl will produce auricular fibrillation in normal patients, (37)

Figure 8. Autonomic Innervation of the Heart.


Superior
Vena Cava
Right Vagus
Capillary
Sympathetic
Nerves
(T1-T7)
S.A.
Node
Left Vagus
Capillary
A.V. Node
and Bundle
Branches

in hyperthyroid patients, [38] and in patients with auricular flutter. [35] The fibrillation that follows the administration of toxic doses of digitalis may be due in part to increased vagal tone. [39]

The evidence to date leaves little room for doubt that increased vagal stimulation does not play an important role in the genesis of auricular fibrillation. Let us therefore examine in some detail, the changes that take place in the auricle when there is a progressive increase in the amount of vagal stimulation or progressively larger doses of a vagomimetic drug are injected (See Figure 14).

1. An increase in auricular conduction time (prolonged P-R interval) is one of the first electrocardiographic changes that take place under the influence of vagal stimulation. With very small doses of intravenous acetylcholine or a slight amount of vagal stimulation, it may be the only noticeable change. [40] Except for the creation of local blocks of irritability as suggested by Di Palma [41] the action of increased conduction time on the mechanisms involved in auricular fibrillation is not fully understood. It is recognized, however, that almost all cases of clinical and experimental auricular fibrillation are preceded by an increase in the P-R interval. [22, 27, 28, 30]

2. Formation of A-V blocks is the next electrocardiographic change observed when acetylcholine is injected

into the experimental animal. Slightly larger doses are required to produce A-V block than are necessary to effect a prolongation of the P-R interval. (40) This change in almost all cases precedes complete inhibition of impulse formation at the sino-auricular node and probably represents but one of the several progressive changes that lead eventually to auricular fibrillation if the dose of acetylcholine is large enough.

3. Slowing of impulse formation at the sino-auricular node seems to depend chiefly on the right vagus, although it is possible that some fibers from the left vagus also innervate the sino-auricular node. (42, 43) Grant in 1949 reported that stimulation of the right vagus was six times as effective as the left vagus in producing auricular fibrillation in dogs. The innervation of the sino-auricular node by the right vagus may well explain this observation. (44) The importance of the inhibition of the sino-auricular node is recognized but not fully understood. We have shown in the section on experimental auricular fibrillation that one of the most significant electrocardiographic changes that takes place when auricular fibrillation is induced in dogs is first slowing and then complete inhibition of the normal P waves. This has been observed by other workers (41, 44) and is well recognized as one of the changes that precedes clinical and experimental auricular fibrillation. (22, 27, 28, 30) We have suggested previously that

initiation of auricular fibrillation depends upon the existence of effective ectopic foci in the auricles and complete inhibition of stimulus formation at the sino-auricular node.

4. The production of auricular fibrillation is the terminal change that takes place in the auricle if there is a sufficient increase in the vagal tone or if large enough doses of acetylcholine are injected into dogs (40, 43) or human subjects. (37, 38) As was mentioned previously, the mechanism by which auricular fibrillation is initiated has been the subject of much investigation and speculation. Present controversies center chiefly around the question of whether fibrillation is initiated and perpetuated by a single, rapidly firing focus, multiple foci, or a self-sustaining re-entrant phenomenon termed the circus movement.

Recently, Prinzmetal and his group have by a series of experiments where they cut or block the hypothetical path (39) disproved for all practical purposes the circus movement theory of auricular fibrillation. Acceptance of these tenets leaves us with no other alternative than to accept the ectopic foci theory.

It has been demonstrated that when experimental auricular fibrillation is produced by a single focus or two separate foci, the electrocardiographic picture is the same. (39) These workers concluded that as there was no difference

in the electrocardiographic and cinematographic recordings of these two experiments, that auricular fibrillation is, therefore, initiated and perpetuated by a single ectopic focus.

While we accept the ectopic foci theory as the basis of initiation and perpetuation of auricular fibrillation, we do not believe there is conclusive evidence in favor of the single focus over the multiple foci. It is difficult to see how one can postulate a single focus when a drug such as aconitine is injected into the auricular tissue. What is to prevent the drug from spreading to other parts of the auricle, either directly or hematogenously, thereby giving rise to multiple foci? For this reason we will assume in this paper that auricular fibrillation is due to the presence of ectopic foci. However, the views which we will present can just as well be applied to the single focus if subsequent experimental evidence disproves the multiple ectopic foci theory.

As was previously mentioned, two factors must be present before ectopic foci in the auricle may become responsible for auricular arrhythmias. First the foci must be present in the auricle and second, the stimulatory threshold of the auricle must be low enough so that these foci are able to produce effective stimuli. Normally the threshold of the auricle is high enough so that even if ectopic foci are present, the sino-auricular node is the only focus

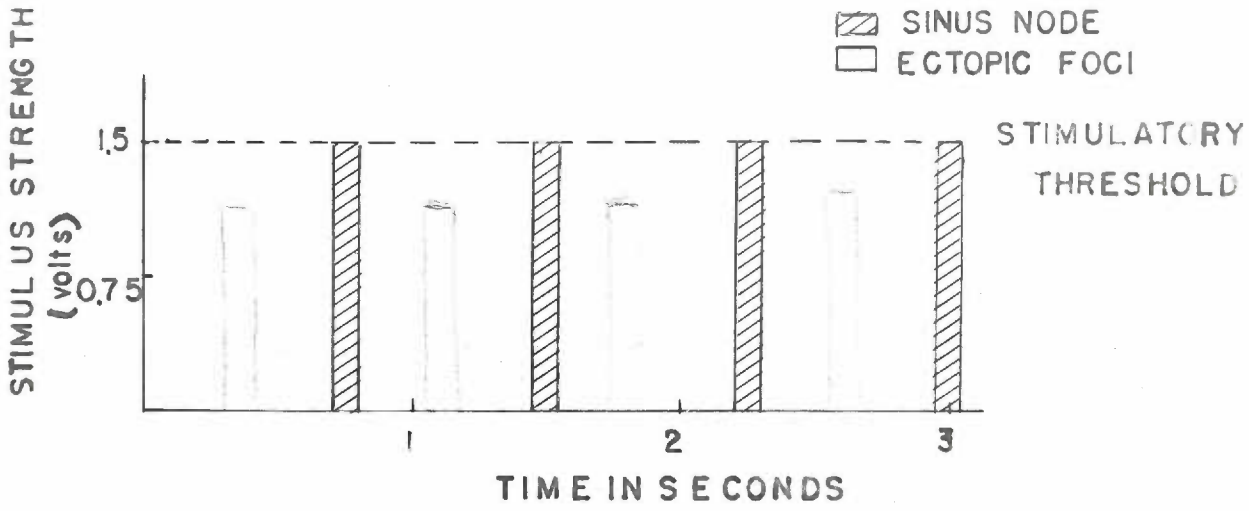
capable of producing potentials of sufficient intensity to be a pacemaker. We suggest that the role of the vagus in the genesis of auricular fibrillation is to lower the stimulatory threshold of the auricle so that these ectopic foci if present under certain circumstances, are now brought into play. It has been shown experimentally that acetylcholine or vagal stimulation frequently leads to the discharge of volleys of rapid impulses in isolated auricular tissue.(46, 47) Two possible explanations are offered for these observations. First it may indicate that the foci were present all the time and were sub-threshold until acetylcholine lowered the stimulatory threshold, or it may well be that the presence of the foci as well as the lowered stimulatory threshold were a result of the drug. Most of the available evidence points to the former as being the most probable explanation. The presence of the ectopic foci in the auricular tissue is more than likely due to another factor such as thyrotoxicosis (38) or epinephrine release.(39)

Lewis and Master in 1925 provided us with experimental evidence that the sensitivity of specific tissue such as the S-A and A-V nodes are less influenced by the vagus than is the auricular muscle.(48) Weston and McCawley in 1952 observed that this increase of auricular sensitivity (lowered stimulatory threshold) is directly proportional to the rate of impulse formation.

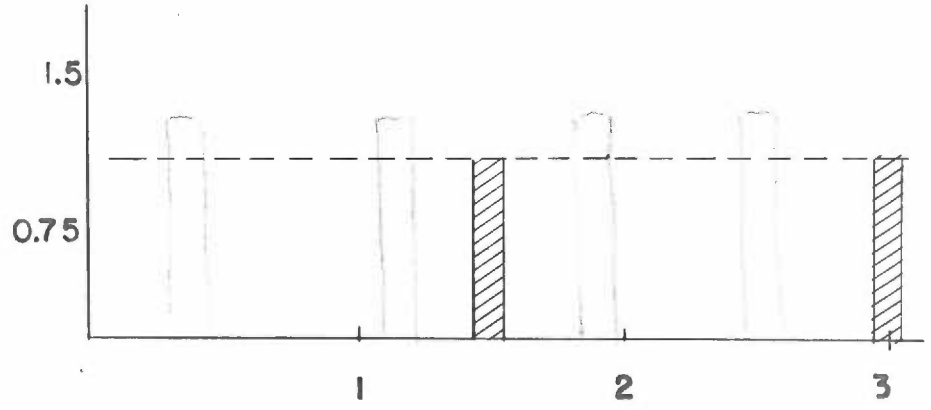
Scherf has shown that it is possible to produce auricular tachycardia of approximately 200-300 beats per minute by the injection of 0.03 c/c of a 0.05% solution of aconitine into the walls of the auricles. (50, 51) The tachycardia appeared within a period of two minutes and lasted about sixty minutes. Faradic stimulation of the vagus during this time increased the rate by as much as 100 per cent and caused some animals to fibrillate. Cooling abolished the tachycardia and changed the fibrillation into a tachycardia. They were unable to terminate this auricular tachycardia with strong faradic stimulation of the vagus. While clinically one is able to terminate nodal auricular tachycardia with vagal stimulation, it is impossible to break auricular flutter or fibrillation. The reason for this lies in the fact that auricular tachycardia, if it is of sino-auricular origin, is slowed due to the inhibitory effect of the vagus upon the sino-auricular node but flutter and fibrillation, which are due to ectopic foci, are not inhibited by vagal stimulation but seem to be perpetuated by it. Figure 9 shows the action of vagal stimulation on the stimulatory threshold and S-A node of the isolated rabbit's auricle.

It may be observed that normally the sino-auricular node (shaded area) beats at 60-90 beats per minute and that the stimulatory threshold is approximately 1.5 volts.

Figure 9. The Effects of Acetylcholine on the Normal Stimulatory Threshold and the S-A Node of the Isolated Rabbit Auricle.



CONTROL



ACETYLCHOLINE

Any ectopic foci (unshaded area) that may be present are unable to stimulate the auricle because they are not of threshold intensity. Under the influence of vagal stimulation or vagomimetic drugs the sino-auricular node is slowed. The stimulatory threshold of the auricle is decreased so that ectopic foci if present are now of threshold intensity and auricular arrhythmias result. It might be assumed then, that if we wished to produce auricular fibrillation experimentally or clinically, all that would be required is an increase in vagal tone (carotid sinus massage, ocular pressure, induction of vomiting, Valsalva or allied procedures) or the intravenous injection of acetylcholine.

Unfortunately, the problem is not as simple as that. In our experience, only about 30-40 per cent of dogs will fibrillate when doses up to 1-2 mg/kg of acetylcholine are given intravenously. (40) When doses larger than this are given, the animals usually die of respiratory arrest. This is in agreement with Horlick and Surtshin (45) who produced fibrillation in about 50% of dogs tested with similar doses of acetylcholine. Nahum and Hoff (37) were able to produce auricular fibrillation in three of seventeen normal human subjects with acetylcholine. However, Starr et al (52) did not report a single instance of auricular fibrillation in humans who were given methacholine.

Therefore we are faced with the logical conclusion

that there is a second factor concerned with auricular fibrillation which seems to be missing in most normal humans, and approximately fifty per cent of normal dogs but is present in thyrotoxic humans.(38) in hyperthyroid dogs (53), in anemic humans (27) and in anemic dogs. (45)

Nahum and Hoff have suggested the term "Excitatory Factor" for this second influence involved in auricular fibrillation, (38). They suggest that it might be thyroid hormone, while Grant offers the more acceptable thesis that it is the action of epinephrine. (44)

SYMPATHETIC INFLUENCE

While it was shown by De Elio in 1947 that epinephrine will lower the stimulatory threshold slightly and decrease the refractory period, it is not as effective as vagal stimulation or vagomimetic drugs, therefore, its role in the genesis of auricular fibrillation must be entirely different than the vagus. (54) As its absence almost precludes the possibility of inducing experimental fibrillation, (45) we would like to postulate a major role for the cardiostimulatory nerves in the genesis of that arrhythmia.

Grant et al (44) have shown that epinephrine will greatly increase the likelihood of discharge from ectopic foci. The importance of an ectopic foci in the genesis of auricular fibrillation has been demonstrated by

Prinzmetal et al [18, 19, 39] and Van Dongen.[55, 56] The latter in his work has shown that the antifibrillatory potency of the various drugs is directly proportional to their capacity to prevent heterotopic rhythms rather than with their ability to alter the refractory period or conduction time of auricular muscle. This fact has been substantiated by McCawley et al. [57] It is therefore only reasonable to assume that if drugs which inhibit heterotopic rhythms are antifibrillatory drugs, then those drugs which facilitate discharges from ectopic foci such as epinephrine, are fibrillatory drugs. Thus while vagal stimulation with a lowering of stimulatory threshold is one factor in the genesis of auricular fibrillation, a second, and equally important factor, is the initiation or facilitation of ectopic foci by the cardio-accelerator nerves.

Admittedly, we have no direct proof as to this supposed action of the sympathetic fibers to the heart, but let us examine the indirect proof which is available to us. It has been demonstrated that auricular fibrillation is in some way related to tachycardia.[18, 19, 39] It is usually found clinically in individuals who, if they were not fibrillating, would have a tachycardia. [58] (Patients with cardiac failure, mitral valve disease, anemia, and thyrotoxicosis all fall into this category and account for most of the clinical auricular fibrillation.) It has been suggested that those individuals without organic heart

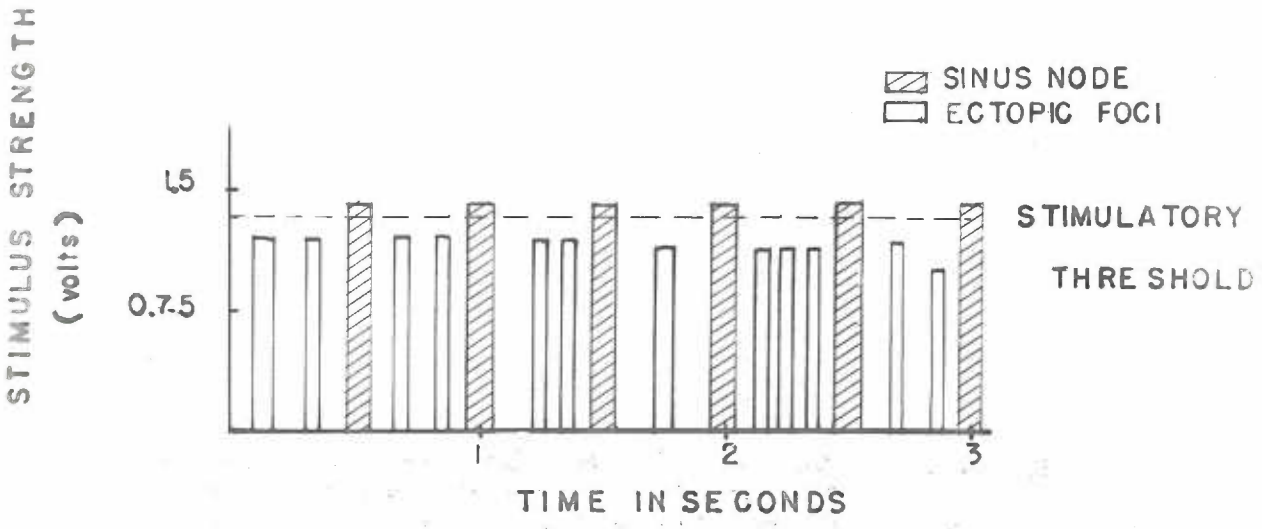
disease, but who have auricular fibrillation, are for the most part, emotional individuals with fast heart rates. (41)

Smith and Moody in 1923 reported epinephrine-incited fibrillation in two patients who had previously exhibited either auricular fibrillation or extrasystoles on forced breathing (vagal stimulation). (59) Otto in 1927 showed that intravenous injections of very small doses of epinephrine frequently caused fibrillation in normal elderly patients. (60) Atropine would prevent this, so the vagus nerve also was involved. Cowan and Ritchie list epinephrine as one of the many agents which may induce auricular fibrillation in intact animals or in perfused hearts. (61) Winterberg was unable to induce auricular fibrillation by means of electrical stimulation of the vagus in cats, but when he added epinephrine or calcium salts, he was quite successful. (62) In 1949 Grant reported on a series of experiments with dogs in which he found that the most effectual method of producing experimental auricular fibrillation was to combine an intravenous epinephrine drip with simultaneous vagal stimulation. (44) When the vagus was blocked with atropine, he was unable to get fibrillation and in only one case was he able to get fibrillation with vagal stimulation alone. In 1933, Rosenblum et al showed that hyperthyroid dogs and rabbits would fibrillate when given small intravenous doses of epinephrine. The dose of epinephrine was so small that it did not disturb the cardiac rhythm in normal animals. (63)

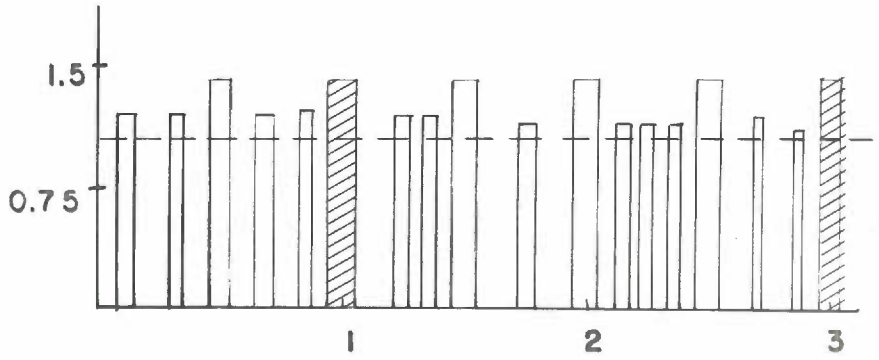
Recently it was shown that vagal stimulation (64, 65) or tissue anoxia (65) will cause the release of an epinephrine-like substance in the heart. Therefore, it is exceedingly difficult to separate the influences of parasympathetic and sympathetic innervation of the heart, but at this time, the evidence is irrefutable that both play major roles in the genesis of auricular fibrillation. In review, it appears that auricular fibrillation depends upon the co-existence of sympathetic and parasympathetic influences on the auricle. While either acetylcholine or epinephrine will produce isolated cases of auricular fibrillation, the consistant production of auricular fibrillation in experimental animals depends upon acetylcholine or vagal stimulation in addition to epinephrine or epinephrine-potentiating states such as thyrotoxicosis.(44, 45, 49

We have attempted to show the action of epinephrine in the genesis of auricular fibrillation by a series of experiments with isolated rabbits' auricles, (see Figure 10). It was demonstrated, that while epinephrine lowered the stimulatory threshold slightly, it was much less effective than similar concentrations of acetylcholine, (see Figure 9). The rate of firing of the S. A. node was increased from a normal of 60-80 to 140 per minute. Visually there were no other changes in the auricle. We postulated, however, that there were some changes present that we were unable to measure at that time, namely the initiation of

Figure 10. The Effects of Epinephrine, Alone and in Combination with Acetylcholine on the Normal Stimulatory Threshold, Electrical Activity, and the S-A Node of the Isolated Rabbit Auricle.



EPINEPHRINE



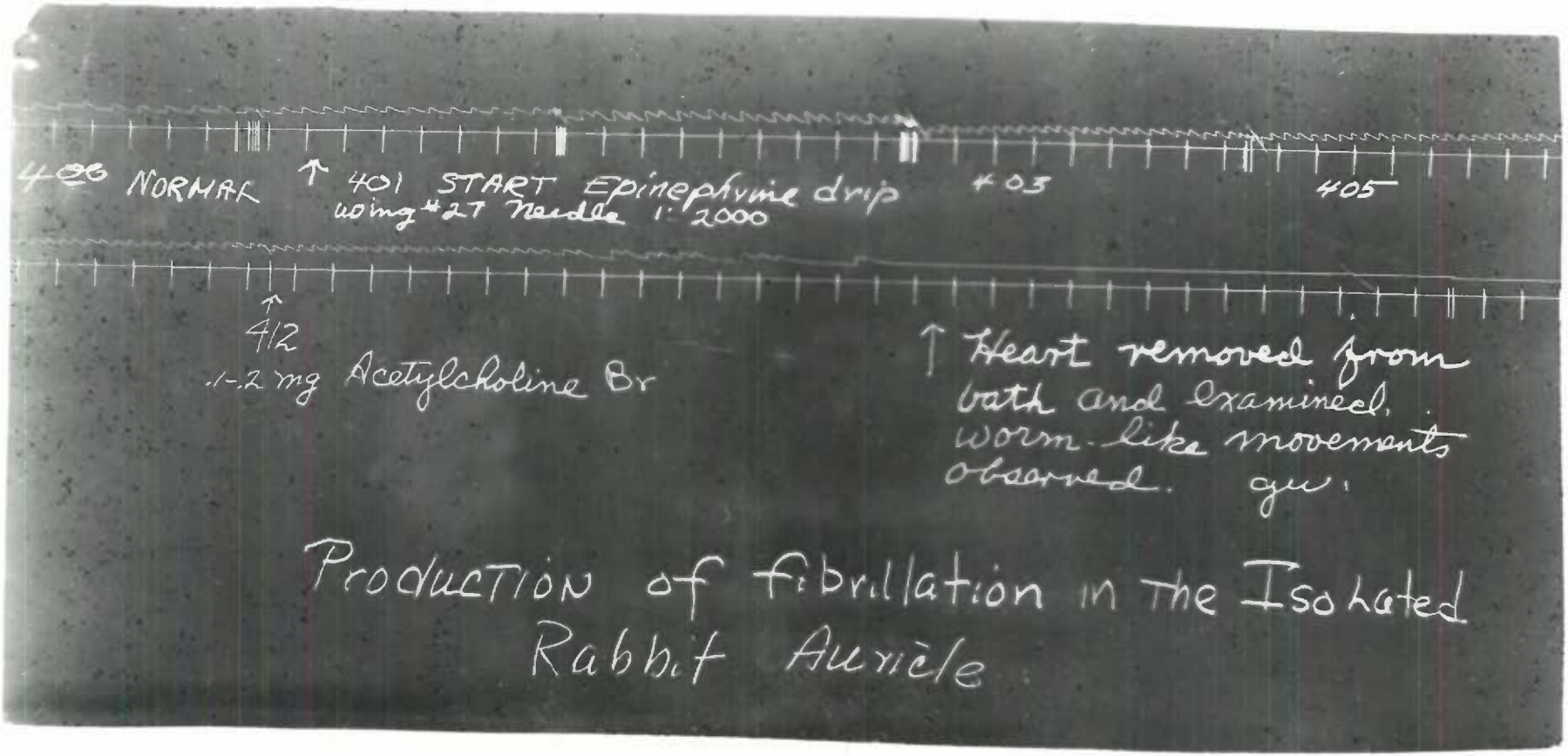
ACETYLCHOLINE + EPINEPHRINE
(auricular fibrillation ?)

subthreshold stimuli from ectopic foci. In order to prove that these subthreshold stimuli were present, it became necessary to devise a method of making them threshold. This was done by adding acetylcholine to the bath in order to lower the stimulatory threshold (see Figure 11).

At the onset of the experiment, the auricle was contracting at a regular spontaneous rate of 80-85 per minute. When the normal rate and rhythm had been determined, epinephrine was dripped into the bath at a constant rate. Within a period of 5-6 minutes this rate was increased to 168 per minute. Eleven minutes after the start of the continuous epinephrine drip, 0.1 to 0.2 mg of acetylcholine were added to the bath. The rate became slower and the rhythm irregular. (Notice the similarity between this rhythm and clinical auricular fibrillation). After a few seconds of irregular irregularity, the effective contractions of the auricle ceased. When the auricle was then removed from the bath and observed with a magnifying glass, it was found to be in a chaotic state of motion quite similar to that described by Prinzmetal et al (39) when they produced auricular fibrillation by means of an aconitine focus.

The chaotic movement of the isolated auricle (auricular fibrillation) could not be produced by the individual action of either epinephrine or acetylcholine, but required the simultaneous action of these two

Figure 11. Fibrillation Produced in the Isolated Rabbit Auricle by Acetylcholine and Epinephrine.



Production of fibrillation in the Isolated Rabbit Auricle

neuroeffector-hormones. We are, therefore, left with the conclusion that each hormone has a separate and equally important role in the genesis of auricular fibrillation. The vagal hormone (acetylcholine) lowered the stimulatory threshold of the auricle while the cardio-stimulatory hormone (epinephrine) initiated multiple ectopic foci.

It is of interest that Prinzmetal et al (39) reported minute complexes of an estimated 50 to 500 millimicrovolts in amplitude which occur at the astonishingly high frequency of 7,000 to 20,000 per minute in direct lead oscillograms of experimentally produced auricular fibrillation. They state that such deflections are too weak and too rapid to be recorded by standard electrocardiographic equipment. Larger deflections corresponding to the "f" waves of the electrocardiogram varying from 3 to 10 milli-microvolts in potential are inscribed at rates of 500 to 1000 per minute. Smaller intermediate complexes are described and it is felt that this form of electrical activity probably is responsible for the irregular baseline of the "f" waves seen in the limb leads of clinical auricular fibrillation. None of the above electrical activity is found in normal tracings, thus must result from one of the initiating factors of auricular fibrillation. Knowing the stimulatory effect of epinephrine on the pacemaker and the fact that epinephrine often is the cause of ventricular fibrillation (41) it is not difficult to postulate that the same

stimulatory effect is present in auricular tissue and accounts for these ectopic foci.

Stotler and McMahon [43] have demonstrated that in addition to the sympathic nerve endings in the S-A and A-V nodes, there are fibers that terminate around the capillaries scattered throughout the myocardium. Stimulation of the sympathetic nerves to the heart results, therefore, in stimulation of the S-A and A-V nodes and in addition, epinephrine is released around the myocardial capillaries. It is not difficult to picture these multiple intravascular injections of epinephrine into the myocardium as a source of ectopic stimuli. In all probability under normal circumstances, these stimuli are subthreshold and remain unsuspected and asymptomatic. But in conditions where there is concomitant vagal stimulation to lower the stimulatory threshold of the auricle they become threshold stimuli and auricular fibrillation develops.

THE ROLE OF TEMPERATURE, ANOXIA, ANEMIA, DIGITALIS, THYROTOXICOSIS AND ORGANIC HEART DISEASE IN THE GENESIS OF AURICULAR FIBRILLATION

It is well recognized that temperature, anoxia, digitalis, thyrotoxicosis and organic heart disease are important factors in the genesis of clinical and experimental auricular fibrillation. Each of these factors will be discussed separately and in some cases experimental evidence will be included with the discussion. Most of the experiments we have done are on the isolated rabbit auricle and a description of the methods used will be found in the section on methods of testing antifibrillatory drugs.

HYPOTHERMIA

Dill and Forbes have shown that human subjects which are chilled to 30° C often have runs of spontaneous auricular fibrillation.(67) They were unable to demonstrate that the fibrillation was a result of the hypothermia and finally concluded that it was not the hypothermia, but the concomitant anoxia that was responsible for the initiation of the auricular fibrillation. Clinical fibrillation in hypothermic conditions also has been reported by Wayburn (68), Talbott (69) and Grosse-Brockhoff.(70) Most of these workers agree that cold per se does not potentiate the auricle for fibrillation but that anoxia or reflex vagal stimulation are the responsible factors. While

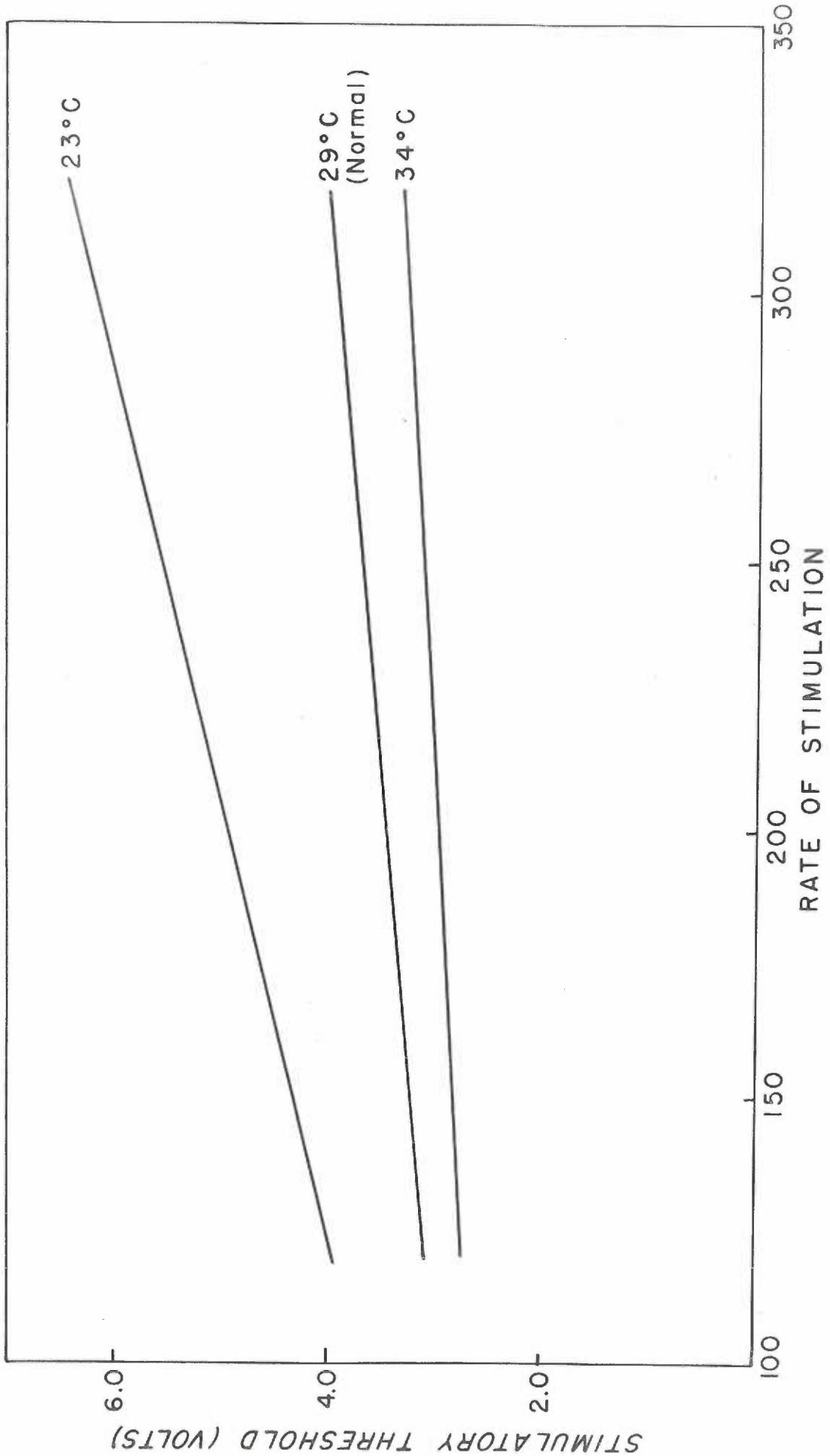
Grosse-Brockhoff [70] favors the hypothermia of auricular muscle as the inciting cause of the fibrillation, his views are not in agreement with the experimental evidence offered by other workers in this field.

It has been demonstrated that cooling auricular muscle prolongs its relative refractory period [71] and that experimental fibrillation may be terminated by perfusing the heart with a cool fluid [13] or by cooling an ectopic foci with CO_2. [19, 39, 50, 51]

The effects of temperature on the auricle cannot be measured with any degree of accuracy in the experimental animal because of the uncontrollable effects of blood supply and autonomic innervation. The isolated auricle technique obviates these interferences and is therefore the method of choice in experiments of this type.

Figure 12 shows the effect of hypothermia and hyperthermia, on the stimulatory threshold of the isolated rabbit auricle. The normal threshold measured at 29° C varies from 3-4 volts depending on the stimulatory rate. This threshold is increased when the bath is cooled to 23° C and decreased when bath is warmed to 34° C. This readily explains why Scherf [50, 51] and Prinzmetal et al [19, 39] were able to slow the rate of discharge from an aconitine ectopic focus by cooling and increasing the rate by warming, for as we have shown before, the rate of firing of an ectopic focus depends on the stimulatory threshold of

Figure 12. The Effects of Temperature Changes On the Normal Stimulatory Threshold of the Isolated Rabbit Auricle.


23°C
29°C
(Normal)
34°C
6.0
4.0
2.0
STIMULATORY THRESHOLD (VOLTS)
100
150
200
250
300
350
RATE OF STIMULATION

the surrounding auricular muscle.

In review, the action of hypothermia may be direct depression of an auricular muscle with an elevation of the stimulatory threshold or it may in the intact animal cause a reflex vagal stimulation and a decrease in the stimulatory threshold.

HYPERTHERMIA

The mode of action of hyperthermia is just the opposite of hypothermia and quite similar to vagal stimulation. Scherf (50, 51) produced a tachycardia of 200-300 beats per minute in the dog's heart by injecting aconitine into the auricle. The rate was increased by as much as 100 per cent by vagal stimulation or by warming the auricle. It was abolished by cooling the auricle.

ANOXIA

In the genesis of auricular fibrillation the action of anoxia is two-fold. Anoxia results in reflex stimulation of the autonomic nervous system in the intact animal and in man, (31) while mild anoxia of the isolated rabbit auricle results in a decrease of the stimulatory threshold much in the same manner as hyperthermia, digitalis, vagal stimulation and acetylcholine. (40) Prolonged anoxia, however, will result in a progressive increase in the stimulatory threshold until the auricle stops beating.

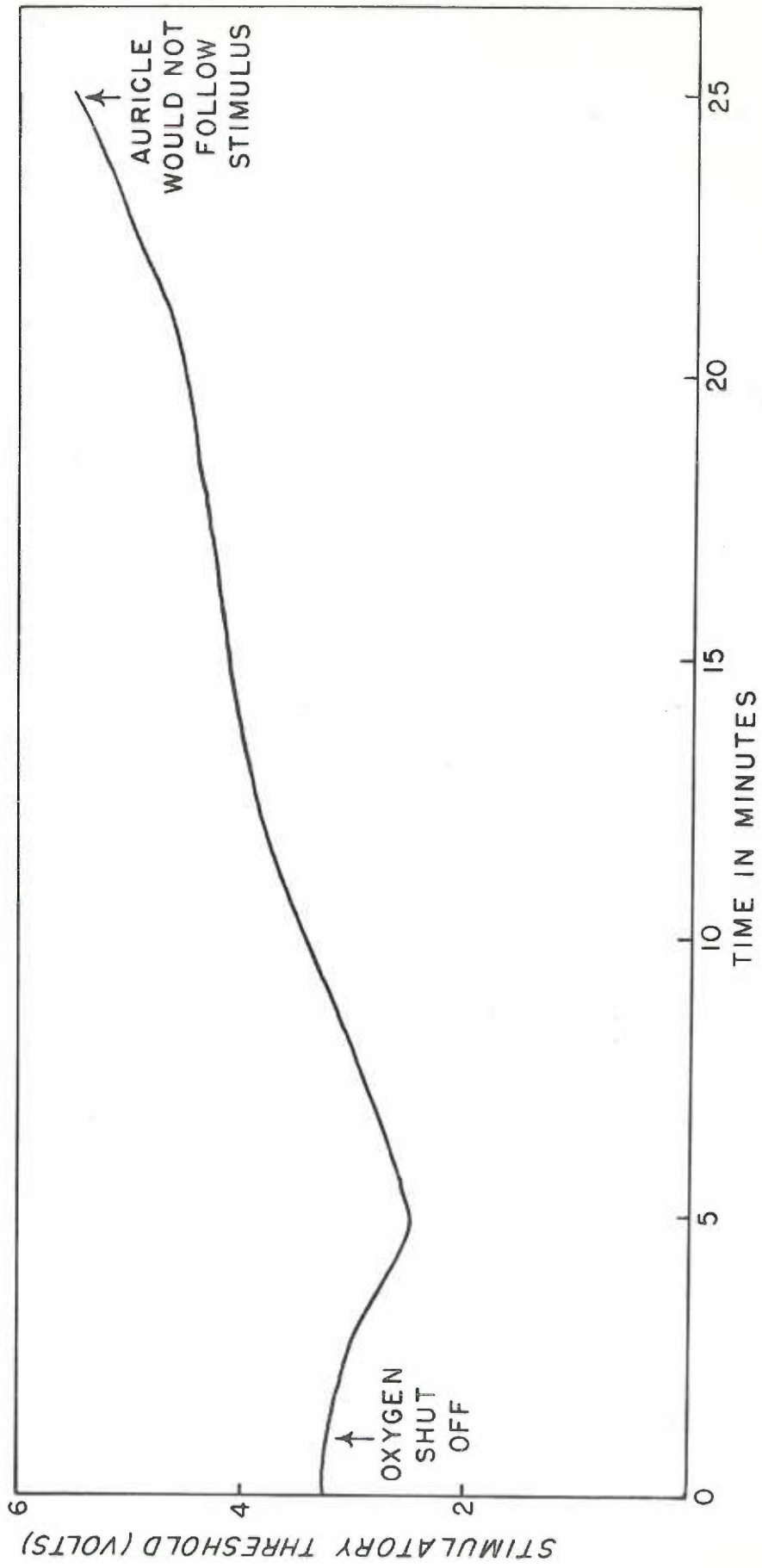
Figure 13 shows the effect of mild and severe anoxia on the isolated rabbit auricle. The normal threshold was determined for a rate of 240. At this point the oxygen supply was shut off. Initially it is observed that the threshold decreased for a period of 5-10 minutes but as the anoxia became more complete the threshold increases until the auricle ceases to follow the stimulus. This may explain why fibrillation may be present in those clinical conditions which show a moderate degree of anoxia such as anemia and cardiac failure.

It has been observed that in the experimental animal, anoxia frequently converts aconitine-induced flutter into fibrillation. (39) The presence of anoxia has also been found to favor the production of auricular fibrillation by cholinergic drugs (37) and may be a factor in post-operative fibrillation. The fact that auricular fibrillation is uncommon in patients with anoxia resulting from severe emphysema or congenital heart disease may be attributable to the chronicity of the anoxia in such instances.

ANEMIA

Schlichter in 1949 reported several cases of induced auricular fibrillation in anemic patients which were tested with small intravenous doses of acetylcholine.(27) This fibrillation reverted to normal sinus rhythm in all

Figure 13. The Effects of Anoxia on the Normal Stimulatory Threshold of the Isolated Rabbit Auricle.


AURICLE
WOULD NOT
FOLLOW
STIMULUS
OXYGEN
SHUT
OFF
STIMULATORY THRESHOLD (VOLTS)
6
4
2
0
5
10
15
20
25
TIME IN MINUTES

patients when the red blood count was returned to normal by transfusion with whole blood. This was the first reported evidence that anemia plays an important role in the genesis and perpetuation of auricular fibrillation.

During the same year, Horlick and Surtshin demonstrated that it was possible to standardize the minimal intravenous dose of acetylcholine necessary to produce second degree A-V block in unanesthetized dogs. (29) While there was wide variability in the "block dose" among different animals, the "block dose" for any one animal remained constant over long periods of time. When they injected doses of acetylcholine 10-20 times larger than the "block dose" they were able to produce auricular fibrillation in about fifty per cent of the animals.

After the animals were made anemic, the "block dose" and the fibrillating dose of acetylcholine were both reduced. Half of those animals who previously did not fibrillate with 10-20 times the "block dose", fibrillated when that dose was given, after the development of anemia. In all cases the "minimal block dose" paralleled the hemoglobin concentration of the blood.

Smith and Wilson perfused the coronaries of dogs with anoxic blood and at the same time injected intravenous Mecholyl. By this method they were consistently able to produce auricular fibrillation where previously neither procedure used alone had succeeded. (66) The only logical

conclusion is that anemia or tissue anoxia potentiates the action of the vagus or vagomimetic drugs in producing auricular fibrillation. We have previously shown that a mild degree of tissue anoxia decreases the stimulatory threshold of the isolated rabbit auricle. This is in complete agreement with Resnik (72) who feels that early anoxia predisposes to auricular fibrillation but that severe anoxia inhibits fibrillation. This is substantiated by the fact that persons dying anoxic deaths do not regularly develop terminal fibrillatory states. (22) It is also reasonable to assume that anoxia in the fringe area of myocardial infarction must have some effect on the irritability and production of ectopic foci. (73)

In summary, anemia through the mechanism of tissue anoxia is an important factor in the genesis of some cases of auricular fibrillation as it lowers the stimulatory threshold of the auricle. Ideally the treatment of such cases would be the treatment of the anemia and not the use of antifibrillatory drugs.

DIGITALIS

The use of digitalis is the treatment of auricular fibrillation and flutter is based chiefly on emperical observations of the pulse rate and not on sound application of the known pharmacological actions of the drug. It is known that digitalis stimulates the vagus and Figure 6 shows

that its action on the stimulatory threshold of the isolated auricle is similar to acetylcholine. It has been suggested by DiPalma (22) that the antifibrillatory action of digitalis is due to its effect on conduction time and refractory period, however, the antifibrillatory action of digitalis probably stems from a different source.

Digitalis is given most often in circumstances where the heart muscle is fatigued, stretched and anoxemic. These conditions are the ideal stage setting for auricular fibrillation. (66) It is surprising then, that digitalis which supplies positive inotropic effects, the relief of dilatation, better coronary perfusion pressure and oxygenation of the heart, often stops fibrillation? Digitalis simply removes the factors which were responsible for the initiation of the auricular fibrillation and is not an antifibrillatory drug per se. Proof of this is obtained indirectly by the observation that many patients who are fibrillating and decompensated, but who are not given digitalis, revert to regular sinus rhythm with bed rest, sedation, oxygen and diuretics. Here again the conditions favoring the fibrillation are removed and the establishment of a regular sinus rhythm is made possible.

The use of digitalis should be limited to those cases in which anoxia and cardiac failure are present. If we give the drug to a patient who is in flutter or fibrillation from some other cause, e.g. thyrotoxicosis, then the

flutter will be changed to fibrillation and the fibrillation will be perpetuated. This may readily be explained through its action of decreasing the stimulatory threshold of auricular muscle.

THYROTOXICOSIS

As was mentioned previously, thyrotoxicosis is responsible for 14.1 per cent of all clinical auricular fibrillation. Auricular fibrillation is the most frequent cardiac arrhythmia seen in hyperthyrodism. It usually disappears permanently when the hyperthroidism is relieved. Thus it would seem that there is some factor in hyperthyroid patients which favors the production of auricular fibrillation.

There is some evidence that thyroid extract directly affects the myocardium producing increased activity and sensitivity. Yater in 1931 demonstrated that the isolated perfused hearts of thyrotoxic rabbits beat at a much faster rate than similar preparations from normal rabbits. (74) This was substantiated by the work of Lewis and McEachern (75) who worked with isolated rabbits' auricles and obtained similar results. Their reports also indicated that there is an increased reaction of the heart of the thyrotoxic animals to epinephrine. In all probability, it is this latter action of thyrotoxicosis which is most important in the production of auricular fibrillation in

hyperthyroid patients. Aumann and Youmans in 1940 experimentally analyzed the sensitization of the cardiac accelerator mechanism by thyrotoxicosis in dogs. (76) Their conclusions were that thyrotoxicosis markedly potentiates the action of epinephrine on the heart.

Nahum and Hoff produced auricular fibrillation in hyperthyroid patients by the injection of 0.75 mg/kg acetylcholine. (38) This same dose of acetylcholine did not produce fibrillation in normal persons. Weston and McCawley (77) working with experimentally induced hyperthyroidism in dogs were able to show that smaller doses of acetylcholine were needed to produce auricular fibrillation in the thyrotoxic dogs than in control animals.

In view of the clinical and experimental data at hand, the importance of thyrotoxicosis in the genesis of auricular fibrillation cannot be denied. While the precise mechanism of action is not known, it must be a combination of sensitization of the myocardium and a potentiation of the heart to the action of normally circulating epinephrine.

ORGANIC HEART DISEASE

The relationship or organic heart disease will be discussed from a theoretical aspect using what information we have at this time regarding the mechanism of auricular fibrillation and the influence of anoxia, vagal stimulation, and sympathetic stimulation on this mechanism.

1. Mitral stenosis with resulting left auricular dilatation is a common cause of auricular fibrillation. It has been shown that reflex vagal stimulation results from an increase in pressure in the aorta, (78) in the auricles and great veins (79) and mechanical dilatation of the auricle. (80) The vagus in turn not only lowers the stimulatory threshold of the auricle but results in reflex stimulation of the cardio-accelerator nerves (44) with a release of epinephrine. The latter conceivably stimulates or initiates ectopic foci and the combination of these two neurohormones results in auricular fibrillation. Evidence for sympathetic stimulation in this condition is offered by Boas and Goldschmidt (58) who observed that obstruction of the mitral valve would result in a tachycardia. The role of the cardiac stimulatory nerves in this tachycardia was proved when the heart rate returned to normal and remained stable in a patient after his left stellate ganglion was removed. Previous to the removal of the ganglion he had an uncontrollable tachycardia.

2. Congestive failure with auricular dilation, tachycardia and anoxia is a condition where one or more factors are present that initiate increased vagal tone and sympathetic stimulation of the auricular tissue.

3. Rheumatic fever not only may result in congestive failure and mitral disease due to endocarditis, but it has been suggested by Altschule (30) that the inflammatory

response in the auricle may sensitize nerve endings in auricular tissue and result in hyperactive reflex vagal stimulation. It has been suggested that the increased P-R interval seen characteristically in rheumatic fever represents increased vagal stimulation (36) as it is well known that vagal stimulation, chemically or electrically will result in prolongation of the P-R interval.

4. Coronary disease and infarctions result in tissue anoxia with potentiation of normal vagal stimuli. In addition they are usually complicated by tachycardia which means increased stimulation by the sympathetic nerves.

5. Hypertension with its resulting tachycardia, cardiac failure, and cardiac dilation also brings into effect increased stimulation of the heart by the autonomic nervous system.

EXPERIMENTAL AURICULAR FIBRILLATION IN NORMAL AND THYROTOXIC DOGS

The need for an experimental method of producing auricular fibrillation consistently in animals becomes apparent when one attempts to evaluate antifibrillatory drugs. (81, 82) It is true that certain isolated heart techniques may be used in the initial screening process but in the final evaluation, there is an incontrovertible need for trial on auricular fibrillation that is similar to the clinical disease.

It has been demonstrated that acetylcholine will produce fibrillation in 20-30 per cent of a series of control dogs. (29, 40, 44) By combining the action of anemia and intravenous acetylcholine in dogs, Horlick and Surtshin were able to produce auricular fibrillation in 75 per cent of them. (29) The disadvantages of this method include the production of anemia by venipuncture or the use of drugs and the need for doing blood counts and hemoglobin determinations daily.

Various other methods have been used to produce experimental auricular fibrillation. They include hyperthyroidism and epinephrine, (63) sub-epicardial injections of aconite, (51) simultaneous application of Mecholyl and faradic stimulation of the auricle, (44) and application of Mecholyl directly to the surface of the auricle in the

region of the sinus node. (38) The results obtained with these procedures are not predictable nor are they consistant enough to be used as an index for testing antifibrillatory drugs.

There are many open chest techniques where the auricles of the dog are stimulated chemically or electrically but they are complicated by the problems of artificial respiration, surgery, and atelectasis. These problems practically obviate this technique for extensive experimental use.

The use of the intact thyrotoxic animal was first suggested by Rosenblum (63) but he limited his studies to the effect of epinephrine on experimentally induced hyperthyroidism. In view of the known effects of vagal stimulation and vagomimetic drugs on auricular fibrillation, we decided to use this factor in addition to thyrotoxicosis to produce fibrillation in dogs.

Horlick and Surtshin, (29) in their work with anemic dogs determined the minimal "heart block dose" and "fibrillating dose" of intravenous acetylcholine for each control animal. It was found that these doses remained constant for each animal for long periods of time. After the animals were made anemic, the heart block and fibrillatory doses were again determined. It was shown that the minimal amount of acetylcholine required to produce either heart block or auricular fibrillation was less in anemic animals

than in the controls. They also showed that 50 per cent of the animals that did not fibrillate with acetylcholine alone would fibrillate when they combined anemia and intravenous acetylcholine. Their conclusions were that anemia and acetylcholine have an additive effect in producing auricular fibrillation in experimental animals.

The object of these experiments is to determine if induced thyrotoxicosis and acetylcholine injections have additive effects in the production of heart block and auricular fibrillation in the experimental animal and to determine whether such a procedure will consistently produce an auricular fibrillation that can be used to screen antifibrillatory drugs.

METHOD

Twenty-three mongrel dogs weighing from six to twelve kilograms were used in these experiments. The animals were anesthetized with pentobarbital given intraperitoneally. After they were anesthetized they were secured to an animal board and a needle was inserted into the saphenous vein. This was held in place by using a three-way stop cock attached to a 50 c/c syringe which was clamped to a ring stand. Injections could then be made by connecting a second syringe containing the drug into the third opening of the stop-cock.

As soon as the needle was placed into the vein

the animals were given ½ - ¾ c/c of Heparin solution. (1000 U.S.P. units per cc.) This prevented clots from forming in the injecting system. Standard Lead II electrocardiograms were recorded before and after each injection.

Acetylcholine was made up fresh on each experimental day in solutions containing 1 mg per c/c and 10 mg per c/c. A small dose of acetylcholine (about 0.6 c/c of the solution containing 1 mg per c/c) was then injected into the vein. If it caused 2:1 heart block the dose was decreased until a dose was determined that represented the smallest dose that could be given consistently to produce a 2:1 heart block. When this was determined, 2 c/c of acetylcholine (5 mg per c/c) was given rapidly, if fibrillation was produced, then the minimal fibrillating dose was determined by decreasing the dose with subsequent injections. Higher doses of acetylcholine may be given but the animals often die of respiratory arrest. If the animal would not fibrillate on approximately 1 mg/kg then the fibrillating dose was regarded as being 1 mg/kg +.

When this data was obtained it was recorded and the animals returned to the animal room. The same procedure was repeated two or three times on each animal to make certain that these doses remained constant as reported by Horlick and Surtshin. (29) As soon as the control heart rates, "minimal block dose" and "minimal fibrillating dose" had been determined, the animals were then ready for thyroid

powder feedings.

Eleven of the animals received 1-2 grams of thyroid powder U.S.P. per day for a variable period of time, 10-90 days, depending on the onset of signs of thyrotoxicosis. These signs described by Youmans and Aumann in 1940 (76) included tachycardia, vomiting and diarrhea, and thirst. Control heart rates were determined for each animal and then followed after the animal was placed on thyroid powder. The average control rate was 100. After the onset of vomiting and diarrhea, the animals were considered toxic and the average heart rate at this time was 153. This compares favorably with the results of Youmans and Aumann (76) who reported control rates of 112 and thyrotoxic rates of 161 after 14-21 days of thyroid powder feedings.

It is significant to note that the two animals which failed to fibrillate after being placed on thyroid powder for 45 days still did not show vomiting and diarrhea and probably were not toxic at the time the experiments were done. The possibility of hypothroidism or buffering action by the thyroid gland may be entertained as explanations but temporal obligations precluded any further experiments at that time.

RESULTS

1. Effect of intravenous acetylcholine on the cardiac rhythm of normal dogs. Four of the twenty-two

control animals which received maximal doses of 1-2 mg/kg acetylcholine showed electrocardiographic evidence of auricular fibrillation. The fibrillating dose in these animals ranged from 0.0125 to 1.3 mg/kg. The P-R interval was determined in all animals and except for one animal the P-R interval was ~~0.8-0.9~~ 0.08-0.09 seconds. However in the animal that fibrillated on 0.0125 mg/kg acetylcholine, the P-R interval was 0.19 sec. The probable significance of this observation will be covered in the discussion. For a comparison of the effects of acetylcholine on normal and thyrotoxic dogs see Table 3.

2. The effect of thyrotoxicosis on the minimal heart block dose and the minimal fibrillating dose of acetylcholine. Eight of the ten dogs which received thyroid powder developed auricular fibrillation when tested with maximal doses of approximately 1.0 mg/kg acetylcholine. The two animals which failed to fibrillate at the time the experiments were conducted did not show vomiting or diarrhea and possibly were not as toxic as the other animals in this series.

The effect of thyrotoxicosis on the minimal block and fibrillating doses is simmarized in Tables 3 and 4.

DISCUSSION

1. The production of auricular fibrillation in normal dogs with intravenous acetylcholine. Certain animals

Table 3: The Effect of Intravenous Acetylcholine on the Cardiac Rhythm of Normal and Thyrotoxic Dogs.

Number of animals and dose of acetylcholine	Normal	Thyrotoxic
Total animals tested	22	10 (1)
Total animals with auricular fibrillation	4	8
Animals fibrillating on less than 0.25 mg/kg	2	2
" " " " " 0.5 mg/kg	2	4
" " " " " 0.75 mg/kg	3	6
" " " " " 1.0 mg/kg	3	6
" " " " " 2.0 mg/kg	4	8

(1) Includes two dogs on thyroid for 45 days without showing vomiting or diarrhea.

Table 4: The Effect of Thyrotoxicosis on the Minimal Heart Block Dose and Fibrillating Dose of Intravenous Acetylcholine in the Intact Dog.

	Control			Thyrotoxic		
Animal Number	Heart Rate	Block Dose mg/kg	Fibrill. Dose mg/kg	Heart Rate	Block Dose mg/kg	Fibrill. Dose mg/kg
1	90	–	1.0 +	148	–	0.4
1A	92	–	"	150	–	0.5
2A	132	–	"	164	–	0.1
3A	106	–	"	144	–	0.1
12	97	–	"	180	–	1.0
12A	96	0.04	"	165	0.06	0.5
13A	120	0.07	1.3	188	0.08	0.42
14A	96	0.09	1.25+	134	0.15	1.0
15A (1)	80	0.04	1.0+	148	0.04	1.0+
18A (1)	75	0.03	"	148	0.03	"

(1) These animals did not show vomiting or diarrhea.

(+) Indicates that while the fibrillating dose was not determined, it was greater than the dose shown.

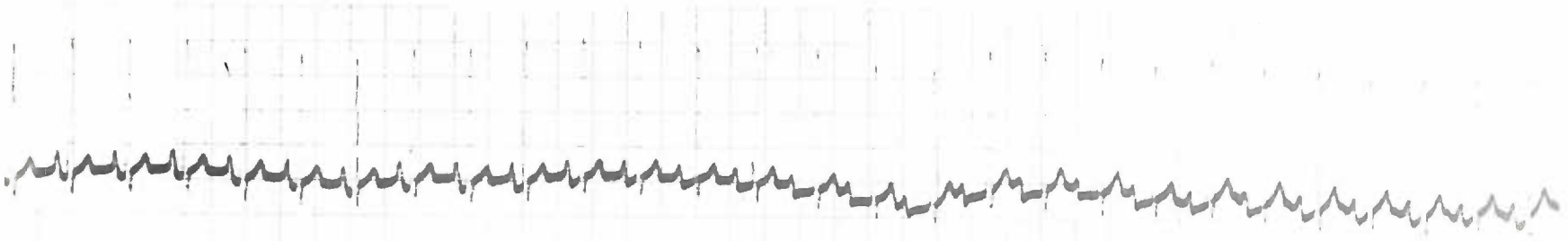
were found which seemed to fibrillate quite easily on small intravenous doses of acetylcholine. All of the animals which fibrillated were much smaller than those which did not. As a general rule, it was the small scrawny dog, not the large, healthy animals which seemed to fibrillate most easily. When injections of intravenous acetylcholine are given to these animals in increasing dosages, an interesting sequence of events takes place (see Figure 14). Small doses of acetylcholine produced a temporary increase in the P-R interval. Larger doses produced a prolongation of the P-R interval followed by 2:1 heart block. Still larger doses of acetylcholine resulted in complete heart block with complete inhibition of P waves and QRS complexes which is then followed by fibrillation of the auricles and irregular QRS complexes. When the effect of the drug wears off, the rhythm returns to normal.

In most cases the fibrillation lasts one to three minutes but the period of fibrillation could be prolonged to fifteen to thirty minutes by the use of physostigmine in addition to the acetylcholine. The action of the physostigmine is to prolong the action of the acetylcholine thus lengthening the period of fibrillation.

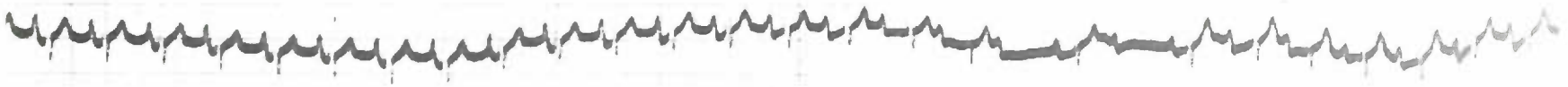
Only one of the four control animals that fibrillated showed electrocardiographic evidence of increased vagal tone before the experiments began. This particular animal had a P-R interval of 0.19 seconds compared to the normal

Figure 14. The Effects of Increasingly Larger Doses of Acetylcholine on the Cardiac Rhythm of the Intact Dog, showing the Relationship between Prolonged P-R Interval, 2:1 Heart Block and Auricular Fibrillation.

EFFECT OF INTRAVENOUS ACETYL CHOLINE ON CARDIAC RHYTHM OF INTACT DOG



ACETYL CHOLINE 0.04 mg/Kg PROLONGED P-R INTERVAL



ACETYL CHOLINE 0.05 mg/Kg 2:1 HEART BLOCK

ACETYL CHOLINE 0.5 mg/Kg AURICULAR FIBRILLATION

P-R interval of 0.08 seconds. It is significant to note that this animal with evidence of increased vagal tone fibrillated with a dose of acetylcholine of 0.0125 mg/kg while the other three animals required doses of 0.08 mg/kg, 0.5 mg/kg and 1.3 mg/kg.

Clinically and experimentally it has been demonstrated that prolongation of the P-R interval is due to increased vagal tone. (36, 83, 84) Altschule has reported a high correlation between the occurrence of partial heart block (increased P-R interval) and auricular fibrillation in rheumatic heart disease. In a series of twenty-five patients with prolonged P-R intervals, twenty-one developed auricular fibrillation. (30) A similar correlation was found between the occurrence of a prolonged P-R interval and auricular fibrillation following myocardial infarction. (85) Electrocardiographic records showing the development of auricular fibrillation indicates that in almost every case, a prolongation of P-R interval with heart block precedes the initiation of auricular fibrillation. (29, 86, 87)

If we assume that increased vagus activity is responsible for the increased P-R interval, formation of 2:1 heart block, and initiation of auricular fibrillation by decreasing the stimulatory threshold of the auricle, then we might expect drugs which block or reverse the effect of the vagus to have antifibrillatory activity.

Starr has reported that quinidine will block the

bradycardia which normally follows the injection of acetylcholine. (88) More recently Weston and McCawley have shown that diphenhydramine has a similar action. (89) At the same time they have shown that antifibrillatory drugs will increase the amount of acetylcholine required to produce prolongation of the P-R interval, 2:1 heart block and auricular fibrillation. These observations form the basis for a new method of testing antifibrillatory activity which is discussed in the second part of this paper.

2. <u>The effect of thyrotoxicosis on the minimal heart block dose and the minimal fibrillating dose of acetylcholine</u>. The higher incidence of fibrillation in the thyrotoxic animals over the controls would be expected on the basis of our clinical knowledge of the increased incidence of auricular fibrillation in thyrotoxic states. While only nineteen per cent of the control animals fibrillated on 1-2 mg/kg acetylcholine, eighty per cent of the thyrotoxic animals fibrillated with similar doses of the drug.

In three of the five animals which had minimal heart block doses determined before and during thyrotoxicosis, there was a slight increase in the minimal block dose. In the two animals which probably were not completely thyrotoxic as indicated by their lack of vomiting and diarrhea, there was no significant change in the minimal heart block dose. This would indicated that while thyrotoxicosis makes dogs more susceptible to acetylcholine-induced

auricular fibrillation, the mechanism is not merely potentiation of the vagus. If the mode of action was potentiation of the vagus as is true with anemia in dogs (29) then we would expect the minimal heart block dose to be appreciably decreased in thyrotoxicosis. On the other hand, if the action of thyrotoxicosis is the potentiation of a second factor in the genesis of auricular fibrillation; namely, sympathetic activity, then we would expect larger doses of acetylcholine to be required to nullify the sympathetic action on the auricular tissue and sinus node in order to produce heart block. Youmans and Aumann have demonstrated that thyroid hormone sensitized the adrenergic neuroeffector system of the dog's heart. (76) The effect of this sensitization is reflected in the action of thyrotoxicosis on the minimal heart block dose and the minimal fibrillating dose of acetylcholine.

SUMMARY

1. It has been shown that intravenous acetylcholine in doses of 1 mg/kg will produce auricular fibrillation in nineteen per cent of control dogs.
2. The dog requiring the least acetylcholine to produce auricular fibrillation had electrocardiographic evidence of increased vagal activity before experiment began.
3. It has been shown that 100 per cent of dogs fed thyroid powder in doses of 1-2 ~~grains~~ grams daily until signs of toxicity develop, will fibrillate with intravenous acetylcholine in doses of 1 mg/kg.
4. It has been shown that the "minimal heart block dose" of acetylcholine is greater in thyrotoxic animals than in the controls.
5. It has been shown that the "minimal fibrillating dose" of acetylcholine is less in thyrotoxic animals than in control animals.

THE MECHANISM OF ACTION OF ANTIFIBRILLATORY DRUGS

It has been emphasized previously in this paper that auricular fibrillation in most cases is a symptom of organic heart disease, anemia, thyrotoxicosis or hypertension, and should be primarily treated as such. It should be treated much in the same manner that we would treat any other symptom e.g. headache due to hypertension. We may give analgesics for the headache but our main concern is with restoring the blood pressure to near normal limits.

In all cases of fibrillation the underlying pathology such as anemia, thyrotoxicosis or congestive failure should be diagnosed and treated. If this is not done, then treatment with antifibrillatory drugs such as quinidine is as useful and as effective as treating the headache of hypertension with aspirin!

Lewis [15] on the basis of his circus movement theory decided that the perfect antifibrillatory drug would have two actions. It would increase the conduction time and decrease the refractory period. Quinidine lowered the refractory period of the auricle but it decreased the conduction time. Fortunately this discrepancy was not a vital one for quinidine was and remains one of the most effective antifibrillatory drugs that we have.

Up to the present time, most investigators have considered the change in refractory period to be the key to

the antifibrillatory action of quinidine. However, Van Dongen [56] considers the ability of a drug to suppress heterotropic rhythms to be a more accurate measurement of antifibrillatory activity. In view of the experimental results of Prinzmetal et al [18, 19, 39] it would seem that this view is the correct one. If we accept the "ectopic foci" origin of auricular fibrillation then we would define an antifibrillatory drug as one that abolishes ectopic foci. This might be accomplished by one of two mechanisms. First the drug might abolish the foci or the factors that initiated it. Secondly it might increase the stimulatory threshold of the auricle to a level where the foci could not fire or would fire so slowly that the regular pacemaker would take over.

1. <u>Drugs that might abolish or inhibit the ectopic foci</u> might also be expected to block the action of cardio-accelerator action of epinephrine on the heart, as we believe that sympathetic stimulation is responsible for this aspect of auricular fibrillation. Krayer has shown by use of the heart-lung preparation in dogs that epinephrine-induced tachycardia can be inhibited by quinidine. [90] Diphenhydramine and certain other antihistaminics have this same effect on the epinephrine-inducted tachycardia in isolated rabbits' auricles. [91] They are also known to block the epinephrine-induced hyperglycemia in rabbits. [92] Adrenergic-blocking agents such as Dibenamine and the ergot

derivatives have not been tested for antifibrillatory activity but it would not be surprising to find that they were antifibrillatory drugs.

2. Those drugs which would increase the stimulatory threshold of auricular tissue would also be expected to block the vagus nerve which is probably responsible for the decreased stimulatory threshold seen in experimental tachycardia. (15, 40) In 1936 Starr demonstrated the antagonism between acetylcholine and quinidine on cardiac rate. (88) Normally acetylcholine will slow the cardiac rate and lower the blood pressure of anesthetized dogs. However, when animals are protected with quinidine, acetylcholine will not cause a change in heart rate although it does cause a fall in blood pressure! Weston and McCawley in 1952 demonstrated that diphenhydramine has a similar action. (89) Atropine, antihistamines, atabrine, Pronestyl, Banthine and Demerol which inhibit vagal stimulation are drugs that have been used as antifibrillatory drugs or suggested as possible antifibrillatory drugs.

In summary, it would seem that those drugs which inhibit the action of the vagus and sympathetic stimulation of the heart are the most effective antifibrillatory drugs. In addition, the elevation of stimulatory threshold is a third and equally important characteristic.

Such drugs as quinidine and diphenhydramine meet all three of these requirements. Their limitations lie in

their toxicity, idiosyncrasy and short duration of action. At the present, studies are being made on drugs which are apparently as effective as quinidine but whose duration of action is much longer.

Up to the present time the surgical treatment of auricular fibrillation has not been attempted. However, in view of the role which the vagus nerve plays in the genesis of auricular fibrillation, it is not beyond the realm of reality to expect that section of the right vagus nerve could convert auricular fibrillation to normal sinus rhythm or to a sinus tachycardia.

SUMMARY OF PART ONE

1. The present theories regarding the genesis of auricular fibrillation are discussed. We feel that the most acceptable one is the "ectopic foci" theory and that auricular fibrillation depends upon the presence of ectopic foci which are rapidly stimulating the surrounding auricular tissue for its initiation and perpetuation.
2. In all probability the presence of these foci depends upon increased activity of the cardio-accelerator nerves, although there is the alternative possibility that these foci are present all the time but remain latent as they are of subthreshold intensity.
3. It has been shown that increased vagal activity as evidenced by shortened relative refractory period, increased P-R interval, and 2:1 heart block is responsible for decreasing the normal stimulatory threshold of the auricle in the dog. A similar action of acetylcholine on the isolated auricle has been demonstrated.
4. The conversion of subthreshold stimuli to threshold stimuli is brought about by a decrease in the stimulatory threshold. This decrease in stimulatory threshold is also responsible for allowing ectopic foci to fire their excitatory discharges at a faster rate. A vicious cycle exists here as the faster a foci fires, within limits, the more effective it makes the vagus in lowering

the stimulatory threshold.

5. Other factors such as digitalis, anoxia and hyperthermia decrease the stimulatory threshold and probably contribute to clinical and experimental auricular fibrillation in that manner.
6. Thyrotoxicosis potentiates the action of epinephrine in producing auricular fibrillation in conjunction with vagal stimulation (acetylcholine).
7. The mode of action of antifibrillatory drugs is to act directly on the auricular muscle to increase the stimulatory threshold and block the action of sympathetic and parasympathetic neurohormones upon the heart.

PART TWO

METHODS OF TESTING ANTIFIBRILLATORY DRUGS

INTRODUCTION

Although the available methods for screening drugs for antifibrillatory activity are legion, they may be conveniently divided into two main groups; those which utilize experimental animals and those which rely on clinical trial on patients.

ANIMAL PRODECURES

In respect to fibrillation in animals, it is obvious that there is a marked species difference. Fibrillation is easily produced in dogs, but difficult to produce in cats. Rabbits lie somewhere in between. In appropriate conditions, the reptile heart is useful. Sheep and pigs have been used by some investigators. Ideally, dogs constitute the best experimental animal, but in large-scale screening tests, the expense is prohibitive. For this reason cats and rabbits have served as the most economically useful animal in antifibrillatory studies.

A further classification in the use of animals concerns technique. Some investigators have used an isolated technique, others an intact technique. The intact technique varies greatly under conditions of anesthesia and open chest with artificial respiration to no anesthesia and induction of fibrillation by esophageal and intracardiac leads. Representative methods of each type of investigation

will be discussed in some detail.

1. Isolated technique: Strips of rabbit auricle are suspended in oxygenated Locke's solution and driven by a suitable stimulator. The fastest rate at which the particular strip can be driven without skipping beats is determined. This serves not only as an index of the refractory period but also as a threshold level. Ordinarily in this preparation the auricular strip can be driven at rates up to 260/min. With quinidine in a concentration of 1/100,000 added to the bath, the rate at which the auricular strip can be driven without irregularity falls to 180/min. This presumably indicates that the refractory period is lengthened. (93)

This method has the advantage of simplicity, small expense and considerable precision. It has the disadvantage common to all bath techniques, notably, complete separation from all reflexes, including nervous, chemical and hormonal common to the organism as a whole. We have applied this method to certain antihistaminic drugs. The results will be discussed later.

2. Intact technique, open chest. Perhaps the most commonly used method is that of direct application of electrodes to the auricles of an intact animal with open chest and with artificial respiration. Most often an induction coil is used as the source of current and the inter-coil distance necessary to just produce fibrillation serves

as a threshold level. Most investigators now agree that the induction coil is very unsatisfactory as a source of current since its output both as to wave form and amplitude varies widely at identical settings. (73) The use of a condenser discharge or square wave generator such as the Wein bridge stimulator, makes it possible for application of currents which are predictable as to wave form and frequency. In this method fastidious attention to the proper application of electrodes is necessary as it is indeed in any method using electrical stimulation. The common difficulties encountered are short-circuiting and polarization effects. The former may be solved by proper anchoring and suspension of the auricle, the latter by use of non-polarizable electrodes. Attention must also be directed to the use of anesthesia which does not unduly depress autonomic reflexes and a rate of respiration just sufficient to prevent anoxemia but not cause overventilation. When the proper source of current is used and attention to the above points followed, this method is practical. The results can be expressed in terms of milliampers of current at a certain frequency (600/min) just necessary to produce fibrillation.

Perhaps the most accurate method is that in which the electrical stimulus is applied only during a certain portion of the heart cycle. This requires that the sinus node be removed or concainized and the heart driven by a motor-driven device which also adds a second stimulus at a

given interval. (94) Another method consists of triggering the circuit of a vacuum tube stimulator with the QRS potential so as to interject a stimulus at a given interval. (34) The threshold is the amount of current necessary to produce fibrillation at a certain point in the heart cycle. While this method is superior it is difficult technically and probably not necessary in the screening of drugs. It is the only method which yields direct evidence of the length of refractory period and of the vulnerability at various times in the heart cycle.

With the chest open, various procedures may be utilized to enhance the onset of fibrillation or to perpetuate it. It is now well known that a pledget of cotton soaked in Mecholyl and applied to the region of the sinus node will insure continuous auricular fibrillation in the dog and will render it more producible in the cat. Another method recently described is that of injecting aconite subepicardially in the auricle of the dog. (51)

3. Intact technique, closed chest. The heart may be fibrillated by strong AC or DC shocks applied to the limbs or trunk of an animal. This resembles accidental death from electrocution in humans. Strong currents must be used and ventricular rather than auricular fibrillation often ensues.

An esophageal electrode combined with an intra-auricular electrode introduced through the jugular vein makes

possible the production of fibrillation with electrical stimulation. The disadvantages of this method lies in the fact that the intra-auricular electrode position cannot be maintained stationary and rather large amounts of current must be used. The advantage is obviously that no anesthesia need be used. (95)

HUMAN TRIAL

While it is obvious that the final proof of the value of a drug is the restoration of a sinus mechanism in humans, it is rarely feasible to assay drugs by this method. Often patients who are fibrillating stop spontaneously. Moreover, fibrillation is often complicated by congestive failure. In some patients with advanced mitral stenosis and a markedly dilated left auricle, it is impossible to stop the fibrillation by any method or drug. Therefore, it should be obvious that no conclusion is possible if a supposed anti-fibrillatory drug is given to a patient with fibrillation and the fibrillation does not stop. On the other hand if it does stop this may only be accidental.

METHODS USED IN THIS SERIES OF EXPERIMENTS

At the present time we have no ideal method for measuring the antifibrillatory activity of new drugs. In the past, many of the tests used were based upon certain principles involved in the circus movement theory. The

effect of antifibrillatory drugs on the refractory period is a widely used test at the present time. It has been used in this paper to demonstrate antifibrillatory activity in the antihistaminic drugs.

Van Dongen, (55, 56) as well as ourselves, has criticized the use of the refractory period as the only index of antifibrillatory action. He has demonstrated that antifibrillatory activity is more closely associated with the ability to suppress ectopic foci. In order to measure this property of drugs, we have modified the measurement of sensitivity in the isolated rabbits' auricle and used it as a screening test for antifibrillatory activity on certain antihistaminic drugs. (96)

Horlick and Surtshin have shown that certain conditions such as anemia which predispose the animal to auricular fibrillation, will decrease the amount of acetylcholine normally required to produce at 2:1 heart block in dogs. (29) We have shown that antifibrillatory drugs have the opposite action. They increase the "minimal heart block dose." We have applied this method of testing drugs for antifibrillatory activity to several antihistaminic and anti-vagal drugs. This test is not only useful in comparing the effectiveness of the various drugs, but it gives a good index of onset and duration of action.

Finally, we have utilized the property of some drugs to break or prevent the formation of experimentally

produced auricular fibrillation in dogs as an index of antifibrillatory activity. The method is used here chiefly to show the action of certain antihistaminic drugs on induced auricular fibrillation.

THE REFRACTORY PERIOD MEASURED IN THE ISOLATED RABBIT AURICLE AS AN INDEX FOR TESTING CERTAIN ANTI-HISTAMINIC DRUGS FOR ANTIFIBRILLATORY ACTIVITY

In previous experiments, McCawley and others (9, 97) have shown that benzhydryl ether antihistaminics prevent the formation of ventricular arrhythmias in animals. Based on these experiments we have undertaken an experimental study to find out whether or not these compounds might be applicable to the treatment of clinical auricular fibrillation. Clinical auricular arrhythmias and ventricular arrhythmias do not usually respond to the same drug and any study of a drug on both types of arrhythmia might be established on the assumption of a common physiological mechanism for myocardial "excitability and conduction of propagated myocardial disturbance." Furthermore, the antihistaminics possess many of the chemical and pharmacological characteristics of both procaine and quinidine.

Clinical experience with quinidine in therapy of auricular fibrillation has indicated a need for a substitute which may be used intravenously, which will not induce marked hypertension, and which, in itself, will not provoke ventricular arrhythmias. We have endeavored to obtain evidence that such a drug may be found among certain antihistaminic compounds.

The antihistaminics available for therapy of allergic conditions, and for study of antifibrillatory

properties, may be classified into three chemical groups viz: a.) aminoethyl ethers, b.) propylamines, and c.) ethylene diamines. All are dialkylaminoalkyl derivatives jointed to isocyclic or heterocyclic nuclei. The atom joining the two portions of the molecule, a.) oxygen, b.) carbon or c.) nitrogen, is the basis for the chemical classification. When significant activity was found among the aminoether group, additional derivatives were included in the study.

The structural relationships between the various antihistaminics is shown in Figure 15.

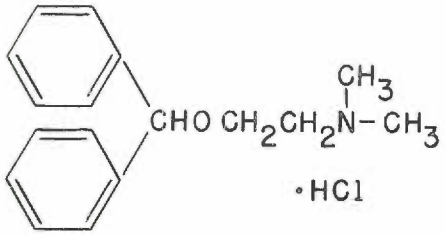
A prolongation of refractory period of the isolated auricle for assay of antifibrillatory substances. Dawes has devised a method for evaluating antifibrillatory drugs based on a prolongation of refractory period of the isolated rabbit auricle. (24) The refractory period in this procedure is measured indirectly. The auricles are driven by repetitive stimuli. As the frequency of this stimulus is gradually increased, a rate will be reached where the auricles no longer respond to every stimulus applied; this is recorded as the "maximal response rate." The refractory period is taken as the reciprocal of the maximal response rate. It has been found that this "maximal response rate" is lowered by quinidine. The magnitude of reduction of the maximal response rate serves as a basis for evaluation of antifibrillatory drugs.

Figure 15. Chemical Classification of Antihistaminics.

Table I

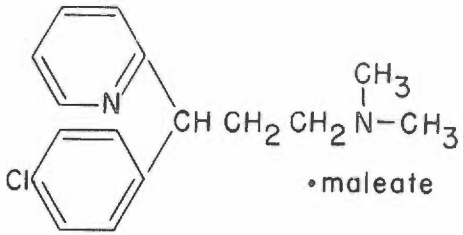
CHEMICAL CLASSIFICATION OF ANTIHISTAMINICS

Aminoethyl ether a)



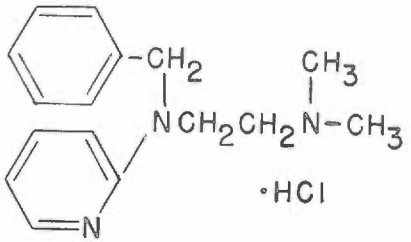
diphenhydramine
Benadryl (R)
dimethylamino ethyl
benzhydryl ether

Propylamine b)



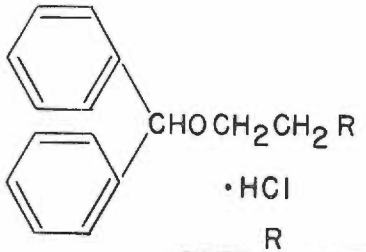
chlorprophenpyridamine
Chlortrimeton (R)
1-para chlorophenyl 1-
(2-pyridyl) 3-dimethyl-
amino propane

Ethylene diamine c)

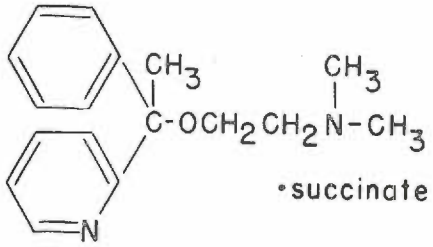


tripelennamine
Pyribenzamine (R)
N-dimethyl N'
benzyl, N' pyridyl
ethylene diamine

Diphenhydramine analogs



		R
ethyl analog	(S-45)	-diethylamine
piperidyl analog	(S-49)	-piperidyl
Linadryl (R)	(A446)	-morpholine
Ambodryl (R)	(AH890)	-dimethylamine, para bromo



doxylamine
Decapryn (R)
dimethylamino ethoxy methyl
benzyl pyridine

METHODS

We used young rabbits three to four months old. They were killed by a blow on the occiput and the heart immediately removed and placed in a dish of warmed saline. After washing the chambers free of blood, the auricles were coarsely trimmed from the ventricle and blood vessels, taking care not to injure tissues near the sinus node.

The auricles were then quickly affixed to a heart lever and immersed in an oxygenated Tyrode's solution containing 0.2 per cent glucose, with the bath temperature maintained at 29° C. Rectangular wave impulses, provided by a Bioelectronics model S-III stimulator, were used for stimulating the auricles using the electrode arrangement as described by Dawes.

The ohmic resistance of the tip of the auricle between the electrodes was measured at the beginning of each experiment (12,000-21,000 ohms) and was used to detect any occurrence of electrode shorting by the Tyrode's solution. Using a three millisecond pulse duration and with a frequency of approximately 300/min., the voltage was increased until the auricular contractions followed each stimulus. We found that this just "supra threshold" stimulus intensity remained constant at frequencies between 200/min. and 400/min. The mean stimulus current required under these conditions was 0.76 milliamperes.

For our assay of antifibrillatory drugs, we found it necessary to allow each auricle preparation to remain in the bath for a sixty-minute equilibration period. After this equilibration period, the mean maximal response rate was 328/min.; range 270-378/min. The auricles contracted spontaneously with a mean rate of 96.6/min.; range 76-122/min. for the fifty auricles used.

After measuring the change in maximal response rate produced by a drug, the auricles may be washed with fresh tyrode's solution and another drug or different concentration of the same drug may be tested. However, if a reduction by more than 30 per cent of the initial maximal response rate has been produced, the auricle usually does not recover completely; i.e., the next drug tested will elicit an unpredictable response. Even with this limitation, repetition of the same concentration of quinidine yielded the same extent of action only for the second and third times applied. Until additional study of the method is made, confidence cannot be placed in results obtained on more than two drug applications to a single auricle preparation.

RESULTS

Quinidine sulfate was used as the standard of comparison and its effects on the refractory period of the isolated auricle were studied at several concentrations.

The maximal response rate is lowered when quinidine sulfate is added to the bath to yield concentrations from 10-7 to 10-4. When the maximal response rate was determined at five-minute intervals after the addition of quinidine it was found that at least twenty minutes were required for the full effect to develop. Certain of the antihistaminics required longer periods of time (sixty minutes) for their maximum effect. Comparison between drugs was made after each had developed its maximum effect. If comparison had been made at any arbitrary time period, there would have been confusion of the drug's potency of action with its rate of diffusion into auricular tissue.

The curve relating dosage to drug action of prolongation of refractory period for quinidine sulfate is illustrated in Figure 16. An analysis of variance did not disprove a linear regression of effect with log of concentration; this observation has been reported by other investigators. (24, 99, 100)

The dose-effect curves obtained for the antihistaminics show linear relationship between logarithm of dose and reduction of maximal response rate. The combined slope of the several dose-effect curves obtained (see Figure 17) was, however, steeper than that of quinidine. Thus in comparing the antihistaminics with quinidine, the relative potencies will vary with the absolute dosage level at which they are compared, since their slopes are not parallel. An

Figure 16. Action of Quinidine on the Refractory Period of the Isolated Rabbit Auricle.

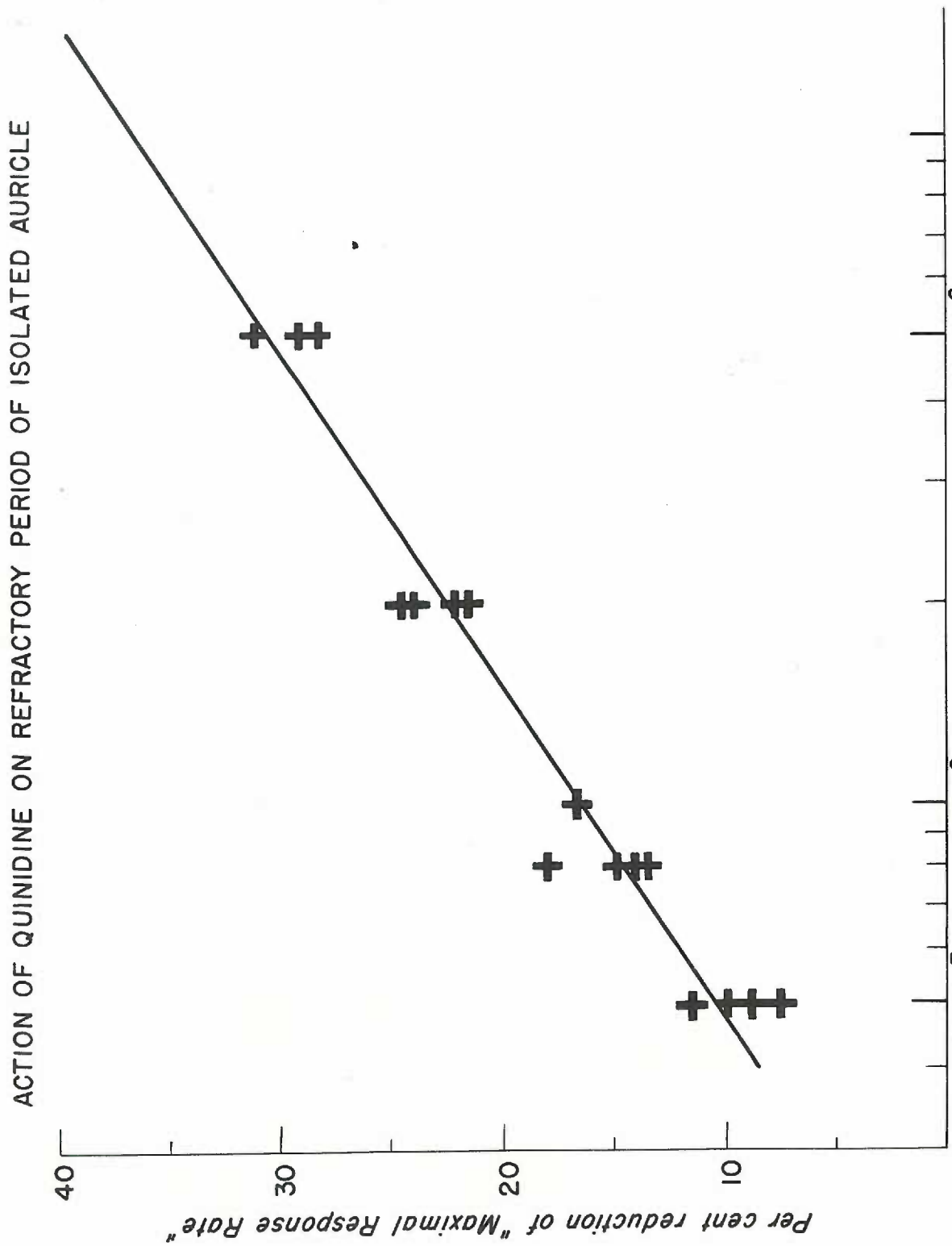

ACTION OF QUINIDINE ON REFRACTORY PERIOD OF ISOLATED AURICLE
Per cent reduction of "Maximal Response Rate"
40
30
20
10
5 x 10-7
1 x 10-6
5 x 10-6
Concentration of Quinidine Sulfate

FIG. 1

Figure 17. Action of Antihistamines on the Refractory Period of the Isolated Auricle.

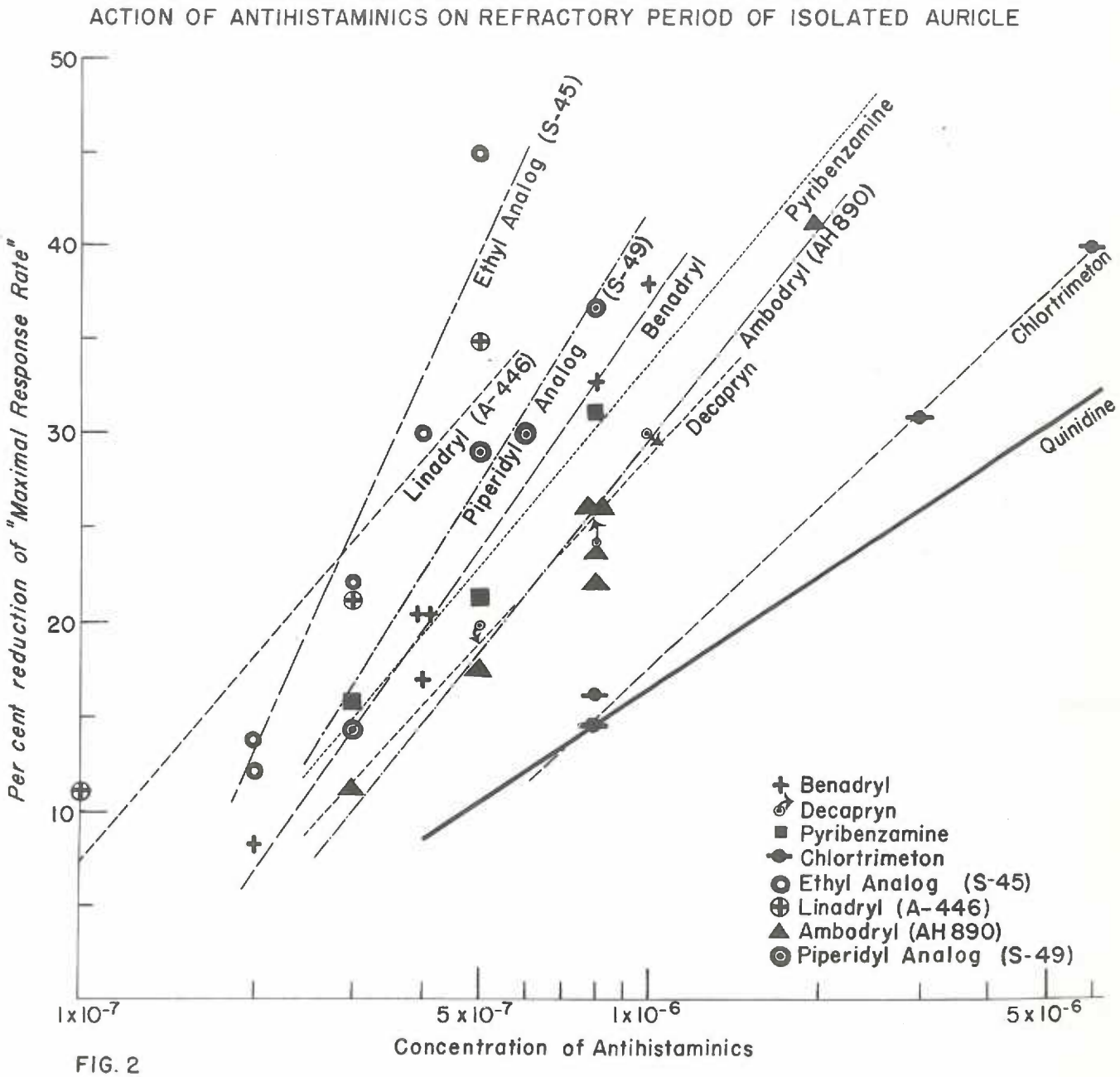

ACTION OF ANTIHISTAMINICS ON REFRACTORY PERIOD OF ISOLATED AURICLE
Per cent reduction of "Maximal Response Rate"
50
40
30
20
10
Ethyl Analog (S-45)
Linadryl (A-446)
Piperidyl Analog (S-49)
Benadryl
Pyribenzamine
Decapryn
Ambodryl (AH890)
Chlortrimeton
Quinidine
Benadryl
Decapryn
Pyribenzamine
Chlortrimeton
Ethyl Analog (S-45)
Linadryl (A-446)
Ambodryl (AH890)
Piperidyl Analog (S-49)
1x10-7
5x10-7
1x10-6
5x10-6
Concentration of Antihistaminics

FIG. 2

additional factor deterring any accurate comparison of potencies exists because of conditions imposed by isolated tissue studies. The chlorprophenpyridamine was used as the maleate salt, while doxylamine was available as the succinate salt. These anions may reduce the calcium ion concentration available to the isolated auricle. DeEllo has indicated that alteration of the calcium ion concentration may affect the activity of isolated auricular tissue. (25) A similar situation is suspected with compound A-446 as stock solutions of the hydrochloride salt will form precipitates with Tyrode's solution. Further dilution to the concentrations used in these experiments did not cause precipitation, but it may be suspected that some form of ionic imbalance may have resulted.

Although comparison of individual drug potencies with our small samples, rough inferences are suggested by inspection of Figure 17, it appears that chlorprophenpyridamine has about the same potency as quinidine. The other antihistaminics of different chemical classifications show greater activity; S-45 and A-446 are the most potent.

The results obtained with compounds of the amino ethyl ether series were reasonably homogeneous and permit some observations on the relationship between chemical structure and prolongation of refractory period of the isolated auricle. A survey of this information indicates

that the dimethylamine radical of diphenhydramine may be changed to diethylamine (S-45), to the morpholine (A-446), to piperidine (S-49) and the same high order of activity on the heart is retained. Substitution of bromine on the one benzene ring (AH890) does not alter the effect of diphenhydramine on the atrial refractory period. Even greater changes in chemical structure may be made without significantly affecting action on the heart; with doxylamine, pyridine replaces one benzene ring and a methyl radical substitutes for hydrogen on the carbon atom joining the two aromatic rings.

There was no obvious relationship between antihistaminic activity and an action of prolonging the relative refractory period among the compounds tested. The comparative antihistaminic potencies have been rated by Loew et al (101) as follows: Diphenhydramine, 1.0; S-49, 1.0; A-446, 0.5; and S-45, 0.125. On the isolated heart it can be seen from Figure 17 that all of these latter derivatives were more potent than the parent diphenhydramine. There was, moreover, no correlation with toxicity (lethal dosage) and activity on the refractory period. The LD_{50}s calculated from the results of intra-abdominal injection in rats are: S-45, 55 mgm./kgm.; S-49, 80 mgm./kgm.; diphenhydramine 82 mg./kgm.; and A-446, 185 mgm./kgm. Linadryl, or A-446, appears particularly of interest for additional studies as the low order of toxicity in animals is borne out

with a low incidence of side effects reported in patients. (102)

The production of fibrillation in isolated auricles and conversion to a normal rhythm by antihistaminics. In the process of estimating the "maximal response rate" of the isolated auricle, the electrical stimulus was applied for a few seconds at each increase in frequency until the auricle failed to follow each impulse. At the end of the few seconds' train of impulses, the auricle exhibited a few irregular beats of great force and then returned to its normal spontaneous rhythm of 90-128 beats/min. It was observed that an occasional auricle preparation would continue to contract at a rapid rate (292-324/min), the amplitude of each contraction being quite small. It is possible that manipulation of the auricle or trimming away of excess tissue had produced a focus of trauma which acts as an ectopic pacemaker. The fibrillation state can be deliberately produced by crushing the tip of an appendage into the electrode holder, with sufficient regularity for assay purposes. When the auricles were in this "fibrillation" state, there was no spontaneous recovery; after two to three hours all activity ceases, perhaps because the fibrillating auricle is unable to take up sufficient oxygen. Fibrillation cannot be produced with all auricles, as after excessive trauma, the auricle fails to contract spontaneously at all. It will be noted that the fibrillating isolated auricle has most of the characteristics

of clinical auricular fibrillation.

Quinidine sulfate was added to the bath solution with the fibrillating auricle to yield a concentration of 5 X 10-6. It was found that no change occurred in the rate of contraction of the auricle for about fifteen minutes at which time the rhythm suddenly changed from a rate of 318/327 min. to 90-100 min. On close inspection of the kymographic recording it was observed that the conversion of fibrillation to a slow rhythm of 90-100/min. had occurred in the brief span of time of four to six seconds. The tracings also revealed that after conversion the contractions of the auricles were greater in strength than during the "fibrillation" state and approached the vigor of the pre-fibrillatory auricle.

Diphenhydramine also was evaluated using the "fibrillating" isolated auricle. The bath concentration used was 8 X 10-7 and conversion to a normal rhythm occurred after a twenty-minute lag period. Greater concentrations of diphenhydramine, 1 X 10-6 to 1 X 10-5, were able to shorten this delay or lag period only by a few minutes (fourteen minutes at the highest concentration). Although the data are few, they would tend to support the idea that the lag period represents the time required for an effective concentration to be reached in the auricular myocardium. The lag period time should vary with concentration of drug according to the Mass Law hypothesis.

An experimental "fibrillation" state in the isolated auricle can be routinely produced by the application of the alkaloid, aconitine. Aconitine has been known for some time to induce abnormal stimulus formation in the heart. (50) If this alkaloid is applied to the atria in an open chest preparation, an auricular tachycardia appears which has many characteristics of clinical auricular flutter. In our experiments, aconitine, as a 0.5 per cent solution in benzene, was injected into an appendage of the isolated rabbit auricle preparation. Within two minutes the spontaneous contraction rate has risen from 105 to over 300 with the amplitude of contractions being very small. Quinidine and diphenhydramine added to the bath in concentrations of 10-7 to 10-5 lowered the frequency of contractions to 69-100/min. within a seventeen to twenty-minute period of time. Part of the action of aconitine still is manifest as the rhythm of contractions was now very irregular, both as to rate and strength of contractions. Apparently, quinidine or diphenhydramine restore a slow rhythm to an auricle in fibrillation or flutter from aconitine, but they cannot impose a regular rhythm in a slowly beating auricle.

DISCUSSION

Attention has been focused in the search for anti-fibrillatory drugs on an ability to prolong the refractory

period of the heart. There is really no need to cavil over the terms absolute, effective or relative refractory period as there is no real proof that a prolongation of refractory period is responsible for the therapeutic effectiveness of a drug in clinical auricular fibrillation. To be sure, there is also no proof that a drug which cannot prolong refractory period will "convert" a patient with auricular fibrillation. Studies of a drug's ability to prolong refractory period will in any event provide useful information. A drug which will prolong the refractory period should, by an extension of this "pharmacological" action, be able to cause toxic atrioventricular blocks and bundle branch conduction disturbances.

It is noteworthy in the experiments using the "fibrillating isolated auricle" that after the addition of quinidine or diphenhydramine, there occurred a sudden decrease of the auricular contraction rate of 190-200 beats/min. Conversion to a normal sinus rhythm in patients with auricular fibrillation also occurs suddenly and is an example of an all or none response to drugs. Drug action in prolonging the auricular refractory period (measured experimentally as a decrease in maximal response rate), has in contrast, an incremental type of drug response. It is conceivable that auricular fibrillation cannot continue when the refractory period has been prolonged to a certain threshold value; when this threshold is reached through drug

action, the fibrillation process would then necessarily stop. If the same concentrations of quinidine and diphenhydramine mentioned above are applied to the non-fibrillating isolated auricle, there is a reduction in the maximal response rate (reciprocal of refractory period) of 50-60 beats/min. Note that the decrease in auricular contraction rate on conversion of the isolated fibrillating auricle was 190-200 beats/ min. and there is thus a 140-150 beats/min. change that cannot be accounted for on the basis of prolongation of refractory period alone. These observations would suggest that the therapeutically useful antifibrillatory action of quinidine cannot be entirely one of a prolongation of refractory period.

An explanation for the necessity of an hour's equilibration period of the isolated auricle preparation before one can test an antifibrillatory drug is to be found in the work of De Elio.(54) He reported that epinephrine added to the isolated auricle preparation caused first an increase in the "maximal response rate" followed by a decrease in this measurement and its eventual return to the initial value. The biphasic direction of change and time course observed by us closely parallels De Elio's findings with 20 microgm. epinephrine. It thus appears possible that epinephrine is released to the isolated auricle as the animal is being sacrificed and the auricles removed and trimmed.

It would appear that a variety of experimental

techniques must be applied to a potentially useful antifibrillatory drug before it can be given a clinical trial. (82) Our present state of knowledge concerning the genesis of clinical auricular fibrillation is insufficient to permit us to select one alteration of critical function of the auricle for study with antifibrillatory agents.

SUMMARY

1. The isolated auricle technique of Dawes has been used to study the action of a series of antihistamine compounds on the myocardial refractory period. The antihistaminics studied prolong the refractory period at concentrations lower than that required by quinidine. Diphenhydramine appeared worthy of clinical trial in patients with auricular fibrillation.
2. Fibrillation or "flutter" has been evoked in the isolated auricle preparation by a localized traumatic infarct or by aconitine. Quinidine and diphenhydramine can produce a sudden conversion to a slow rhythm in these preparations.

CHANGES IN THE STIMULATORY THRESHOLD OF THE ISOLATED RABBIT AURICLE AS AN INDEX FOR TESTING VARIOUS DRUGS FOR ANTIFIBRILLATORY ACTIVITY

The need for other methods of screening antifibrillatory drugs became apparent when some of the drugs reported by McCawley and Weston (96) to possess effective antifibrillatory activity failed to convert some cases of clinical auricular fibrillation, which were readily converted with small doses of quinidine. With our views on the mechanism of fibrillation and the importance of stimulatory thresholds in relationship to the rate of discharge from an ectopic foci, it was only to be expected that we might attempt to use this factor as an index to measure antifibrillatory activity.

METHOD

We used young rabbits three to four months old. They were killed by a blow on the occiput and the heart immediately removed and placed in a dish of saline. The auricles were coarsely trimmed from the ventricle and great vessels. The auricle was then affixed to a heart lever and immersed in an oxygenated Thyrode's solution containing 0.2 per cent glucose. The temperature of the bath was maintained at 29° C. Stimulation was fractionated through a three-stage decade box and coupled to the electrodes through a cathode follower so that the impedance of the stimulus did not vary as the voltage was changed. The electrodes were connected

to the auricles in the manner shown in Figure 18.

The threshold voltage was determined at the following frequencies: 112, 118, 128, 144, 165, 234, 370 and 312. When normal values were obtained, drugs were added to the bath to give various known concentrations. At variable periods of time, depending on the rate at which the drug acted on the auricle, the threshold voltage was again determined. When the drug had reached a maximum effect, usually 20-40 minutes, the solution was drained and fresh Tyrode's was added. A second determination was done on the auricle if the stimulatory threshold returned to normal after the first determination. No more than two determinations were done on any auricle.

RESULTS

A total of 27 auricles were tested with various drugs at different concentrations. A summary of these results appears in Figure 19. The abscissa shows the rate at which the auricle was stimulated. The ordinate indicates the percentage increase of the normal stimulatory threshold of the auricle for that particular stimulatory rate.

DISCUSSION

The significance of a lowered stimulatory threshold in the mechanism of auricular fibrillation has been emphasized several times in this paper. If auricular

Figure 18. Apparatus Used for the Experiments on the Isolated Rabbit Auricle.

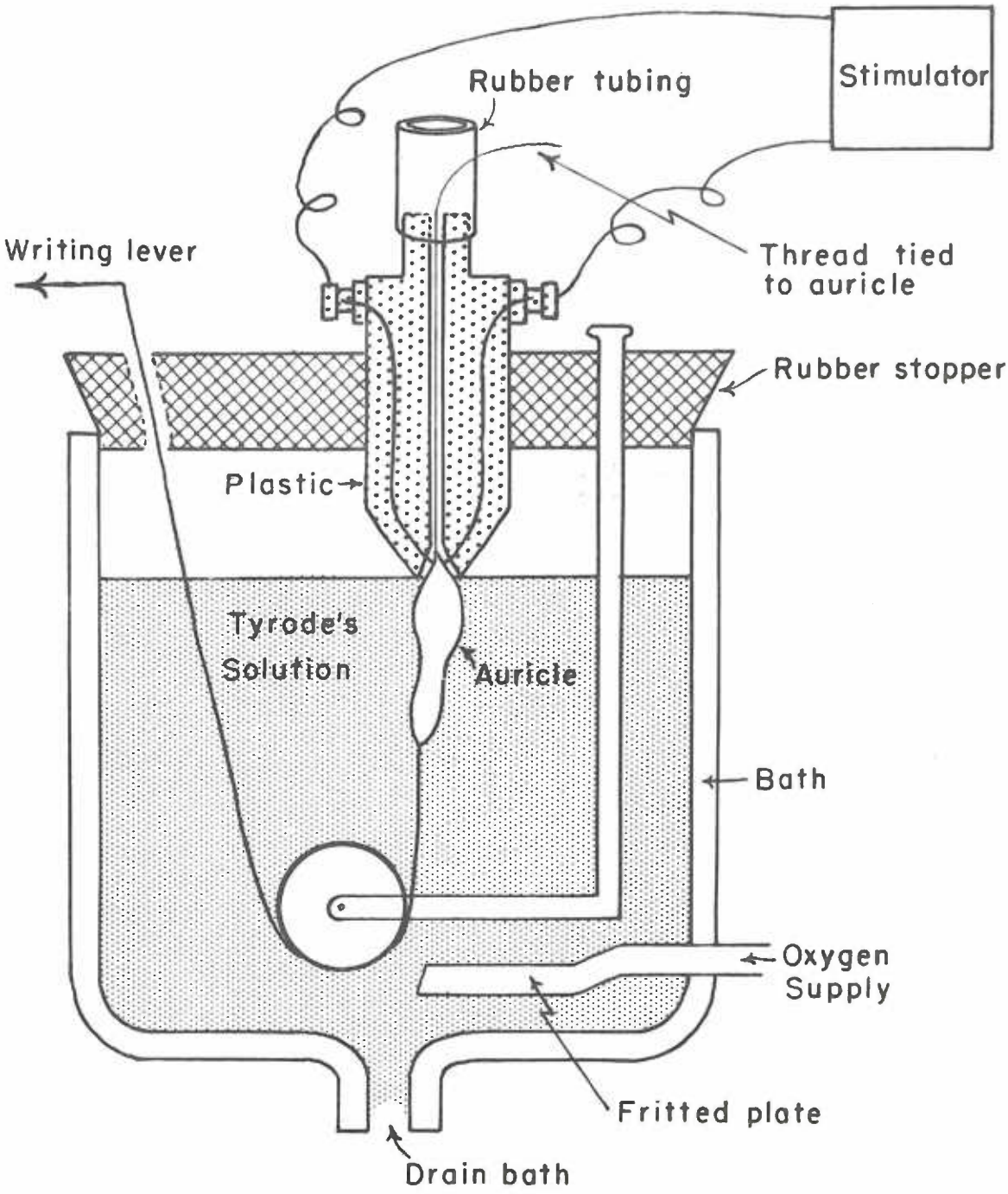
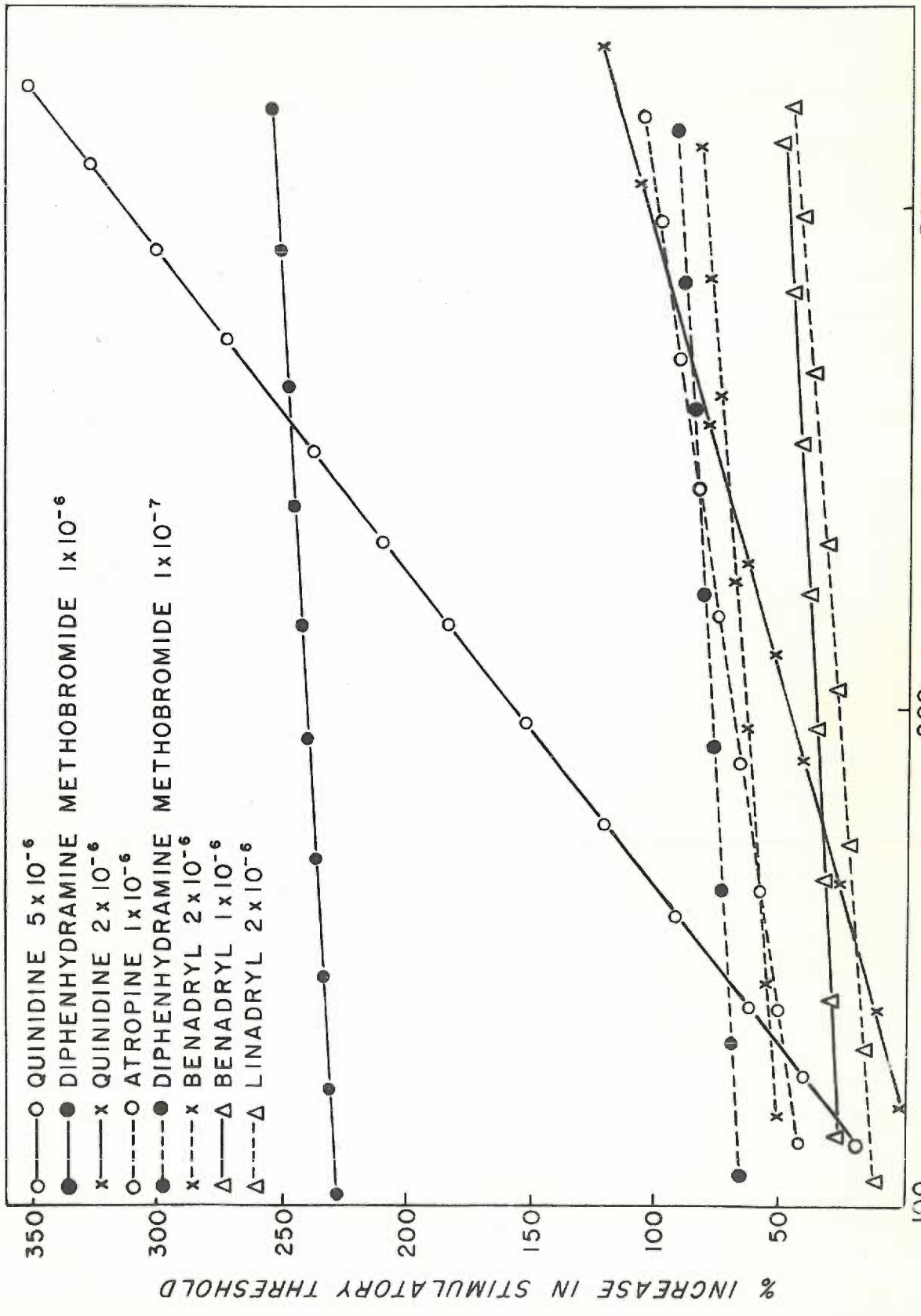

Stimulator
Rubber tubing
Writing lever
Thread tied
to auricle
Rubber stopper
Plastic
Tyrode's
Solution
Auricle
Bath
Oxygen
Supply
Fritted plate
Drain bath

Figure 19. The Effects of Quinidine, Atropine and Selected Antihistamines on Stimulatory Threshold of the Isolated Rabbit Auricle.


QUINIDINE 5 x 10⁻⁶
DIPHENHYDRAMINE METHOBROMIDE 1 x 10⁻⁶
QUINIDINE 2 x 10⁻⁶
ATROPINE 1 x 10⁻⁶
DIPHENHYDRAMINE METHOBROMIDE 1 x 10⁻⁷
BENADRYL 2 x 10⁻⁶
BENADRYL 1 x 10⁻⁶
LINADRYL 2 x 10⁻⁶
% INCREASE IN STIMULATORY THRESHOLD
350
300
250
200
150
100
50
100
200
300
RATE OF STIMULATION

fibrillation is dependent on increased auricular sensitivity either to perpetuate ectopic foci or enhance circus movement then a drug which will decrease this sensitivity would have definite antifibrillatory properties.

Quinidine was used as a control in this experiment, the object being to compare other drugs, in this case, selected antihistaminic drugs, with quinidine. Examination of Figure 19 shows that the efficiency of quinidine increases not only as the concentration increases but also as the rate of stimulation increases.

Diphenhydramine (Benadryl) seems to be just as effective as quinidine in depression of sensitivity. This is borne out by clinical trial of this drug in auricular fibrillation. (103)

Atropine seems to be about twice as effective as either diphenhydramine or quinidine. Lewis reports that atropine was used to treat auricular fibrillation but that it was not always effective. (15) A possible explanation is the difference in the dosages of quinidine and atropine that the patient can tolerate. Linadryl (A-446) showed a marked antifibrillatory action based on its effect on the refractory period (Figure 17). Clinical trial of this drug was discouraging and was in part responsible for the development of this method for testing antifibrillatory drugs. (103) The reason for the clinical failure is readily explainable on the basis of its action on the sensitivity of the auricle.

It is less than half as effective as diphenhydramine or quinidine and therefore could not be expected to have the antifibrillatory action of those drugs regardless of what it does to refractory period.

Diphenhydramine-methobromide (S-92) was suggested as an antifibrillatory drug because it was thought that its action would be more prolonged than quinidine or Benadryl. Preliminary tests have shown that it is 10-20 times as effective as quinidine and has a longer duration of action which is an additional advantage. However, toxicity tests are not completed on this drug and it has not yet been released for clinical trial.

SUMMARY

1. The isolated rabbit's auricle was used to study the effect of quinidine and certain antihistaminic drugs on the stimulatory threshold of the auricle at different rates of stimulation.
2. All of the antifibrillatory drugs tested increased the stimulatory threshold of the auricle. The efficiency of the drug was increased as the rate of stimulation was increased.
3. S-92 appears to be 10-20 times as effective as quinidine and should be worthy of clinical trial as soon as it is released for clinical use.

THE USE OF THE "MINIMAL HEART BLOCK DOSE" OF ACETYLCHOLINE IN DOGS AS AN INDEX FOR TESTING VARIOUS DRUGS FOR ANTIFIBRILLATORY ACTIVITY

The experimental and theoretical basis for this method of testing drugs for antifibrillatory activity was discussed in the section on "Experimental Auricular Fibrillation."

METHODS

Mongrel dogs weighing approximately 10 kilograms each were anesthetized with pentobarbital given intraperitoneally. After they were anesthetized they were secured to an animal board and a needle was inserted into the saphenous vein. This was held in place by using a three-way stop cock attached to a 50 c/c syringe which was clamped to a ring stand. Injections could then be made by connecting a second syringe containing the drug into the third opening of the stop cock.

As soon as the needle was placed into the vein the animals were given ¼-½ c/c of Heparin solution. (100 U.S.P. units per c/c) This prevented clots from forming in the injecting system. Standard Lead II electrocardiograms were recorded before and after each injection.

Acetylcholine was made up fresh on each experimental day in solutions containing 1 mg per c/c and 10 mg per c/c. A small dose of acetylcholine (about 0.6 c/c of the

solution containing 1 mg per c/c)was then injected into the vein. If it caused 2:1 heart block the dose was decreased until a dose was determined that represented the smallest dose that could be given to consistently produce a 2:1 heart block. When this was determined, 2 c/c of acetylcholine (5 mg per c/c) was given rapidly, if fibrillation was produced then the minimal fibrillating dose was determined by decreasing the dose with subsequent injections. Higher doses of acetylcholine may be given but the animals often die of respiratory arrest.

When this data was obtained, the animal was given the test drug and the "minimal heart block dose" determined every five minutes until the drug had produced its maximal effect. Then the minimal heart block was determined every 15 minutes until it returned to normal.

RESULTS

Figure 20 shows the effects of Benadryl, Linadryl and quinidine on the "minimal heart block dose" of the same animal. This data was collected during three experiments. Each time the control block dose was determined as 0.015 mg/kg before the injection of the test drugs.

Figure 21 shows the comparative effects of Bathine, Benadryl and S-92 on different animals. This accounts for the differences in the control "heart block dose." The results are computed as per cent increase in the minimal

Figure 20. Elevation of 2:1 Heart Block Dose of Intravenous Acetylcholine by Anti-fibrillatory Drugs in the Intact Dog.

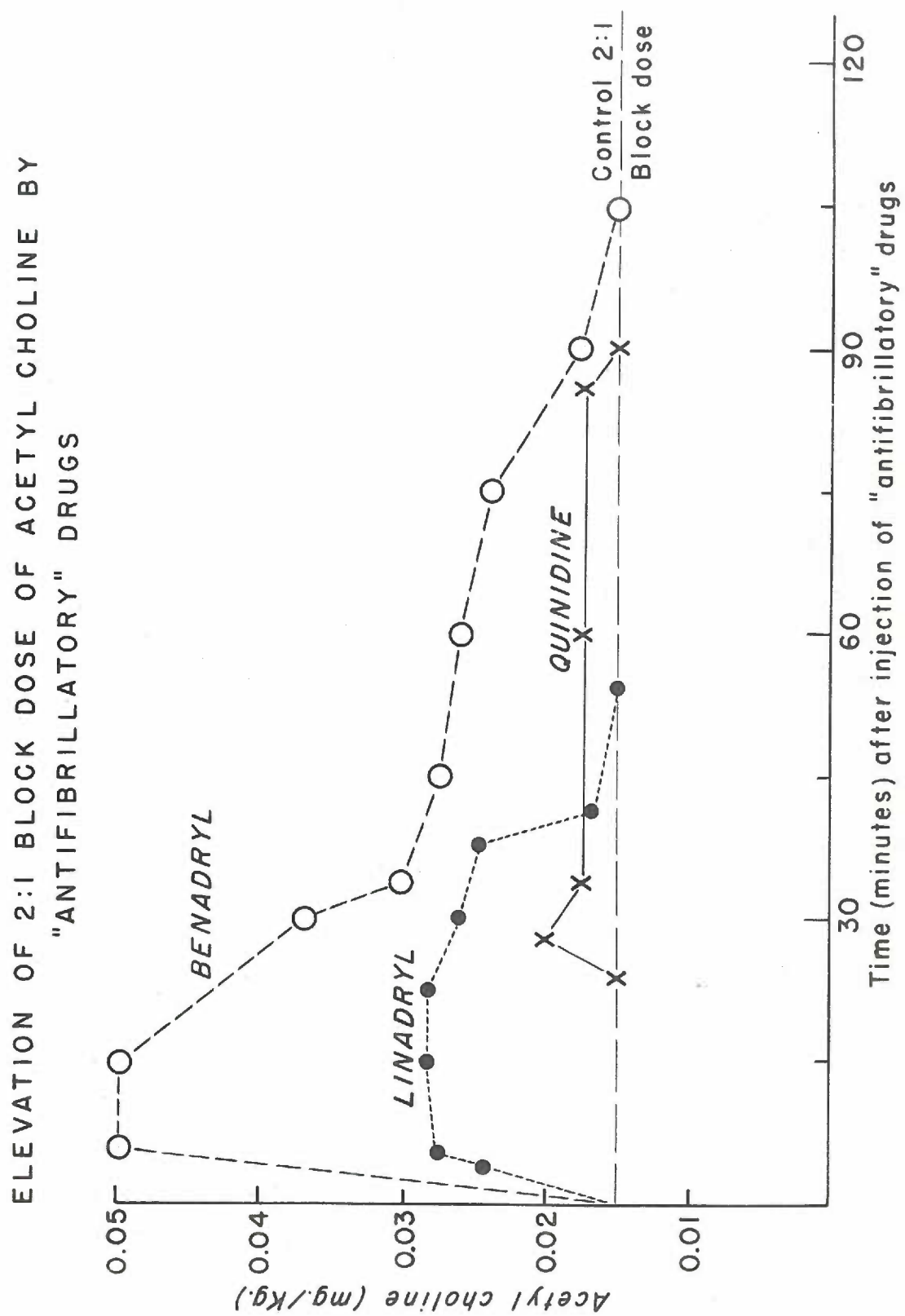
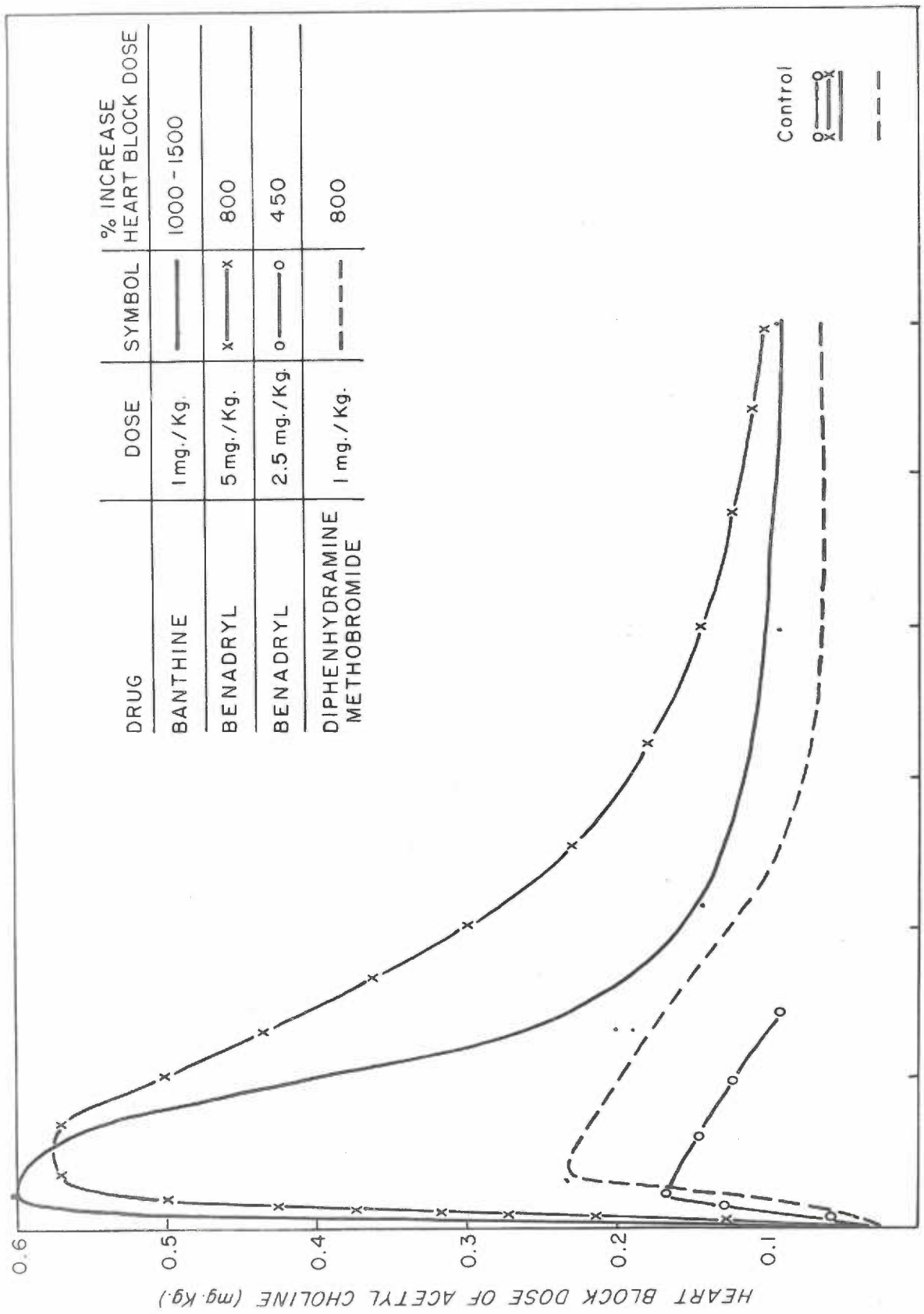

ELEVATION OF 2:1 BLOCK DOSE OF ACETYL CHOLINE BY "ANTIFIBRILLATORY" DRUGS
BENADRYL
LINADRYL
QUINIDINE
Control 2:1
Block dose
Acetyl choline (mg./kg.)
0.05
0.04
0.03
0.02
0.01
30
60
90
120
Time (minutes) after injection of "antifibrillatory" drugs

Figure 21. Elevation of 2:1 heart Block Dose of Intravenous Acetylcholine in Intact Dog by Banthine.


DRUG
DOSE
SYMBOL
% INCREASE HEART BLOCK DOSE
BANTHINE
1 mg./Kg.
1000 - 1500
BENADRYL
5 mg./Kg.
800
BENADRYL
2.5 mg./Kg.
450
DIPHENHYDRAMINE METHOBROMIDE
1 mg./Kg.
800
Control
0.6
0.5
0.4
0.3
0.2
0.1
30
60
90
120
150
180
HEART BLOCK DOSE OF ACETYL CHOLINE (mg./kg.)
TIME (MINUTES) AFTER INJECTION OF "ANTIFIBRILLATORY" DRUGS

heart block dose.

Figure 20 clearly demonstrates the difference in the onset of action of intravenous quinidine and the antihistaminics. The antihistaminics have an immediate action while the action of quinidine is delayed for 20-30 minutes. This is upheld by the clinical observation that intravenous quinidine (104) usually does not affect auricular fibrillation until 20-30 minutes after injections. The duration of action of both drugs is about the same and explains why they must be repeated every three to four hours. (104)

On a mg/kg basis Benadryl apparently is much more effective than quinidine, however the equalizing factor clinically is the dosages of each drug that may be safely administered. White has reported daily oral dosages of quinidine as high as 6 grams without undue side effects. (5) On the other hand, Mackmull (105) indicates that intravenous doses of 200-300 mg of Benadryl will cause dizziness, tingling and thickened speech in 100 per cent of the patients tested. Fifty to seventy-five per cent of these patients will also complain of sleepiness and blurred vision. Tremors and nausea were found in twenty-five per cent of these patients. However, doses as high as 400 mg intravenously have been given to some patients with auricular fibrillation without severe toxic effects. (103)

Figure 20 indicates that such drugs as Banthine and S-92 might be more effective in smaller doses than

Benadryl. At the present time intravenous Banthine is being tested clinically as an antifibrillatory drug but S-92 has not yet been released for clinical trial. However, the results of this and other experiments indicate that it should receive a clinical test as soon as it becomes available. Its advantages lie not only in its increased effectiveness, but experiments have shown that it will protect the auricle from the effects of acetylcholine for periods of 6-8 hours.

DISCUSSION

As was shown in a previous section in this paper, the action of vagal activity or vagomimetic drugs is essentially three-fold. Slowing of the sinus node, increased sensitivity of the auricular musculature and prolongation of the P-R interval are all obvious and measurable actions. Starr (88) has used quinidine to block the bradycardia of vagal stimulation. More recently we have shown that Benadryl has the same action. (91) As yet this factor has not been used to test other antifibrillatory drugs but undoubtedly it will be exploited in the future. Preceding experiments have shown that antifibrillatory drugs decrease the sensitivity of the auricular musculature. However the effects of vagal activity on the conduction time as manifest by prolongation of the P-R interval or production of 2:1 heart block have not previously been utilized as an index of

antifibrillatory activity. The results of this study clearly indicate that antifibrillatory drugs protect the auricle from the effects of acetylcholine on its conduction mechanism. We are not in a position at the present time to evaluate the exact role of conductive defects in the production of auricular fibrillation, but it has been indicated that almost all clinical and experimental auricular fibrillation is preceded by a conduction defect, either prolongation of the P-R interval or heart block. (29, 85, 86, 87) The probable explanation for the above observations is that conduction defects indicate vagal activation but do not in themselves play an important role in the mechanism of fibrillation.

The occurrence of heart block in these experiments is used as an indication of a vagomimetic drug acting on the auricle. We feel that if we can prevent this block with drugs such as quinidine or diphenhydramine, then we are protecting that auricle from the effects of a fibrillatory influence, in this case, acetylcholine.

SUMMARY

1. The minimal heart block dose of acetylcholine has been determined in the dog and used as an index of antifibrillatory activity.
2. These studies indicate that quinidine, Benadryl, Banthine and diphenhydramine-methobromide are all

effective drugs in increasing the "heart block dose" of acetylcholine.

3. The effectiveness of Banthine and S-92 in this respect warrant their clinical trial as antifibrillatory drugs.

THE USE OF EXPERIMENTALLY PRODUCED AURICULAR FIBRILLATION IN DOGS AS AN INDEX FOR TESTING DRUGS FOR ANTIFIBRILLATORY ACTIVITY

The nearest approach to actual clinical trial of the antifibrillatory drugs that we are able to make in the laboratory is the prevention or blocking of experimentally induced auricular fibrillation in animals. The various methods used to produce fibrillation in animals were discussed in the introduction. This series of experiments will be limited to acetylcholine-induced fibrillation in normal and thyrotoxic dogs.

METHODS

The animals used in this series of experiments were selected because they would fibrillate when given small intravenous doses of acetylcholine. Some of these animals were normal animals, others were thyrotoxic animals. The thyrotoxicosis was produced by daily feedings of 1-2 gms of thyroid powder U.S.P. daily for a variable period of time. The animals were anesthetized with pentobarbital given intraperitoneally. A needle was introduced into the saphenous vein for the purpose of injecting drugs. (See section on Experimental Auricular Fibrillation for details) Standard Lead II electrocardiograms were recorded before and during the injection of the acetylcholine. Solutions of acetylcholine were made up fresh each day in strengths of

1 mg/cc and 5 mg/cc. In all of the animals a dose of acetylcholine was established that would consistently produce a short period of auricular fibrillation. The animals were then protected with the antifibrillatory drug and again challenged with the control fibrillating dose of acetylcholine. This was repeated every fifteen minutes until auricular fibrillation was again produced. In addition to measuring antifibrillatory activity, this gives an estimate of the duration and onset of action of the test drug.

This method was varied in some animals. As soon as the control fibrillating dose was determined, the animals were given 0.25 to 0.5 mg of physostigmine intravenously. When acetylcholine was injected after the physostigmine, the resulting auricular fibrillation instead of lasting for several seconds, lasted as long as thirty minutes in some cases. As soon as the fibrillation was well established, the antifibrillatory drug was given and if the arrhythmia was broken shortly afterward, it was assumed that this was due to the injection of the antifibrillatory drug.

RESULTS

Table 5 summarizes the results obtained in this series of experiments. Three antifibrillatory drugs were tested. Benadryl and quinidine were equally effective in intravenous dosages of 2.5 mg/kg. The onset of action was delayed in quinidine but was immediate in Benadryl. The

Table 5. The action of various drugs on experimental auricular fibrillation in the intact dog.

DRUG	DOSE	BLOCKS FIBRILLATION	ONSET	DURATION
S-92	1 mg/kg	Yes	Immediate	5-6 hrs
Quinidine	2.5 mg/kg	Yes	10-15 minutes	1-2 hrs
Benadryl	~~1 c/c/kg~~ 2.5 mg/kg	Yes	Immediate	1-2 hrs
Saline	10 c/c	No	--	--

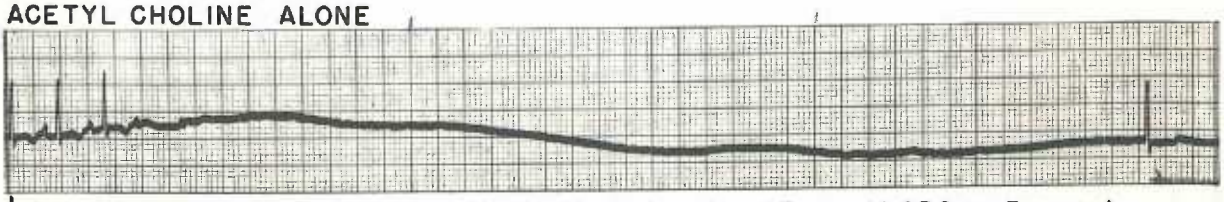
duration of action of both agents was about the same. S-92 a drug that has shown promise in isolated auricle techniques and in the minimal "heart block dose" technique was effective for a period of 5-6 hours in a dose of 1 mg/kg.

Figure 22 demonstrates the manner in which Benadryl protects the animal from induced auricular fibrillation. Auricular fibrillation was consistently produced in this animal when 1 mg/kg acetylcholine was injected intravenously. The first noticeable effect was a prolongation of the P-R interval followed by approximately thirty seconds of cardiac arrest. An irregular ventricular bradycardia with no P waves then was produced, followed by thirty to forty seconds of typical auricular fibrillation. The rhythm then reverted to sinus rhythm with a prolonged P-R interval. When the full effect of the acetylcholine had passed the rhythm returned to normal. This complete cycle usually lasted about three to five minutes. If preceding injections of physostigmine had been made, then the duration of auricular fibrillation was much longer. After the fibrillating dose was determined, the animal was protected with 2.5 mg/kg Benadryl and again challenged with acetylcholine. This time instead of fibrillation there is only prolongation of the P-R interval and complete auricular-ventricular block. As the effects of the Benadryl disappear in one to two hours, the duration of the block increases, then progresses to complete cardiac asystole, and eventually auricular

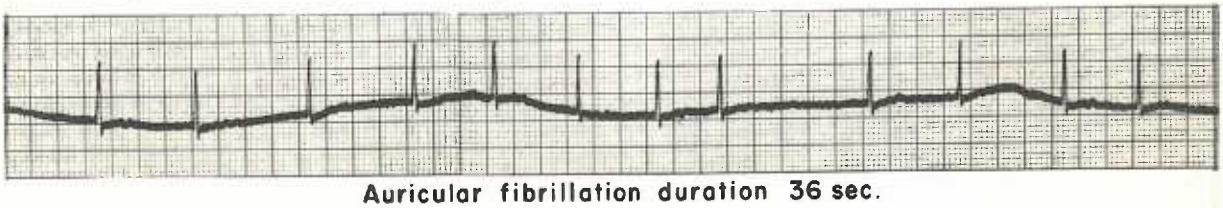
Figure 22. Prevention with Benadryl of Auricular Fibrillation Induced in an Intact Dog with Acetylcholine.

PREVENTION WITH BENADRYL OF AURICULAR FIBRILLATION INDUCED IN AN INTACT DOG

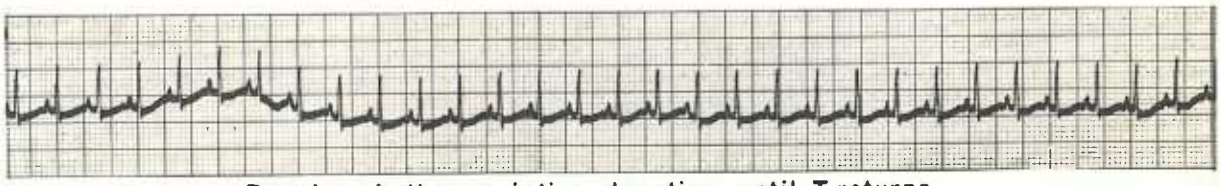
ACETYL CHOLINE ALONE



↑ 1 mg./Kg. acetyl choline Heart block duration 27 sec. (8 QRS no P waves)

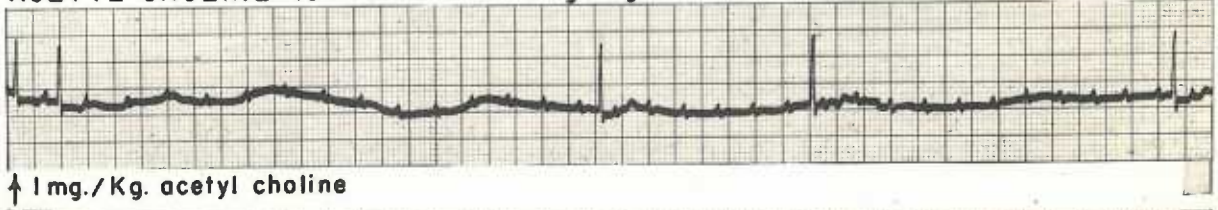


Auricular fibrillation duration 36 sec.

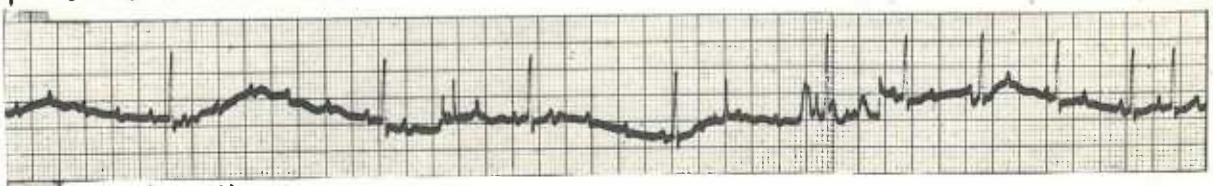


Regular rhythm variation duration until T returns

ACETYL CHOLINE 10 minutes after 5 mg./Kg. BENADRYL HYDROCHLORIDE



↑ 1 mg./Kg. acetyl choline



record continues

fibrillation is produced. Quinidine and S-92 produced similar effects. Control experiments with normal saline had no effect on acetylcholine-induced auricular fibrillation.

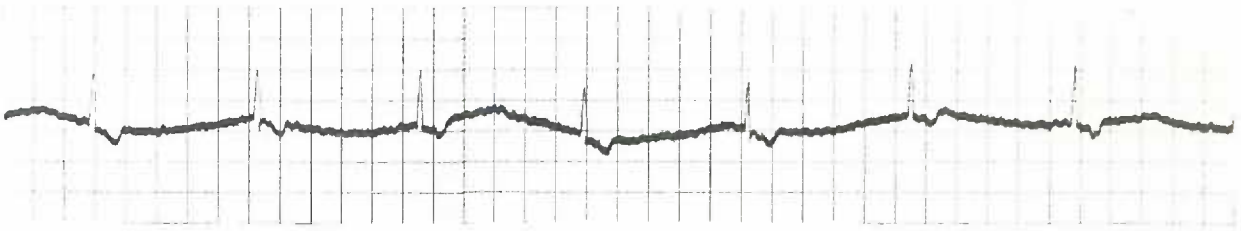
Figure 23 shows the electrocardiographic record of an animal with prolonged auricular fibrillation produced by a combination of physostigmine and acetylcholine. The effects of intravenous Benadryl are shown at various intervals after the injection of the drug. The first effect is an increase in the rate of ventricular contractions with a decrease in the R wave and an increase in the S wave. Later the R wave becomes elevated and the S wave disappears. Large inverted T waves are now found and approximately seventy-five seconds after the injection of Benadryl, the rhythm radically changes to that of 2: 1 heart block; three minutes later there is sinus tachycardia, which is later followed by a normal cardiac rhythm.

DISCUSSION

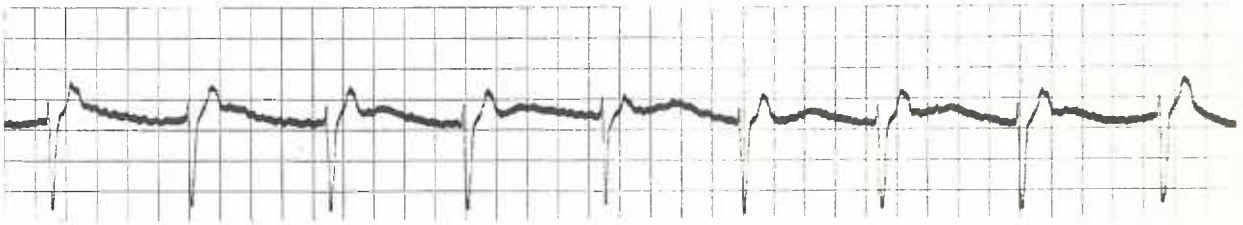
The method of producing auricular fibrillation with acetylcholine is not a new one (44, 45, 87) but merely re-emphasizes the role of vagal influences in the genesis of auricular fibrillation. But we have been able to modify the technique using intravenous physostigmine in conjunction with the acetylcholine. This prolongs the duration of the induced fibrillation by prolonging the action of the

Figure 23. Conversion with Benadryl of Auricular Fibrillation Induced in an Intact Dog with Acetylcholine and Physostigmine.

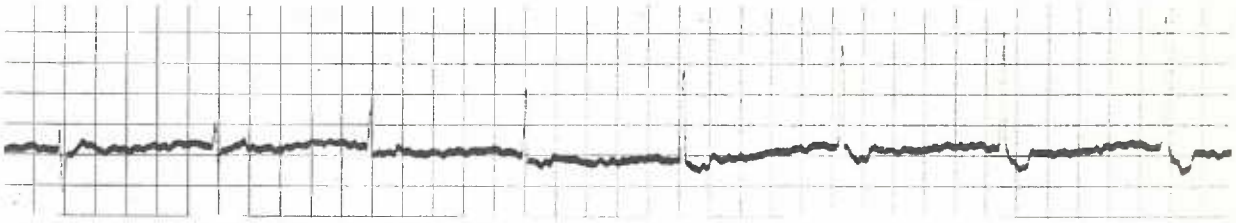
CONVERSION WITH BENADRYL OF AURICULAR FIBRILLATION INDUCED IN AN INTACT DOG WITH ACETYL CHOLINE AND PHYSOSTIGMINE



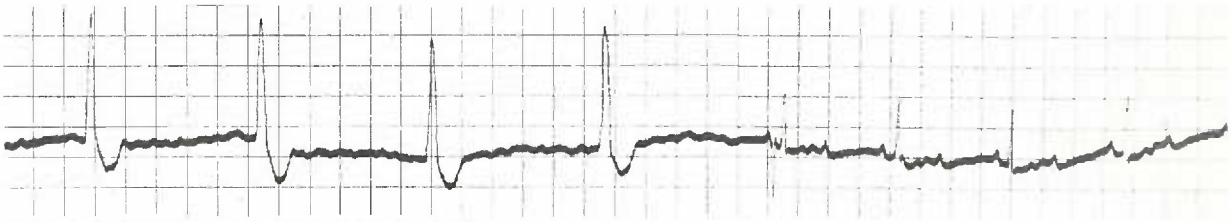
AURICULAR FIBRILLATION



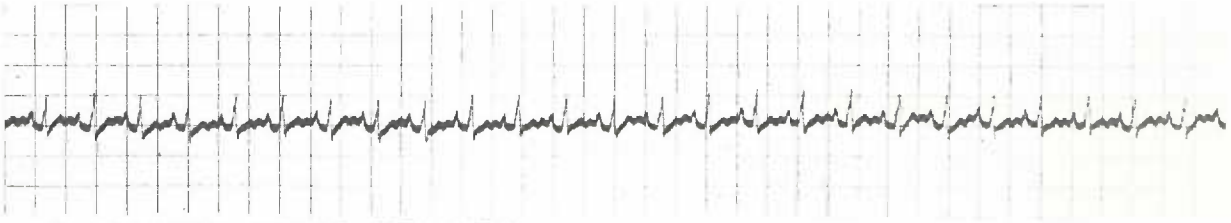
15 SECONDS AFTER 5mg/Kg BENADRYL



45 SECONDS AFTER BENADRYL



75 SECONDS AFTER BENADRYL



180 SECONDS AFTER BENADRYL

acetylcholine and provides us with auricular fibrillation that is second only to clinical fibrillation for the evaluation of antifibrillatory drugs. Needless to say, this is not meant to be a screening technique as the number of normal dogs that will fibrillate on acetylcholine alone is only 20-50 per cent. (45, 49) However, if a colony of animals is fed thyroid powder until the signs of thyroid toxicity are produced, then 80-100 per cent of the animals will fibrillate on acetylcholine. This technique (89) represents the most recent and effective method of producing auricular fibrillation in the intact dog that is available at the present time.

The ability of such drugs as quinidine, Benadryl and S-92 to prevent or break this induced auricular fibrillation probably is a direct result of their effect on the myocardium. It was pointed out in the first part of this paper that all of these drugs decrease the sensitivity and increase the stimulatory threshold of the auricle. If fibrillation is dependent on a lowering of tissue sensitivity by vagal influences, digitalis, or acetylcholine, so that multiple or single ectopic foci may now enter the picture, (10, 19, 39, 55, 56) then the method by which these antifibrillatory drugs protect the auricle becomes apparent. They increase the sensitivity of the auricle to the extent that ectopic foci no longer are able to produce threshold

stimuli and the auricular fibrillation is either broken or prevented.

SUMMARY

1. Auricular fibrillation is produced in normal and hyperthyroid dogs.
2. Physostigmine in combination with acetylcholine prolongs the duration of the induced fibrillation.
3. The induced auricular fibrillation is either broken or prevented by such drugs as quinidine, Benadryl and S-92.
4. It is suggested that S-92 be given a trial on clinical auricular fibrillation as soon as it is released.

SUMMARY OF PART TWO

1. The various methods of producing experimental auricular fibrillation in animals are reviewed.
2. The isolated auricle technique of Dawes has been used to study the action of a series of antihistamine compounds on the myocardial refractory period. The antihistaminics studied prolong the refractory period at concentrations lower than that required by quinidine. Diphenhydramine appeared worthy of clinical trial in patients with auricular fibrillation.
3. Fibrillation or flutter has been evoked in the isolated auricle preparation by a localized traumatic infarct or by aconitine. Quinidine and diphenhydramine can produce a sudden conversion to a slow rhythm in these preparations.
4. The isolated rabbit's auricle was used to study the effect of quinidine and certain antihistaminic drugs on the stimulatory threshold of the auricle at different rates of stimulation.
5. All of the antifibrillatory drugs tested increased the stimulatory threshold of the auricle. The efficiency of the drug was increased as the rate of stimulation was increased.
6. S-92 appears to be 10-20 times as effective as quinidine and should be worthy of clinical trial as soon as it is

released for clinical use.

7. The minimal heart block dose of acetylcholine has been determined in the dog and used as an index of anti-fibrillatory activity.

8. These studies indicate that quinidine, Benadryl, Banthine and diphenhydramine-methobromide are all effective drugs in increasing the "heart block dose" of acetylcholine.

9. The effectiveness of Banthine and S-92 in this respect warrant their clinical trial as antifibrillatory drugs.

10. Auricular fibrillation is produced in normal and hyper-thyroid dogs.

11. Physostigmine in combination with acetylcholine prolongs the duration of the induced fibrillation.

12. The induced auricular fibrillation is either broken or prevented by such drugs as quinidine, Benadryl and S-92.

13. It is suggested that S-92 be given a trial on clinical auricular fibrillation as soon as it is released.

BIBLIOGRAPHY

1. Hering, H. E. Analyse des Pulsus Irregularis Perpetuus, Präger Med. Wchnschr., vol. 28, p. 377, 1903.

2. Cusny, A. R. and Edmunds, C. W. Paroxysmal Irregularity of the Heart and Auricular Fibrillation. Studies in Pathology (edited by Bulloch) Aberdeen, 1906.

3. Rothberger, C. J. and Winterberg, H. Vorhofflimmern und Arythmia perptua. Wiener Klinische Wchnschr., vol. 22, p. 839, 1909.

4. Lewis, T. Auricular Fibrillation, A Common Clinical Condition. Brit. Med. Jour., vol. 2, pp. 15-28, 1909.

5. White, P. D. Heart Disease, Ed. 5, The Macmillan Company. New York, 1931.

6. Frey, W. Klin. Wchnschr., vol. 5, p. 417, 1918.

7. Bellet, S. and Urbach, J. A New Intramuscular Preparation of Quinidine (Gluconate). J. Lab. and Clin. Med., vol. 34, p. 117, Aug. 1949.

8. Burstein, C. L., Marangoni, B. A., Degreff, A. C. and Rovenstine, E. A. Laboratory Studies on the Prophylaxis and Treatment of Ventricular Fibrillation Induced by Epinephrine During Cyclopropane Anesthesia. Anesthesiol., vol. 1, pp. 167-186, 1940.

9. McCawley, E. L. and White, J. M. Protective Agents for Epinephrine Cyclopropane-induced Cardiac Arrhythmias. Am. J. Med., vol. 8, p. 526, 1950.

10. Mac William, J. A. Fibrillary contractions of the Heart. J. Physiol., vol, 8, p. 296, 1887.

11. Garrey, W. E. The Nature of Fribrillary Contraction of the Heart - Its Relation to Tissue Mass and Form. Am. J. Physiol, vol. 33, p. 397, 1914.

12. Mayer, A. G. Rhythmical Pulsations in Scyphomedusae. Papers from the D pt. of Marine Biology of the Carnegie Institute of Washington, p. 115, 1908.

13. Garrey, W. E. The Suppression of Cardiac Fibrillation by Section. Interstate Med. Jour., Chicago, vol. 19, p. 1081, 1912.

14. Mc William, J. A. Fibrillar Contractions of the Heart. J. Physiol, vol. 8, p. 296, 1887.

15. Lewis, T. Auricular Fibrillation. Br. Med. J., vol. 1, p. 590, 1921.

16. Porter, W. T. On the results of ligation of the coronary Arteries. J. Physiol., vol. 15, p. 134, 1894.

17. Englemann, T. W. Über den einfluss der systole auf die Motorische Leitung in der herzkammer, mit bemerkungen zur Theorie Allorhythmischer herzstörungen, Onderzoek. ged. h. physiol. Lab. d. Utreiht 74, 1896.

18. Prinzmetal, M., Corday, E., Brill, I.C., Sellers, A. L., Oblath, R. W., Flieg, W. A., Kruger, H. E. Mechanism of the Auricular Arrhythmias. Circulation, vol. 1, no. 2, Feb. 1950.

19. Prinzmetal, M. O., Oblath, R., Corday, E., Brill, I. C., Kruger H. E., Smith, L. A., Fields, J., Kennamer, R., and Osborne J. A. Auricular Fibrillation. J. A. M. A., vol, 146, p. 14, Aug. 4, 1951.

20. Lewis, T. and Drury, A. N., Revised Views of Refractory Period. Heart, vol. 13, p. 95, 1926.

21. Sagall, E. L., Horn, C. D. and Riseman, J. E. Studies on the Action of Quinidine in Man. Arch. Int. Med., vol. 71, p. 460, 1943.

22. DiPalma J. R. and Schultz, J. E. Antifibrillatory Drugs. Medicine, vol. 29, no. 2, p. 126, May 1950.

23. Roth, I. R. The Mechanics of Auricular Flutter and Fibrillation. J. Mt. Sinai Hosp., vol. 8, p. 965, 1942.

24. Dawes, G. S. Synthetic Substitutes for Quinidine. Brit. J. Pharmacol, vol. 1, pp. 90-112, 1946.

25. De Elio. F. J., Action of Acetylcholine, Adrenaline, and Other Substances on the Refractory Period of the Rabbit Auricle, Brit. J. Pharm. and Chemotherapy. vol. 2, p. 131, 1947.

26. DiPalma, J. R. and Mascatello, A. V., Analysis of the Actions of Acetylcholine, Atropine, Epinephrine and Quinidine on the Heart Muscle of the Cat. J. Pharm. Exp. Thera., vol. 101, p. 3. March 1951.

27. Schlichter, J. G. Etiology of Auricular Fibrillation and the Mechanisms of its Perpetuation, Am. Heart J., vol. 37, p. 674, 1949.

28. Harrison, T. R., Principles of Internal Medicine Blackiston Co. Toronto, 1950.

29. Horlick, and Surtshin A. The Role of Anemia in the Experimental Production of Heart Block and Auricular Fibrillation in the Dog. Am. Heart J., vol. 38, no. 5, pp. 716-731, Nov. 1949.

30. Altschule, M. D. The Relation Between Prolonged P-R Interval and Auricular Fibrillation in Patients with Rheumatic Heart Diseases. Am. Heart J., vol. 18, p. 1, 1939.

31. Fulton, J. F. Howell's Text of Physiology, W. B. Sanders Co. Philadelphia, 1946.

32. Bozler, E. The Initiation of Impulses in Cardiac Muscle. Am. J. Phys., vol. 138, p. 273, 1943.

33. Bozler, E. The Activity of the Pacemaker Previous to Discharge of a Muscular Impulse. Am. J. Physiol, vol, 136, p. 543, 1946.

34. Andrus, E. C., and Carter E. P. Refractory Period of the Normally Beating Dog's Auricle with a Note on Occurrance of Auricular Fibrillation Following a Single Stimulus, J. Exper. Med., vol. 51, p. 357, 1930.

35. Nahum, L. H. and Hoff, H. E. The Prevention of Ventricular Fibrillation Following Electric Shock of Low Voltage by the Injection of Acetyl B-methylcholine Chloride. Am. J. Physiol., vol. 110, pp. 675-680, Jan. 1935.

36. Altschule, J. D., Relation Between Vagal Activity and Auricular Fibrillation in Various Clinical Conditions., N. E. J. Med., Vol. 233 p. 265, 1945.

37. Nahum, L. H. and Hoff, H. E., Production of Auricular Fibrillation by Application of Acetyl-β-Methylcholine Chloride to Localized Regions in the Auricular Surface. Am. J. Physiol. vol. 129, p. 428, 1940.

38. Nahum, L. H. and Hoff, H. E. Auricular Fibrillation in Hyperthyroid Patients Produced by Acetyl-β-methylcholine Chloride with Observations on the Role of the Vagus and some Exciting Agents in the Genesis of Auricular Fibrillation. J.A.M. A., vol. 105, p. 254, 1935.

39. Prinzmetal, M., Corday, E., Brill, I., Oblath, R. W., Kruger, H. E. The Auricular Arrhythmias. 1st. ed. Charles C. Thomas. 1952.

40. Weston, C. A. and Mc Cawley, E. L. Unpublished data.

41. DiPalma, J. R. and Schultz, J. E. Antifibrillatory Drugs, Medicine, vol. 29, no. 2, p. 126, May 1950.

42. Fulton, J. F. Howell's Text of Physiology, W. B. Sanders Co. Philadelphia, 1946.

43. Stotler, W. A., & McMahon, R. A. The Innervation and Structure of the Conductive System of the Human Heart. J. Comp. Neur. vol. 87, no. 2, p. 57, October 1947.

44. Grant, R., Gertler, M. M. Terroux, K. G., Atrial Fibrillation Induced by Epinephrine in Hypothermic Dogs. Am. Heart Jr., vol. 37, no. 7, pp. 1081-1089, June 1949.

45. Horlick and Surtshin A., The Role of Anemia in the Experimental Production of Heart Block and Auricular Fibrillation in the Dog. Am. Heart J., vol. 38, no. 5, pp. 716-731, Nov. 1949.

46. Rosenblueth, A. and Garcia Ramos, S. Arch. Inst. Cardiol. Mexico, vol. 17, p. 302, 1947.

47. Bolger, E. The Initiation of Impulses in Cardiac Muscle. Am. J. Physiol., vol. 138, p. 27, 1922.

48. Lewis T. and Master, A. M. Observations upon Conduction in the Mammalian Heart; A-V Conduction. Heart, vol. 12, p. 209, 1925.

49. Weston, G. A., and Mc Cawley, E. L., Unpublished data.

50. Scherf, D. Studies on Auricular Tachycardia caused by Aconitine Administrat. Proc. Soc. Exper. Biol. and Med., vol. 65, p. 233, 1947.

51. Scherf. D., Romano, F. J., Terranova, R. Experimental Studies on Auricular Flutter and Auricular Fibrillation. Am. Heart J., vol. 36, no. 2, p. 241, 1948.

52. Starr, I., Elsom, K. A., Reisinger, J. A. and Richards, A. N. Acetyl-B. Methylcholine Action on Normal Persons with a Note on Action of Ethyl Ether of B-Methylcholin. Am. J. M. Sc., vol. 186, pp. 313-323, Sept. 1933.

53. Weston, G. A. and Mc Cawley, E. L. Upublished data.

54. De Elio, F. J. Action of Acetylcholine, Adrenaline, and other Substances on the Refractory Period of the Rabbit Auricle. Brit. J. Pharm. and Semotherapy vol. 2, p. 131, 1947.

55. Bijlsma, U. G. and Van Dongen, K. Experimentelle Therapie des Flatterns and Flimmerns des Herzens. Ergebn d. Physiol. vol. 41, p. 1, 1939.

56. Van Dongen, K., and Taal, A. Remarks on the Mechanisms of Heart Fibrillation. Arch. Internat. de pharmacodyn et therap., vol. 81, p. 129, Feb. 1950.

57. Mc Cawley, E. L., Dick, H. L. H., Weston, G. A. Unpublished Data.

58. Boas, E. P., and Goldschmidt, E. F. The Heart Rate. Thomas Publish. Co. Springfield, Ill. 1932.

59. Smith, F. M. and Moody, W. B. The Induction of Premature Contractions and Auricular Fibrillation by Forced Breathing. Arch. Int. Med., vol. 32, p. 192, 1923.

60. Otto, H. L. The Action of Epinephrine upon the Cardiac Rhythms. J. Lab. and Clin. Med., vol. 13, p. 70, 1927.

61. Cowan, J., and Ritchie, W. T. Diseases of the Heart. Edward Arnold and Co. London. 1922.

62. Winterberg, H., Stadien über Herzflimmern. II Mitteilung. Uber die Beeinflussung des Herzflimmerns durch einige Gifte, Arch f.d. ges. Physiol., vol. 122, p. 361, 1908.

63. Rosenblum, H., Hahn R. G. and Levine S. A. Epinephrine, Its Effect on the Cardiac Mechanism in Experimental Hyperthyroidism and Hypothyroidism. Arch. Int. Med., vol. 51, p. 279, 1933.

64. Hoffman, F., Hoffman E. J. Middleton, S., and Talesnik J. The Simulating Effect of Acetylcholine on the Mammalian Heart and the Liberation of an Epinephrine-like Substance by the Isolated Heart. Am. J. Physiol., vol. 144, p. 189, 1945.

65. Mc Dowell, R. J. S. The Production of an Adrenalin-like Substance by the Heart. J. Physiol., vol. 104, p.392, 1946.

66. Smith, J. R. and Wilson, K. S. Studies on the Production and Maintenance of Experimental Auricular Fibrillation. Am. Heart J., vol. 27, p. 176, 1944.

67. Dill, D. B. and Forbes, W. H. Respiratory and Metabolism Effects of Hypothermia. Am. J. Physiol., vol. 132, p. 685, 1941.

68. Wayburn, E. Immersion Hypothermia. Arch. Int. Med., vol. 79, p. 77, 1947.

69. Talbott, J. H. The Physiologic and Therapeutic Effects of Hypothermia. New England J. Med., vol. 224, p. 281, 1941.

70. Grosse - Brockhoff, F., and Schoedel, W. Das Bild der Akuten Unterkuklung in Tierexperiment. Arch. F. Exper. Path. Pharmakol., vol. 201, p. 417, 1943.

71. Drury, A. N. Further Observations upon Intra-Auricular Block Produced by Pressure or Cooling. Heart., vol. 12, p. 143, 1925.

72. Resnik, W. H. Observations on the Effect of Anoxemia on the Heart. J. Clin. Invest., vol. 2, p. 125, 1925.

73. Wiggers, C. J. The Mechanism and Nature of Ventricular Fibrillation. Am. Heart J., vol. 20, p. 399, 1940.

74. Yater, W. M. The Tachycardia, Time Factor, Survival Period, and Seat of Action of Thyroxine in the Perfused Hearts of Thyroxinized Rabbits. Am. J. Physiol., vol. 98, pp. 338-343. Sept. 1931.

75. Lewis, J. K. and Mc Eachern, D. Persistence of an Accelerated Rate in Isolated Hearts and Isolated Auricules of Thyrotoxic Rabbits. Bull. Johns Hopkins Hosp., vol. 48, p. 228, 1931.

76. Aumann, K. W. and Youmans, W. B. Differential Sensitization of the Adrenergic Neuroeffector System by Thyroid Hormone. A. J. Physiol. vol. 131, p. 394, 1940.

77. Weston, G. A., Mc Cawley, E. L., Unpublished data.

78. Ryster, J.A.E. and Hooker, D. R. Direct and Reflex Response of Cardioinhibitory Center to Increased Blood Pressure. Am. J. Physiol., vol. 21, p. 373, 1908.

79. Bainbridge, F. A. Influence of Venous Filling Upon Rate of Heart. J. Physiol. vol. 50, pp. 65-84, 1915.

80. Scherf, D., Scharf, M. M., and Conklen, M. F., Effect of Stretch and Pressure on Stimulus Formation in the Dog's Auricle. Proc. Soc. Exper. Biol. and Med., vol. 70, p. 708, 1949.

81. Mc Cawley, E. L., Weston, G. A., and Dick, H. L. H. Evaluation of Drugs for Possible Use in Therapy of Auricular Fibrillation. Fed. Proc., vol. 1, no. 1, Part 1. March, 1951.

82. Mc Cawley, E. L., Weston G. A., Experimental Evaluation of Drugs for Clinical Auricular Fibrillation. Am.Jr.Med.vol. 10, no 6 pp.766-767 June, 1951.

83. Bruenn, H. G. Mechanism of Impaired Auriculo-ventricular Conduction in Acute Rheumatic Fever. Am. Heart J., vol. 13, pp. 413-425, 1937.

84. Keith, J. D. Overstimulation of Vagus Nerve in Rheumatic Fever. Quart. J. Med., vol. 7, pp. 29-41, 1938.

85. Klainer, J. J. and Altschule, M. D. Prolongation of the P-R Interval in Patients with Paroxysmal Auricular Fibrillation and Flutter Following Myocardial Infarction. Am. J. Med. Sc., vol. 203, p. 215, 1942.

86. Noth, P. H., Essex, H. E., and Barnes, A. R., The Effect of the Intravenous Injection of Acetyl-choline on the Electrocardiogram of the Dog. Proc. Staff Meet. Mayo Clin., vol. 14, pp. 348, 1939.

87. Iglauer, A., Davis, D., and Altschule, M. Auricular Fibrillation in Normal Intact Animals after the Intravenous Injection of Mecholyl. Am. Heart Jr., vol. 22, p. 47, 1941.

88. Starr, I. Antagonism Between the Cardiac Action of Acetyl - β - Methylcholine and Acetylcholine and that of Quinidine. J. Pharm. & Exp. Therp., vol. 56, p. 77, 1936.

89. Weston, G. A. and Mc Cawley, E. L., Unpublished data.

90. Krayer, O. The Antiaccelerator Cardiac Action of Quinine and Quinidine. The J. Pharm. & Exp. Thera., vol. 100, p. 147, Oct. 1950.

91. Weston, G. A. and McCawley, E. L. Unpublished data.

92. Phatak. N. M. Unpublished data.

93. Dawes, G. S. Synthetic Substitutes for Quinidine. Brit. J. Pharm. vol. 1, p. 90, 1946.

94. Wedd, A. M., Blair, H. A., and Gosselin, R. E. The action of Quinidine on the Cold Blooded Heart. Journal of Pharm. and Exper. Thera., vol. 75, p. 251, 1942.

95. Jackson, D. E. Experimental Pharmacology and Materia Medica. (2nd Ed.) C. V. Mosby Co. St. Louis, 1939.

96. Mc Cawley, E. L., Weston, G. A., and David, N. A. Evaluation of Certain Antihistaminics for Use in Auricular Fibrillation. J. Pharmacol. and Exper. Therap. vol. 102, pp. 250-257, 1951.

97. Mc Cawley, E. L., Stanwood, J. E., and David, N. A. Substitutes for Procaine in Ventricular Tachycardia, Fed. Proc., vol. 9, p. 82, 1950.

98. Alles, G. A., and Ellis, C. H. Comparative Actions of Certain Compounds Like Alpha-Pagarine. Jr. Pharmacol. and Exp. Thera., vol. 94, p. 416, 1948.

99. Weston, G. A. and Mc Cawley, E. L. Unpublished Data.

100. Dutta, N. K. Some Pharmacological Properties Common to Antihistamine Compounds. Brit. J. Pharmacol., vol. 4, p. 281, 1949.

101. Loew, E. R., Kaiser, M. E., and Moore v., Synthetic Benzhydryl Alkamine Ethers Effective in Preventing Fatal Experimental Asthma in Guinea Pigs Exposed to Atomized Histamine. Jr. Pharmacol. and Exp. thera., vol. 83, p. 120, 1945.

102. Mc Gavack. T. H. Schulman, P. M. and Boyd, L. J. A clinical Investigation of Beta-Morpholino-Ethyl Benzhydryl Ether Hydrochoride (Linadryl) as an Antihistaminic Agent. J. Allergy, vol. 19,p.141, 1948.

103. Dick, H. L. H., Mc Cawley, E. L., and Weston, G. A. Unpublished Data.

104. Katz, Louis N. Quinidine, J.A.M.A., vol. 136, p. 1028, 1948.

105. Mackmull, G., The Influence of I. V. Administered Benadryl on the Blood Pressure and Electrocardiogram. J. Allergy, vol. 19, pp. 365-370, 1948.