SINOATRIAL FREQUENCY AND CHRONOTROPIC RESPONSE IN THE RAT

REDUCTION OF INTRINSIC SINOATRIAL FREQUENCY AND CHRONOTROPIC RESPONSIVENESS IN THE EXERCISED RAT

By

Richard Lee Hughson, B. Sc. M. Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

April, 1977

DOCTOR OF PHILOSOPHY (1977)
(Medical Science)

McMaster University
Hamilton, Ontario

TITLE: Reduction of Intrinsic Sinoatrial Frequency and Chronotropic Responsiveness in the Exercised Rat.

AUTHOR: Richard Lee Hughson, B. Sc. (University of Western Ontario)
M. Sc. (Univeristy of British Columbia)

SUPERVISORS: Dr. N. L. Jones
Dr. J. R. Sutton
Dr. J. D. Fitzgerald

NUMBER OF PAGES: x, 159

Abstract:

The changes in the physiological properties of the sinoatrial node which follow a programme of exercise training have been examined in the rat. The influence on the intrinsic sinoatrial frequency (ISF) of altered levels of autonomic nervous activity accompanying the physical training has been studied in two series of experiments combining daily pharmacological agents with ten weeks of treadmill exercise. The first series (Study 1) examined the influence on the ISF of the increased parasympathetic activity reported in the trained state by blocking with atropine or stimulating with carbachol the receptors of the parasympathetic system in the heart. The second series (Study 2) examined the influence on the ISF of the increased sympathetic activity which occurs during exercise by stimulating with noradrenaline or isoprenaline, or blocking with propranolol the receptors of the sympathetic system in the heart. The content of potassium (K) and sodium (Na) in the plasma and myocardium were measured in all animals at the completion of the ten week period, and an estimate of electrophysiological changes was derived from the plasma to myocardial K^+ ratio (K_o^+/K_i^+). In addition, the chronotropic response of the sinoatrial node to acetylcholine and noradrenaline was examined *in vitro* for all animals in Studies 1 and 2. The chronotropic responses of the sinoatrial node to exercise and noradrenaline were further explored in the *in vivo* experiments of Study 3.

In Study 1, ISF was reduced with training. Blockade of the parasympathetic receptors with atropine also reduced ISF, and the effects

of exercise and atropine appeared to be additive. Stimulation of the parasympathetic receptors with carbachol had little effect on ISF. In Study 2, stimulation of the sympathetic receptors with either noradrenaline or isoprenaline caused a reduction in ISF, but when combined with exercise, no reduction occurred. Sympathetic receptor blockade with propranolol reduced ISF, and propranolol plus exercise also reduced ISF. It appears that the exercise-induced reduction of ISF was not a consequence of increased parasympathetic activity in the trained state, or of increased sympathetic activity during exercise. The K_o^+/K_i^+ was reduced with training. This was consistent with a more negative maximum diastolic repolarization, and a reduced ISF. The K_o^+/K_i^+ of the drug treated groups indicated that ISF was probably reduced through factors other than a more negative maximum diastolic repolarization. No difference was found in the sensitivity of the atria of any group to acetylcholine *in vitro*. Increased chronotropic sensitivity to noradrenaline was found *in vitro* in the two chronically noradrenaline treated groups. The maximum response to noradrenaline was reduced *in vitro*. The maximum cardiac frequency in response to either exercise or noradrenaline infusion *in vivo* was also reduced. This reduction of maximum frequency in both the atria and the intact heart was observed to be positively correlated with the ISF, thus those hearts with a low ISF obtained a low maximum frequency. This observation led to the proposal that electrophysiological properties of the sinoatrial node which establish the ISF are also involved in imposing a limit on the maximum sinoatrial frequency.

Acknowledgements:

This Thesis could not have been completed without the guidance of my Committee members, Dr. N.L. Jones, Dr. J.R. Sutton, Dr. J.D. Fitzgerald and Dr. M. Todd. The helpful discussions with Dr. J.F. Cade about the initial study design are also gratefully acknowledged. Various technical contributions have come from Mr. Tony Bailey (who allowed me to use the Technicon Autoanalyzer), Mr. David Cragg (who willingly accommodated me in his laboratory area), and Dr. L. Belbeck, Director of Animal Services.

Thank you to Nadia for her patience.

A generous supply of propranolol was received from Mr. H. LeMien of Ayerst Laboratories. The l-levophed bitartrate and isuprel were donated by Dr. J. Abel of Winthrop Laboratories.

This research was supported by a grant from the Ontario Heart Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I: INTRODUCTION

The sinoatrial node is the pacemaker of the heart. In the absence of neural influences on the sinoatrial node, the property of autorhythmicity of the sinoatrial cells maintains cardiac contraction at a frequency determined solely by the intrinsic properties of the sinoatrial cells. This discharge frequency of the denervated sinoatrial node is defined as the intrinsic sinoatrial frequency (ISF).

The ISF can be determined in man by the pharmacological blockade of the receptors for the sympathetic and parasympathetic nervous activity to the sinoatrial node. This was first accomplished by Jose (1966) employing atropine, a muscarinic (parasympathetic receptor blocker) in combination with propranolol, a beta-adrenergic (sympathetic) receptor blocker. In animals, sympathetic and parasympathetic nervous activity to the heart may be interrupted by pharmacological receptor blockade (Jose and Stitt, 1969; Donald and Samueloff, 1966) or by surgical denervation (Cannon, Lewis and Britton, 1926; Cooper et al, 1961). The heart has also been investigated in the isolated organ bath to study intrinsic properties of the sinoatrial node (Bolter, Hughson and Critz, 1973).

In 1967, Sutton et al (1967) studied the ISF in a group of young men by the method of Jose (1966). These investigators observed a wide range of values of ISF and noted the lowest values to be in a group of highly trained endurance athletes. Sutton further observed a significant correlation between the ISF and the maximum aerobic capacity throughout the entire group. To show that ISF was related to the fitness level of an individual, Sutton et al (1967) trained a group of young men and observed

that a reduction in ISF accompanied the anticipated increase in aerobic capacity.

More recent animal experimentation has confirmed the findings of Sutton et al (1967). Lin and Horvath (1972) found a lower ISF in a group of swim trained rats than in inactive controls. Bolter, Hughson and Critz (1973) observed the ISF in the isolated right atrium of swim trained rats to be lower than inactive controls. These findings suggest that some aspect of physical training is responsible for the observed reduction in ISF, but no studies have examined this problem.

In this thesis the question of the reduction of ISF with physical training has been investigated. Raab (1969) postulated that changes in the autorhythmicity (ISF) of the sinoatrial node may result from alterations in the balance of autonomic nervous activity. The relative importance of the parasympathetic or sympathetic nervous activity was not apparent to Raab (1969) from his review of the literature. It was suggested, therefore, that "the autonomic nervous effects of the acts of exercising and the resulting state of being trained"[1] should be studied independently to assess the relative influence of each on the ISF. An increase in resting parasympathetic nervous activity is the major change in autonomic nervous control of the heart associated with the state of being trained (Hall, 1964; Tipton and Taylor, 1965; Bolter, Hughson and Critz, 1973; Ekblom, Kilbom and Soltysiak, 1973), and in increase in sympathetic activity is the major change in autonomic nervous control of the heart associated with exercise (Vendsalu, 1969; Ekblom, Kilbom and

1. Raab, W. (1969) Myocardial electrolyte derangement: crucial feature of pluricausal, so-called coronary, heart disease. Ann. N.Y. Acad. Sci. 147: 629-686 p. 658.

Soltysiak, 1973). Based on these changes in autonomic activity associated with the two components of physical training (the state of being trained and the act of exercise), this thesis first examined the reduction of ISF in terms of altered autonomic nervous balance. Study 1 and Study 2 were designed to investigate the following questions:

(1) Study 1: Does an increase in resting parasympathetic nervous activity in the trained state act on the sinoatrial node to produce the exercise-induced reduction of ISF?

(2) Study 2: Does the increase in sympathetic nervous activity during physical exercise act on the sinoatrial node to produce the exercise-induced reduction of ISF?

These were the first questions asked in this thesis, and will be examined in detail in the Discussion under the headings: "Study 1: Parasympathetic Influences on ISF", and "Study 2: Sympathetic Influences on ISF". The questions were investigated by the combination of treadmill exercise training in the rat, with stimulation or blockade of the parasympathetic receptors of the sinoatrial node in Study 1, and by the combination of exercise training with stimulation or blockade of the sympathetic receptors of the sinoatrial node in Study 2. This thesis has employed treadmill exercise in contrast with the previous investigations employing swimming training of Lin and Horvath (1972) and Bolter, Hughson and Critz (1973) since swimming is associated with possible alterations in autonomic activity and changes in body temperature independent of the exercise, as will be described in the Discussion.

The electrophysiological properties of the sinoatrial nodal cells must change with the exercise-induced reduction of ISF. Raab (1969) had

proposed that alterations in autonomic nervous balance would change the myocardial electrolyte distribution resulting in the reduction of ISF. Although it is clear that electrolyte distribution is a major contributor to the electrophysiology of the sinoatrial node, other properties such as membrane permeability also influence the ISF (Hodgkin and Katz, 1949; Brady, 1974; Noble, 1975). This thesis has employed electrolyte content in the plasma and the myocardium to give an indication of electrolyte distribution and membrane potential. Using this method, the questions can be asked:

(3) Is the exercise-induced reduction of ISF associated with changes in electrolyte distribution between the myocardium and plasma?

(4) Are any changes in ISF produced by pharmacological intervention associated with similar shifts in electrolyte distribution as found with exercise?

These questions will be examined in detail in the Discussion under the heading "Electrophysiology of ISF".

Cardiac frequency in the intact animal is dependent not only upon the ISF, but also upon the influence of the autonomic nervous system. The neurotransmitter of the parasympathetic nervous system, acetylcholine, acts on the sinoatrial node to decrease cardiac frequency. The neurotransmitter of the sympathetic system, noradrenaline, acts on the sinoatrial node to increase cardiac frequency. The balance of parasympathetic and sympathetic activities to the sinoatrial node determines the cardiac freuqency (Grodner et al, 1970). Sensitivity of the sinoatrial node to the neurotransmitters has been reported to change following training. Bolter, Hughson and Critz,

(1973) observed a reduced sensitivity to acetylcholine in swim trained rats. This finding was interpreted as an indication of increased resting vagal activity, based on the inverse relationship between vagal activity and acetylcholine responsiveness established by Bito, Dawson and Petrinova (1970). Sensitivity of the sinoatrial node to noradrenaline has been proposed to decrease following training. In humans, Brundin and Cernigliaro (1976) interpreted a lower cardiac frequency with similar urinary catecholamines post-training to suggest decreased sensitivity to noradrenaline. Ekblom et al (1973) observed a smaller cardiac frequency response in treadmill trained rats to 6-hydroxydopamine, a drug which causes acute release of noradrenaline during terminal axon destruction (Haeusler, 1971; Thoeman and Tranzer, 1968). Ekblom et al suggested a decreased sensitivity to catecholamines could be a part of the adaptation mechanism. Both the study of Brundin and Cernigliaro (1975) and of Ekblom et al (1973) represent indirect assessments of sinoatrial sensitivity to catecholamine. There are no reports of directly measured sensitivity of the sinoatrial node to noradrenaline. Two further questions arise in the study of sinoatrial sensitivity to the natural autonomic transmitters:

(5) Is there a decreased sensitivity to acetylcholine with physical training in the present Studies as in the work of Bolter, Hughson and Critz (1973), and what are the effects of the drugs of Study 1 and Study 2 on acetylcholine sensitivity?

(6) Is there a decreased sensitivity to noradrenaline with physical training as suggested in the literature, and what are the effects of the drugs employed in Study 1 and Study 2 on noradrenaline sensitivity?

The answers to these questions were sought employing a modification of the method of Bolter, Hughson and Critz (1973) for studying the isolated right atrial preparation. The results will be discussed under the headings: "Chronotropic Responses to Acetylcholine in vitro", and "Chronotropic Responses to Noradrenaline in vitro".

In the course of Study 1 and Study 2, it became apparent that the responsiveness of the sinoatrial node of trained rats to noradrenaline was different from the control response. Atria with low values of ISF obtained low maximal frequencies in response to noradrenaline. This observation was felt to be similar to the findings in humans of a decrease in maximum cardiac frequency following a training program (Brorson et al., 1976), and may also be important in view of the findings of Sutton et al (1967) that ISF is reduced by physical training. The in vitro findings of Study 1 and Study 2 led to the formation of the question in Study 3:

(7) Study 3: Does a decrease in ISF result in a reduction of maximum cardiac frequency in vivo?

To answer this question, a group of rats with a wide range of values of ISF were studied for the maximum cardiac frequency responses to exercise and to noradrenaline infusion. The results of Study 3 will be discussed under the heading: "In Vivo Chronotropic Responses".

I-1: Summary

This thesis will examine the reduction of the intrinsic sinoatrial frequency (ISF) with physical training, and also the role of the ISF in establishing cardiac frequency at rest and with maximal sympathetic stimulation. A series of seven major questions were asked and the answers

will be sought in the following studies.

Study 1 will examine question number (1) concerning the role of increased resting parasympathetic activity in the reduction of ISF. In addition the changes in electrolytes described in questions (3) and (4) will be investigated in Study 1, as well as the possible changes in sensitivity to acetylcholine (question (5)) and noradrenaline (question (6)).

Study 2 will examine question number (2) concerning the role of increased sympathetic activity during exercise in the reduction of ISF. The same questions described above concerning changes in electrolytes (questions (3) and (4)) and the sensitivity to acetylcholine (question (5)) and to noradrenaline (question (6)) will also be investigated in Study 2.

Study 3 will investigate the relationship proposed in question (7) between ISF and maximum cardiac frequency <u>in vivo</u>.

II: HISTORICAL OVERVIEW

II-1: Intrinsic Sinoatrial Frequency

"Indeed, one may wonder if the blood or spirit earlier has that throbbing within it ...."[1] This vitalistic assessment of the contraction of the heart was made by William Harvey (1578-1657) while describing the circulation. We now understand that rather than a "spirit" initiating the contraction of the heart, "the heart beat arises in a relatively small area which, because it possesses the property of automaticity to the highest degree, initiates impulses for the rest of the heart."[2] The area initiating the contraction of the heart has been identified as the sinoatrial node (Keith and Flack, 1907).

The property of autorhythmicity in the heart was recognized by the Aztecs. The continued beating of the heart torn from the body of a sacrifice by the Aztec high priest (Figure 1) was a symbol of good fortune (Leake, 1960). William Harvey had noted the continued beating of the heart of an eel placed upon a board (Whitteridge, 1971). In the late 1800's, the study of the intrinsic rhythmicity of the heart was advanced by the development of a physiological salt solution reported by Ringer (1882) in a series of fascinating articles which demonstrated that an isolated heart could be maintained for prolonged periods given the correct

1. Harvey, W. (1963) The Circulation of the Blood, Translated by K. J. Franklin, Dent, London, p. 36

2. Eyster, J.A.E. and W.J. Meek (1921) The origin and conduction of the heart beat. Physiol. Rev. 1:1-43 p. 1.

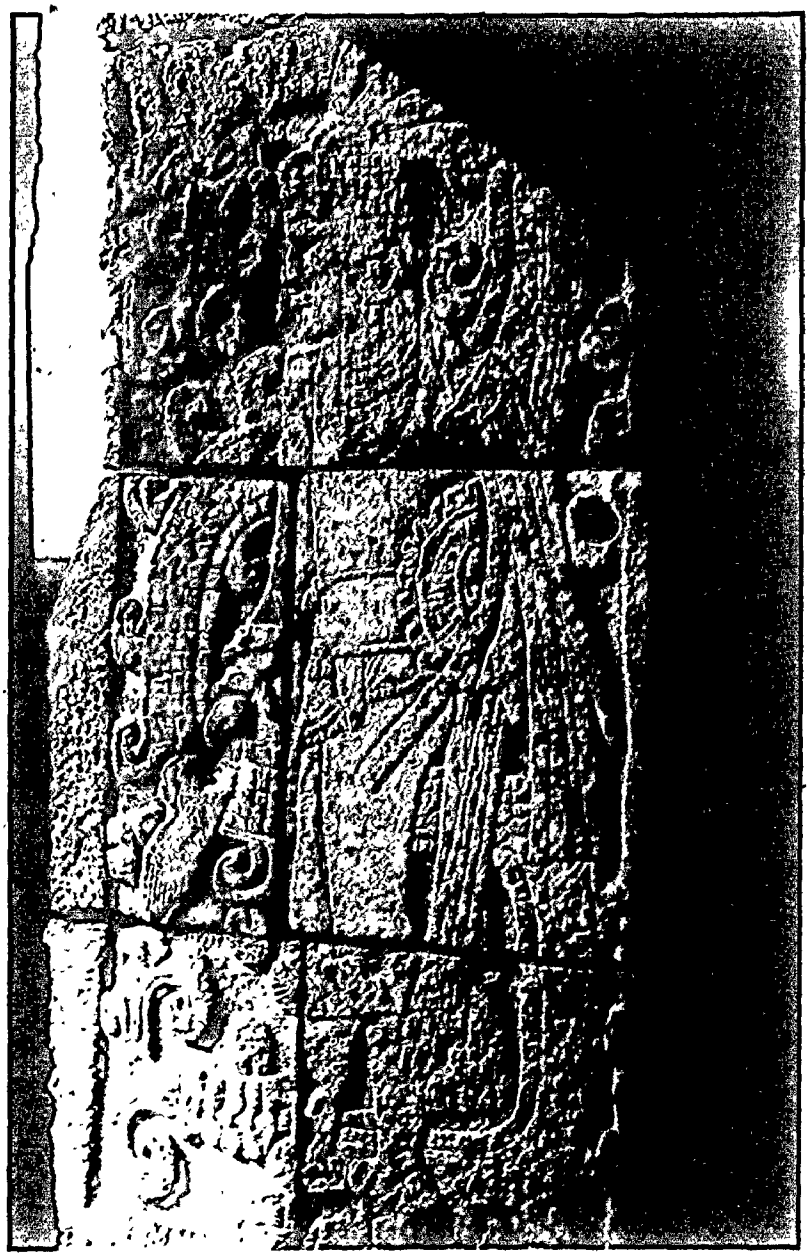


Figure 1: An Aztec High Priest holding a beating heart torn from a sacrifice. (Photograph by Dr. John Sutton)

Cannon, Lewis and Britton (1926) attempted to destroy all neural connections to the heart, but concluded that this was virtually impossible. Cooper et al (1961) succeeded in obtaining total surgical denervation. Donald and Samueloff (1966) employed the method of Cooper et al (1961) to denervate the heart of racing dogs. During exercise, a tachycardia was observed in these animals, however, blockade of the sympathetic receptors of the heart with propranolol eliminated the tachycardia which had resulted from circulating catecholamines. Surgical denervation is a relatively permanent procedure and is not useful for long term studies, or for experimentation in humans. The development of pharmacological receptor blocking agents has advanced the study of ISF making repeated determinations in animals or humans safe and feasible.

The first reports of combined sympathetic and parasympathetic receptor blockade were made in 1966 by Jose (1966) and Robinson et al (1966) in human experimentation and by Donald and Samueloff (1966) in animal studies. Jose (1966) employed combined doses of 0.2 mg/kg propranolol (sympathetic, beta-adrenergic receptor blocker) and 0.04 mg/kg atropine (parasympathetic receptor blocker). This method was employed by Jose and co-workers (Jose, 1966; Jose and Taylor, 1969; Jose and Collison, 1970; Jose, Stitt and Collison, 1970) in studying a wide range of human subjects. These investigators observed reductions in ISF with age and with heart disease. In contrast, Sutton et al (1967) using the same experimental methods in a group of young male subjects, observed a reduction of ISF with increased aerobic fitness. Further support for the finding of a low ISF with high aerobic fitness could be found in the physical training

studies of Sutton et al (1967) and Frick, Elovaino and Somer (1967) in which an increased fitness level was associated with a reduction in ISF. More recent animal training studies have confirmed the exercise-induced reduction of ISF. Lin and Horvath (1972) reported a lower ISF measured by pharmacological receptor blockade in swim trained rats compared with inactive controls. A lower ISF measured in the isolated right atrial preparation was also reported in swim trained rats by Bolter, Hughson and Critz (1973). Reductions in ISF must be associated with changes in the electrophysiological properties of the sinoatrial node. The following section will review the progress in the understanding of the autorhythmicity of the sinoatrial node.

II-2: Electrophysiology of the Sinoatrial Node:

The electrical activity associated with the contraction of the heart has been recognized since the late 1800's when Waller (1887) detected electrical currents on the body surface. Refinements of this observation have led to the development of the electrocardiographic recorder, a very useful tool in modern day medicine. Sir Thomas Lewis had recorded electrical activity from the surface of the heart (Lewis, 1910), but Bozler (1942-43) was the first to detect the slow depolarization typical of cardiac pacemaker cells. Bozler (1942-43) employed a surface electrode and obtained only a crude picture of the membrane properties of pacemaker cells and the slow depolarization which he called the prepotential. With the development of the microelectrode by Ling and Gerard (1949), the study of the membrane potential across a single cell became possible. The first recording of a sinoatrial nodal action

potential was obtained by West (1955) employing the floating microelectrode of Woodbury and Brady (1956). The sinoatrial node exhibits spontaneous rhythmical discharge with a frequency dependent on three primary factors: the maximum diastolic repolarization ("resting membrane potential"); the slope of the prepotential; and the threshold firing level (West, 1972).

In non-pacemaker tissues, the resting membrane potential is dependent on the ionic distribution across the membrane and on the permeability of the membrane to each ion species (Hodgkin and Katz, 1949). In pacemaker cells, the maximum diastolic repolarization level is dependent not only on the same factors as the resting membrane potential, but also on the voltage and time dependent changes in ion permeability (Hall, Hutter and Noble, 1963). Changes in the three major determinants of sinoatrial frequency have been observed. The maximum diastolic repolarization changes with varying concentrations of extracellular ions in the bathing solution of isolated tissues (Vick et al. 1973), or with stimulation of the parasympathetic nervous system and the release of acetylcholine (Hutter and Trautwein, 1956). The slope of the prepotential and the threshold potential have not been observed to change chronically, but do change acutely in response to the autonomic transmitters acetylcholine and noradrenaline (Hutter and Trautwein, 1956).

It was postulated by Bolter, Hughson and Critz (1973), that the ionic balance across the sinoatrial nodal cells may be altered with physical training as suggested by Raab (1969), but concluded that no data on the myocardial electrolyte distribution in the trained mammal was available to support this concept. Since the ionic distribution across the myocardial cell membrane will give an indication of the maximum

diastolic repolarization (resting potential), the electrolyte content of the myocardium and plasma will be examined to yield a clue as to the myocardial electrophysiology.

II-3: Responsiveness of the Sinoatrial Node to Neurotransmitters:

The response of the sinoatrial node to the autonomic neurotransmitters has long been recognized. The slowing of a frog heart perfused with a solution which had passed through another heart during the stimulation of the second heart's vagus nerve provided the initial evidence for a chemical transmitter of the autonomic nervous system (Loewi, 1921, see Burton, 1965). The chemical transmitter released in response to vagal stimulation was acetylcholine. The sympathetic neurotransmitter was identified as noradrenaline by Euler (1946). Noradrenaline increases the frequency of discharge of the sinoatrial node. It has only recently been recognized that changes in the sensitivity to the autonomic transmitters may occur.

Following the report of Bito, Dawson and Petrinovia (1971), of an inverse relationship between the tonic parasympathetic activity to the iris and its responsiveness to acetylcholine, Bolter, Hughson and Critz (1973) examined the effects of swim training on the sinoatrial sensitivity to acetylcholine in the rat. These investigators found a reduced sensitivity in trained animals compared with inactive controls. This finding suggested that tonic vagal activity to the heart had been increased in the trained animals.

The question of altered sensitivity to catecholamines in the hyperthyroid state has been pursued for many years. A review of earlier

experimentation in this area can be found in the study of Cairolli and Crout (1967). These investigators demonstrated that there was no change in sensitivity, but rather an increase in the maximum responsiveness of hyperthyroid animals. Recent investigations have asked the question about the responsiveness of the heart of trained humans or animals to noradrenaline. Brundin and Cernigliaro (1975) observed lower cardiac frequencies in exercising humans following training in spite of similar urinary catecholamine outputs at a fixed work load pre- andpost-training. From these findings, Brundin and Cernigliaro (1975) concluded that the sensitivity to catecholamines decreased with physical training. These investigators did not, however, measure parasympathetic activity and the balance between sympathetic and parasympathetic nervous activity has been shown to be important in establishing cardiac frequency (Grodner et al, 1970). In treadmill-trained rats, Ekblom et al (1973) observed a smaller cardiac frequency response to 6-hydroxydopamine, a drug which acutely stimulates the release of noradrenaline from the terminal axon (Thoenen and Tranzer, 1968). This response was interpreted as an indication of reduced sensitivity to catecholamines. Both of these studies represent indirect assessments of the sensitivity of the sinoatrial node to noradrenaline. A more direct measurement of the sympathetic influence on cardiac frequency during exercise was obtained in trained humans by Ekblom, Kilbom and Soltysiak (1973). The sympathetic response to exercise was determined during parasympathetic receptor blockade with atropine. This study indicated a lower cardiac frequency at all levels of power output following training. These investigators concluded that the activity of the sympathetic nervous system was reduced following training, but made no

conclusions about the sensitivity of the sinoatrial node to sympathetic stimulation.

II-4: Summary

In the "Historical Overview", the development of understanding of the physiology of the sinoatrial node has been briefly reviewed. The experimental methods employed in the present thesis to answer the questions posed in the "Introduction" have been based upon many of these original studies.

III: METHODS:

In the Introduction, seven questions were asked and formulated into three major studies. To answer these questions, it was necessary to employ an animal experimental model. This model allowed for the comparison of control and drug treated animals in exercised and non-exercised groups to study cardiac frequency responses in the intact animal (*in vivo*) and in the isolated atrial preparation (*in vitro*). as well as the measurement of myocardial electrolytes.

III-1: Animals:

The animals used in all experiments were rats of the Sprague-Dawley strain. Male rats were used in Study 1 and Study 2 and female rats in Study 3. The animals were housed in pairs within their own groups, and maintained on a diet of standard rat chow and water *ad libitum*. The environmental conditions were controlled as to light:dark (12:12) and temperature ($22^{o}C$).

III-2: Experimental Design:

The work reported in this thesis consisted of three separate investigations. Study 1: The role of the parasympathetic nervous influences on the intrinsic sinoatrial frequency. Study 2: The role of sympathetic influences on the intrinsic sinoatrial frequency. Study 3: The *in vivo* role of the intrinsic sinoatrial frequency in limiting maximal cardiac frequency.

Study 1 and Study 2 included daily physical exercise and/or drug administration for 10 weeks as set out below. In Studies 1 and 2, in vivo measurements were made of resting cardiac frequency and intrinsic sinoatrial frequency. At the completion of 10 weeks, the animals were anaesthetized and the right atrium was removed for studies of the chronotropic responses to acetylcholine and noradrenaline. The ventricles and a plasma sample were prepared for analysis of electrolyte content.

III-3: Experimental Groups:

III-3-1: Study 1. Role of Parasympathetic Influences

The rats were randomly assigned to six groups each containing 10 animals. Half of the experimental groups were inactive, the other half exercised on a treadmill five days/week. Each pair of inactive and exercised groups received injections five days/week. The first pair acted as a control, receiving injections of 0.2 ml saline (0.9% w/v). The second pair were injected with 5 mg/kg atropine to produce parasympathetic blockade. The third pair received injections of 0.15 mg/kg carbachol to produce parasympathetic stimulation.

III-3-2: Study 2. Role of Sympathetic Influences

The rats were randomly assigned to eight groups of 10 animals. As in Study 1, half of the groups were inactive, the other half exercised on a treadmill five days/week. Each pair of inactive and active groups received injections five days/week. The first pair acted as a control, receiving injections of 0.1 ml carrier medium (lanolin in peanut oil). The second pair was injected with noradrenaline in oil suspension to produce sympathetic stimulation. Pure beta-adrenergic receptor stimulation

was achieved in the third pair by injection of isoprenaline in oil suspension. The fourth pair of animals received sympathetic blockade by administration of propranolol in the drinking water. These latter groups also received sham injections of the carrier medium.

TABLE 1

Experimental Groups

Study 1. Role of Parasympathetic Influences

Drug	Inactive	Exercise
Saline Control (0.2 ml/day)	Inactive Control	Exercise Control
Atropine (5 mg/kg.day)	Atropine Inactive	Atropine plus Exercise
Carbachol (0.15 mg/kg.day)	Carbachol Inactive	Carbachol plus Exercise

Study 2. Role of Sympathetic Influences

Drug	Inactive	Exercise
Control (0.1 ml/day of oil)	Inactive Control	Exercise Control
Noradrenaline (1.0 mg/kg.day in oil)	Noradrenaline Inactive	Noradrenaline plus Exercise
Isoprenaline (0.1 mg/kg.day in oil)	Isoprenaline Inactive	Isoprenaline plus Exercise
Propranolol (5 mg/kg.day orally plus 0.1 ml/day of oil)	Propranolol Inactive	Propranolol plus Exercise

III-4: Physical Training:

The exercised animals followed a ten week physical training programme on the treadmill. A Quinton treadmill was modified to accomodate up to 12 rats at a time by the addition of indiyidual boxes supported by a wooden frame (Figure 2). The animals were observed throughout the training session and were kept running by light prodding when necessary.

Initially the rats exercised at a treadmill speed of 19 m/min. at 0% grade for 15 min. This workload was gradually increased to a final workload of 19 m/min, 5% grade for 45 minutes over the last two weeks of the training programme. Since the animals were randomly assigned to exercised groups, the rate of increase in workload was established by the weakest animals.

III-5: Chronic Drug Administration:

The concentrations of each drug employed for chronic drug administration in Study 1 and Study 2 were determined by preliminary studies as reported in the Appendix.

III-5-1: Parasympathetic Receptor Agonists and Antagonists:

Blockade of the parasympathetic receptors of the heart was obtained with the antagonist atropine (atropine sulphate, B.D.H. Ltd.). Administration of 5 mg/kg atropine dissolved in saline (0.9% w/v) elicited a tachycardia of six hour duration (Appendix 3b). No adverse effects were observed with this concentration.

Stimulation of the parasympathetic receptors of the heart was achieved with the agonist carbachol (carbachol, B.D.H. Ltd.). Administration of 0.25 mg/kg carbachol dissolved in saline (0.9% w/v) caused a

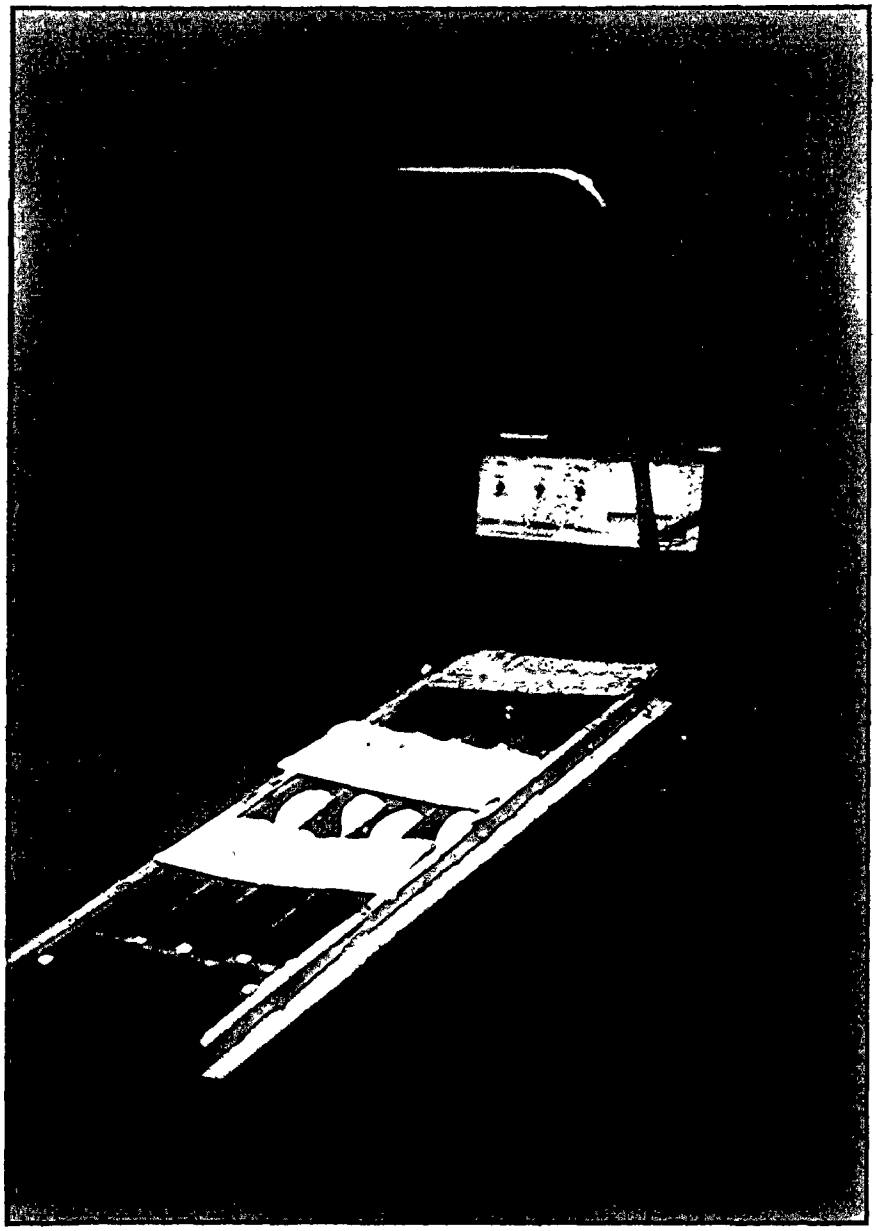


Figure 2. The modified Quinton treadmill used to exercise the rats. Four rats are shown in the centre row of the treadmill. A total of twelve animals could be exercised at one time.

bradycardia of approximately four hours duration (Appendix 3c). This concentration of carbachol also was observed to cause heavy lacrimation and salivation.

The control inactive and exercise groups of Study 1 also received daily injections of 0.2 ml normal saline as a sham control procedure (Appendix 3a).

III-5-2: Sympathetic Receptor Agonists and Antagonists:

Stimulation of the sympathetic receptors of the heart was achieved with the adrenergic agonists noradrenaline (l-levophed bitartrate, Winthrop) and isoprenaline (isuprel, Winthrop). Both drugs were suspended in a lanolin-peanut oil mixture to prolong the duration of activity. A concentration of 1.0 mg/kg noradrenaline suspended in oil has been previously shown to produce a hypertensive response for four hours (Linna et al, 1965), however, the cardiac frequency response observed (Appendix 4a) showed a bradycardia resulting from the baroreceptor reflex. Isoprenaline injected in a concentration of 0.1 mg/kg evoked a marked tachycardia of four hours duration (Appendix 4b).

Blockade of the sympathetic receptors of the heart was achieved with the antagonist propranolol (propranolol hydrochloride, Ayerst). The propranolol was administered in the drinking water in a concentration of 1.0 mg/ml and this water was available for 12 hours per day, with normal drinking water available at other times. The animals received approximately 5 mg/kg per day. Measurement of cardiac frequency showed that a significant bradycardia was achieved by this concentration of propranolol (Appendix 4c). These animals received sham injections as described below.

The inactive and exercise control animals received sham injections with the carrier medium employed in the noradrenaline and isoprenaline administrations. A volume of 0.1 ml of lanolin and peanut oil was injected. In all groups, the injections were subcutaneous, and administered under the skin of the back.

III-6: In vivo measurements:

In all animals in Study 1 and Study 2 resting cardiac frequency and intrinsic sinoatrial frequency were measured before the commencement of the studies and at three week intervals during the studies. Cardiac frequency measurements during the studies were made 48 hours following the last exercise period or drug administration.

III-6-1: Resting Cardiac Frequency

Resting cardiac frequency was obtained from subcutaneously attached small safety pin electrodes. Records were made on a standard electrocardiograph (Cambridge VS5) (Appendix 1). The animals displayed no signs of discomfort from the electrodes. The rats were placed individually into small plastic buckets and following 20 minutes of quiet rest, a cardiac frequency measurement was obtained. Cardiac frequency was obtained from a count of QRS complexes in a 15 second period. Another measurement was obtained at 30 minutes, and the lower of the two measurements at 20 and 30 minutes was taken as the resting cardiac frequency.

III-6-2: Intrinsic Sinoatrial Frequency:

The in vivo ISF was the cardiac frequency measurement obtained 20 minutes after the intraperitoneal injection of 3 mg/kg atropine sulphate (B.D.H. Ltd.) and 8 mg/kg propranolol hydrochloride (Ayerst Laboratories).

These concentrations of atropine and propranolol resulted in a plateau of cardiac frequency between 15 and 30 minutes (Appendix 2a). No further change in cardiac frequency was observed with the administration of additional atropine or propranolol, indicating total autonomic blockade (Appendix 2b).

III-7: In Vitro Studies:

At the completion of the ten week exercise or drug administration programmes, the animals were prepared for in vitro studies. The rats were anesthetized with ether, the chest was opened, and blood was taken from the left ventricle for plasma electrolyte determination. The heart was removed and placed in cooled (2-4^{o}C) physiological saline. The right atrium was dissected free, ensuring an intact sinoatrial node, then attached to bipolar recording electrodes and placed in an organ bath. The ventricles were stored in liquid nitrogen for later analysis of myocardial electrolytes.

Physiological saline of the following composition in g/l was employed: NaCl 6.92, KCl 0.35, $CaCl_2$ 0.28, KH_2PO_4 0.16, $MgSO_4$ 0.15, $NaHCO_3$ 2.1, and glucose 2.0. The organ baths were non-circulating 50 ml vessels immersed in a constant temperature water bath at 30^{o}C. The physiological saline was aerated with a mixture of 95% O_2, 5% CO_2. Similar composition solution aerated with a similar gas mixture was shown previously to have a PO_2 of 211 mmHg, PCO_2 of 36.4 mmHg and a pH of 7.32 when measured at 37^{o}C (Hughson and Ledsome, 1975).

The preparations were allowed to stabilize for 60 minutes prior to studying the chronotropic responses to acetylcholine or noradrenaline. Any preparation which showed an arrhythmia was discarded. Half of the preparations

from each group were studied with acetylcholine first, the other half with noradrenaline first.

The drugs used, acetylcholine chloride (Sigma Chemical) and noradrenaline (l-levophed bitartrate, Winthrop), were dissolved in physiological saline. Drugs were added to the organ bath in 0.1 ml volumes to yield bath concentrations for acetylcholine of 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M, and for noradrenaline of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M.

Chronotropic dose-response curves were constructed for each drug by the addition of increasing concentrations of drug in a cumulative fashion. Preliminary trials with each drug indicated a stable maximum response by three minutes (Appendix 5a, 5b), therefore, the frequency of contraction of the preparation at three minutes was taken as the chronotropic response at that drug concentration. Following the attainment of a maximum response, the preparations were double washed and allowed to stabilize for 30 minutes before the addition of the other drug.

III-8: Electrolyte Determination:

The ventricles were removed from the liquid nitrogen and weighed. The tissue was placed in 4 ml of 0.5 N nitric acid and homogenized using a Polytron. The homogenate was centrifuged for 10 minutes at 10,000 r.p.m. and the supernatant removed for analysis.

The blood taken from each animal was centrifuged immediately and the plasma stored at -20°C until analyzed.

Sodium and potassium contents were determined for both the plasma and myocardium using flame photometry (Technicon Auto Analyzer).

III-9: Maximum Cardiac Frequency Response (Study 3):

To examine the in vivo role of the intrinsic sinoatrial frequency in limiting the maximum cardiac frequency, female Sprague-Dawley rats were studied under exercise and noradrenaline stimulation. The investigation of the reduction of maximum cardiac frequency in relation to the decrease in ISF required a wide range of values of ISF. To achieve this wide range in ISF, the observation from Study 1 that atropine reduces ISF was employed. The animals in the present Study 3 were divided into two groups. One half were injected with 5 mg/kg atropine five days per week for 10 weeks as in Study 1, the other half were untreated. This pretreatment successfully established a range in ISF values with over 100 beats/minute difference. At the completion of 10 weeks, the drug administration was stopped for 72 hours, then the intrinsic sinoatrial frequency was determined as previously described.

III-9-1: Maximum Exercise Cardiac Frequency:

The maximum cardiac frequency response to exercise was determined 48 hours following the measurement of intrinsic sinoatrial frequency. The animals had not previously exercised on a treadmill. Electrodes were attached subcutaneously to the limbs to provide a standard lead II configuration. Cardiac frequency was monitored on a Cambridge electrocardiograph. Following a short period of familiarization with treadmill walking at 19 m/min., the speed of the treadmill was increased to 30 m/min. Maximum exercise was achieved by progressively increasing the grade of the treadmill by 3% every two minutes. The animals were encouraged to continue by gentle prodding until they were unable to match the treadmill speed. Cardiac

frequency was measured during each work load, and the maximum response was taken as the maximum exercise cardiac frequency.

III-9-2: In Vivo Noradrenaline Response:

The maximum chronotropic response to noradrenaline was determined in the same animals used in the study of maximum exercise cardiac frequency. The animals were anesthetized with nembutal, 0.5 ml. Rectal temperature was measured and kept constant at 38°C by a heating pad. Electrodes were attached for monitoring cardiac frequency, and a catheter was placed in the jugular vein for the injection of noradrenaline. Preliminary experiments monitoring blood pressure showed 6 mg/kg hexamethonium to be effective in producing ganglionic blockade (Appendix 6). Noradrenaline was dissolved in 0.9% (w/v) saline and injected via the jugular catheter in total doses of 0.01 μg, 0.1 μg, 1.0 μg, 10 μg and 100 μg. Cardiac frequency was measured continuously and the maximum response was taken as the maximum in vivo cardiac frequency response to noradrenaline.

III-10: Statistical Methods:

The comparison of the mean values of each group to the inactive sham injected control group of Study 1 or Study 2 was made by the computation of a t-statistic employing the group variance obtained by one-way analysis of variance (Armitage, 1971). The appropriate p-values were recorded. This technique was employed for the following measurements: in vivo ISF, resting cardiac frequency, body weight, heart weight, heart weight:body weight, plasma and myocardial electrolytes, in vitro ISF,

maximum noradrenaline chronotropic response, and ED_{50} values for acetylcholine and noradrenaline chronotropic responses. Linear regression equations were calculated for each group for the *in vivo* ISF with respect to time, with 95% confidence limits for slope determined to assess the changes in ISF with time. The relationship between *in vitro* ISF and maximum noradrenaline stimulated frequency was assessed by calculation of the linear regression equation and the correlation coefficient. Similarly in Study 3, the relationship between *in vivo* ISF and either maximum exercise cardiac frequency or maximum noradrenaline stimulated frequency was assessed by calculation of the linear regression equation and the correlation coefficient.

IV: RESULTS

IV-1: Study 1

Study 1 has examined the role of high levels of resting parasympathetic activity in reducing the intrinsic sinoatrial frequency. Measurements have been made of resting cardiac frequency and ISF *in vivo*. The animals were sacrificed and additional information obtained concerning myocardial and plasma electrolytes, especially the K_o^+/K_i^+, and the responsiveness of the sinoatrial node to the neurotransmitters acetylcholine and noradrenaline.

IV-1-1: Resting cardiac frequency:

The resting cardiac frequency showed considerable week-to-week variability (Figure 3). However, the pattern of fluctuation is similar in animals studied at the same time. The first set of animals studied consisted of the groups: inactive control, exercised control, carbachol inactive, and atropine plus exercise. The second set of animals consisted of the carbachol plus exercise and the atropine inactive groups. This cyclic nature of resting cardiac frequency makes interpretation of resting data very difficult. The absolute values of resting cardiac frequency are displayed in Table 2. While caution should be used in interpreting these values, especially of the atropine inactive and the carbachol plus exercise groups, resting cardiac frequency is insignificantly lower than the inactive control in the exercise control and the carbachol inactive groups,

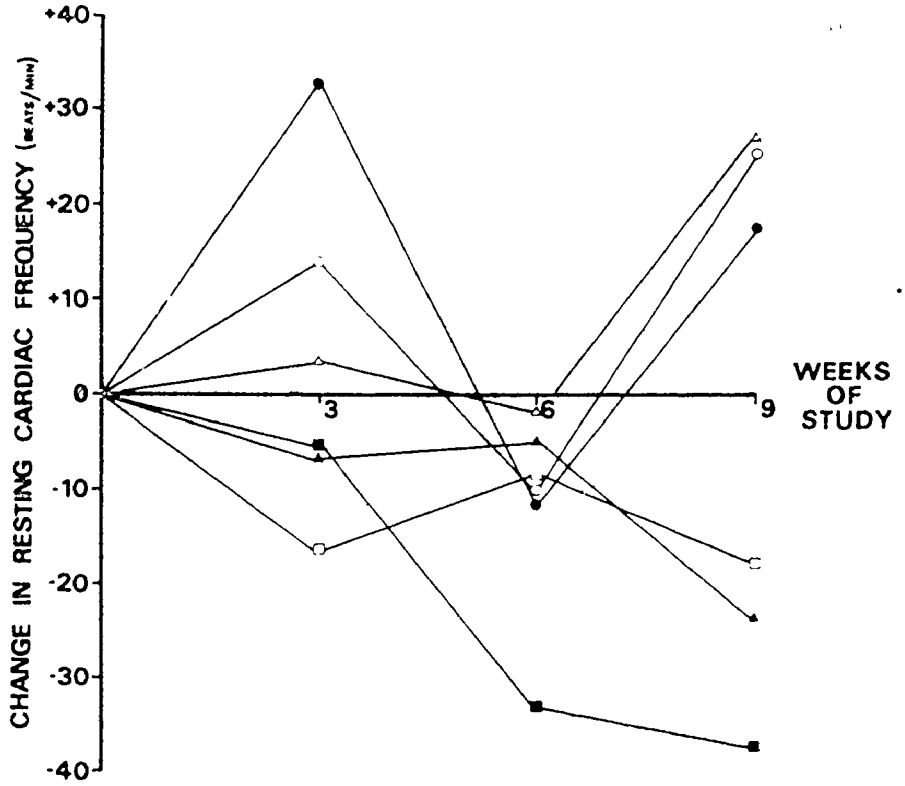


Figure 3. The change in resting cardiac frequency observed during Study 1. Each point represents the mean value for that group. Symbols: Inactive control (○), Exercise control (●), Atropine inactive (□), Atropine plus exercise (■), Carbachol inactive (△) and Carbachol plus exercise (▲).

TABLE 2

Absolute values of resting cardiac frequency in Study 1.

	Pre	3 Wk	6 Wk	9 Wk
Inactive Control	350.2 ± 9.6	363.3 ± 9.2	339.5 ± 7.0	375.3 ± 13.0
Exercise Control	339.1 ± 6.4	368.0 ± 7.8	327.8 ± 12.2	385.5 ± 13.3
Atropine Inactive	353.4 ± 8.2	338.3 ± 6.6	346.3 ± 7.7	339.0 ± 9.8 ($p<0.05$)
Atropine plus Exercise	373.0 ± 7.8	368.8 ± 8.0	341.7 ± 8.2	337.3 ± 12.3 ($p<0.05$)
Carbachol Inactive	328.3 ± 15.0	349.0 ± 10.6	342.3 ± 7.6	355.7 ± 16.0
Carbachol plus Exercise	394.8 ± 12.6	386.4 ± 9.4	390.2 ± 7.6	369.6 ± 8.9

Values are the mean ± 1 S.E.M. Differences in the cardiac frequency from the Inactive Control were assessed at 9 weeks by the computation of the t-statistic. p-values are in brackets beneath the values which differ from the inactive control.

and significantly lower in the two atropine groups.

IV-1-2: Intrinsic sinoatrial frequency in vivo:

All six experimental groups showed a linear decrease in ISF with time. The calculated slope and 95% confidence limits for the slope (Table 4), indicate that the decrease in ISF with time was significant at the 0.05 level or greater. Contrary to the measurement of resting cardiac frequency, no cyclic variation could be observed, and the two sets of animals studied at different times could not be distinguished.

In comparison with the inactive control, the exercised control group had a significantly ($p<0.05$) greater reduction in ISF (Figure 4). The atropine inactive group had a significantly greater ($p<0.05$) reduction in ISF than the inactive control. The atropine plus exercise group demonstrated that the effects of exercise and atropine were additive in reducing the ISF, as this group had the greatest reduction in ISF ($p<0.001$). The carbachol inactive and carbachol plus exercise groups had reductions in ISF which did not differ significantly from the inactive control.

IV-1-3: Electrolytes:

The plasma concentration of potassium was similar to the inactive control animals in all groups (Table 5). Plasma sodium content was significantly lower than the inactive control in the exercise control and the atropine plus exercise groups. In the myocardium, the sodium content, expressed in mEq/100g wet weight of ventricular tissue, was similar in all groups. Myocardial potassium content was significantly greater than the inactive control in the exercised control. The atropine inactive group

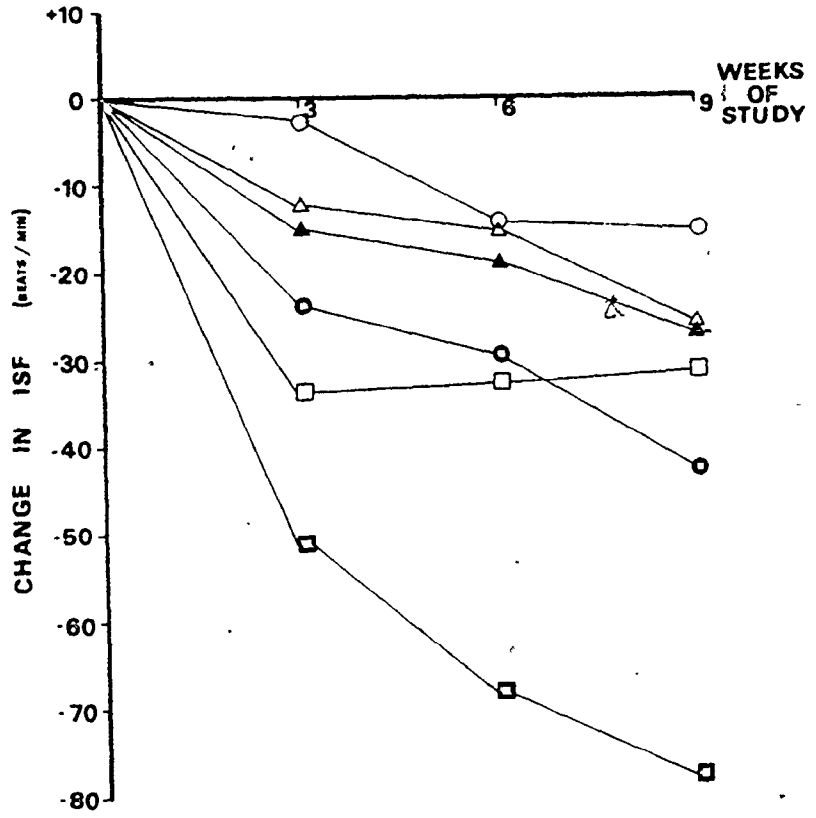


Figure 4. Change in intrinsic sinoatrial frequency during Study 1. Symbols: Inactive control (○), Exercise control (●), Atropine inactive (□), Atropine plus exercise (■), Carbachol inactive (△), Carbachol plus exercise (▲). Differences from the inactive control at week 9: exercise control ($p<0.05$), atropine inactive ($p<0.05$), and atropine plus exercise ($p<0.001$).

TABLE 3

Absolute values of intrinsic sinoatrial frequency in Study 1.

	Pre	3 Wk	6 Wk	9 Wk
Inactive Control	400.3 ± 9.6	404.0 ± 2.9	392.3 ± 4.8	385.0 ± 6.1
Exercise Control	425.0 ± 10.9	391.2 ± 18.7	388.7 ± 12.0	378.6 ± 7.8
Atropine Inactive	397.8 ± 6.4	357.3 ± 9.0	352.8 ± 6.5	363.8 ± 8.4
Atropine plus Exercise	416.7 ± 10.2	368.8 ± 8.0	348.7 ± 7.1	339.0 ± 7.7
Carbachol Inactive	388.3 ± 7.0	376.0 ± 6.3	374.0 ± 7.2	362.3 ± 4.7
Carbachol plus Exercise	386.2 ± 4.4	371.3 ± 6.2	367.6 ± 5.1	360.2 ± 4.7

Values are the mean ± 1 S.E.M.

TABLE 4

Regression lines fitted to change in ISF (Y) against time in weeks (X) of the form Y = mX+b 95% confidence limits for slope in brackets.

Inactive control	Y = -2.19X+0	(-0.37 ≥ m ≥ -3.99)
Exercise control	Y = -4.74X-2.37	(-1.79 ≥ m ≥ -7.69)
Atropine inactive	Y = -3.92X-9.02	(-1.60 ≥ m ≥ -6.23)
Atropine plus exercise	Y = -7.88X-11.53	(-5.30 ≥ m ≥ -10.25)
Carbachol inactive	Y = -2.67X-1.17	(-1.09 ≥ m ≥ -4.25)
Carbachol plus exercise	Y = -2.89X+0	(-1.66 ≥ m ≥ -4.12)

TABLE 5

Myocardial and plasma electrolytes in Study 2.

Group	Plasma (mEq/litre)		Myocardial (mEq/100g wet weight)		K_o^+/K_i^+
	Na	K	Na	K	
Inactive Control	142.0 ± 1.7	5.2 ± 0.3	3.46 ± 0.12	5.98 ± 0.12	0.87 ± 0.05
Exercise Control	136.6 ± 2.7	5.0 ± 0.3	3.62 ± 0.08	6.28 ± 0.14**	0.79 ± 0.06
Atropine Inactive	137.9 ± 7.8	5.0 ± 0.4	3.80 ± 0.11**	5.55 ± 0.14*	0.90 ± 0.07
Atropine plus Exercise	136.7 ± 2.1	5.1 ± 0.3	3.63 ± 0.11	5.86 ± 0.17	0.86 ± 0.06
Carbachol Inactive	138.4 ± 2.8	5.1 ± 0.3	3.56 ± 0.08	6.05 ± 0.18	0.86 ± 0.08
Carbachol plus Exercise	141.2 ± 0.6	5.5 ± 0.5	3.74 ± 0.11	5.99 ± 0.18	0.95 ± 0.12

Values are the mean ± 1 S.E.M. Differences from control were assessed by the calculation of a t-statistic, with the following symbols representing significance levels: * $p<0.1$; ** $p<0.05$.

had a myocardial potassium content significantly less than inactive control, while exercise increased myocardial potassium to a level where atropine plus exercise did not differ from the inactive control. The carbachol inactive group had a myocardial potassium content similar to the control, and exercise produced a small, but non-significant, increase in potassium in the carbachol plus exercise group. The ratio of myocardial to plasma potassium content (K_o^+/K_i^+)(Table 5) was significantly less than inactive control in the exercise control group, and significantly greater in the atropine inactive group. The K_o^+/K_i^+ was similar to the inactive control in the other three groups.

IV-1-4: Heart Weight:Body Weight

The heart weights of all groups were similar to the inactive control (Table 6). However, because the weight gain was less than the inactive control in the exercised animals (exercised control $p<0.01$, atropine plus exercise $p<0.05$, and carbachol plus exercise $p<0.05$), the heart weight to body weight ratio was greater than the inactive control. The heart ratio of the exercise control and of the atropine plus exercise groups were both significantly ($p<0.05$) greater than the inactive control, indicating a relative cardiac hypertrophy.

IV-1-5: Intrinsic Sinoatrial Frequency In Vitro:

The values of the ISF *in vitro* (the spontaneous contraction frequency) are shown in Table 7. The two groups which had the greatest reduction in ISF *in vivo*, exercise control and atropine plus exercise, have the lowest ISF *in vitro*. The ISF *in vitro* was significantly less than

TABLE 6

Body weight, heart weight and heart ratio after 10 weeks of Study 1.

	Body Weight (g)	Heart Weight (g)	Heart/Body Weight (mg/g)
Inactive Control	535.9 ± 9.8	1.29 ± 0.07	2.42 ± 0.14
Exercise Control	478.7 ± 16.0 ($p<0.01$)	1.27 ± 0.12	2.65 ± 0.34 ($p<0.05$)
Atropine Inactive	527.4 ± 20.2	1.30 ± 0.21	2.48 ± 0.18
Atropine plus Exercise	508.1 ± 30.5	1.32 ± 0.21	2.62 ± 0.24 ($p<0.05$)
Carbachol Inactive	542.6 ± 22.1	1.23 ± 0.12	2.38 ± 0.20
Carbachol plus Exercise	514.9 ± 16.2	1.29 ± 0.21	2.51 ± 0.28

Values are the mean ± 1 S.E.M. The p-value obtained from the t-statistic comparing each group with the inactive control is in the bracket beneath the mean. Where no p-value is given, $p>0.05$.

TABLE 7

Intrinsic sinoatrial frequency *in vitro* in Study 1.

Group	ISF (beats/min)
Inactive Control	140.7 ± 7.4
Exercise Control	123.3 ± 6.8*
Atropine Inactive	138.3 ± 7.5
Atropine plus Exercise	119.0 ± 5.0*
Carbachol Inactive	137.4 ± 7.8
Carbachol plus Exercise	136.4 ± 8.5

Values are the mean ± 1 S.E.M. Differences from inactive control were calculated by a t-statistic. The '*' indicates different from inactive control, $p<0.05$.

the inactive control in the exercise control ($p<0.05$) and the atropine plus exercise ($p<0.01$) groups. All other groups had *in vitro* ISF values similar to control. The number in brackets beneath each value of ISF gives the number of atrial preparations which did not develop arrhythmias.

IV-1-6: Acetylcholine Chronotropic Responses:

Assessment of the sensitivity of the chronotropic responses to acetylcholine by calculating ED_{50} values indicated no significant difference from inactive control for any group (Figure 5, Table 8).

IV-1-7: Noradrenaline Chronotropic Responses:

The ED_{50} values for the chronotropic dose-response curves to noradrenaline indicated no significant difference for any group from the inactive control (Figure 6, Table 8). There was, however, a significant reduction in maximum chronotropic response (Figure 6) from the inactive control in the exercise control ($p<0.05$) and the atropine plus exercise ($p<0.05$) groups. The other groups were not significantly different from control. A highly significant relationship was observed between the *in vitro* ISF and the maximum noradrenaline stimulated frequency *in vitro* ($r=0.75$) (Figure 7). The findings displayed in Figures 7 and 8 indicate that atria with a high ISF obtain a higher maximum frequency in response to noradrenaline.

IV-2: Study 2

Study 2 has examined the role of increased sympathetic activity during exercise in causing a reduction in ISF. As in Study 1, measurements

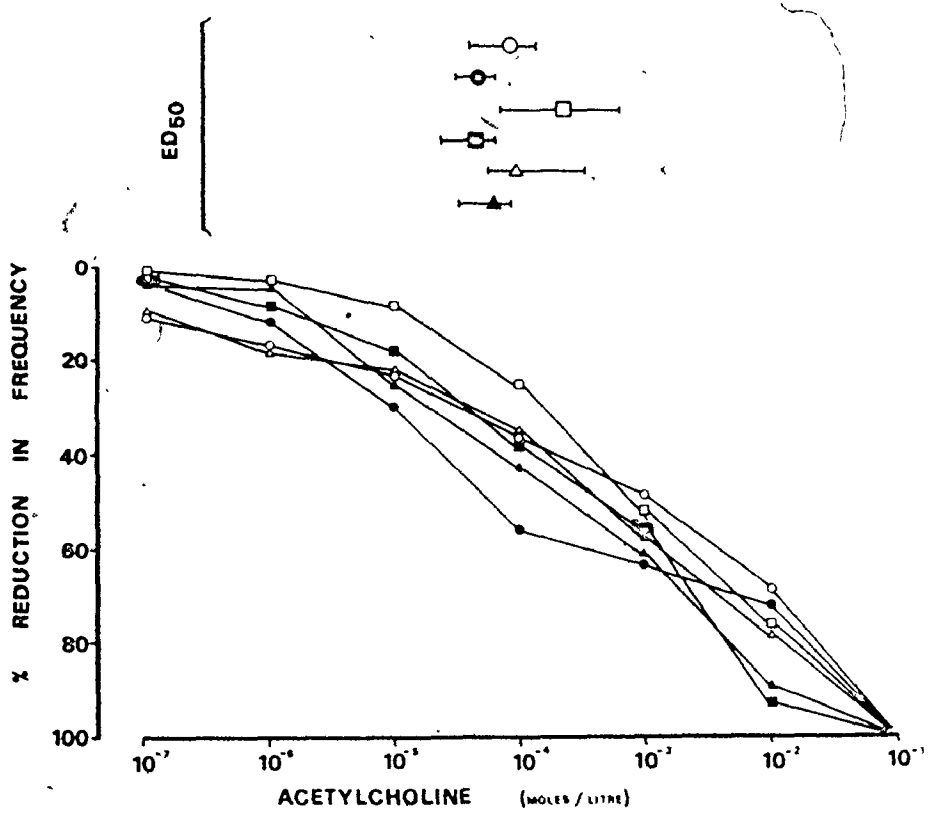


Figure 5. The in vitro acetylcholine chronotropic dose-response curves for Study 1 showing the ED_{50} values. Each point of the dose-response curve is the mean value. The ED_{50} is the mean value, with $\pm$ I S.E.M. shown. Symbols: Inactive control (○), Exercise control (●), Atropine inactive (□), Atropine plus exercise (■), Carbachol inactive (△), and Carbachol plus exercise (▲). There was no difference from the inactive control for the ED_{50} value of any group.

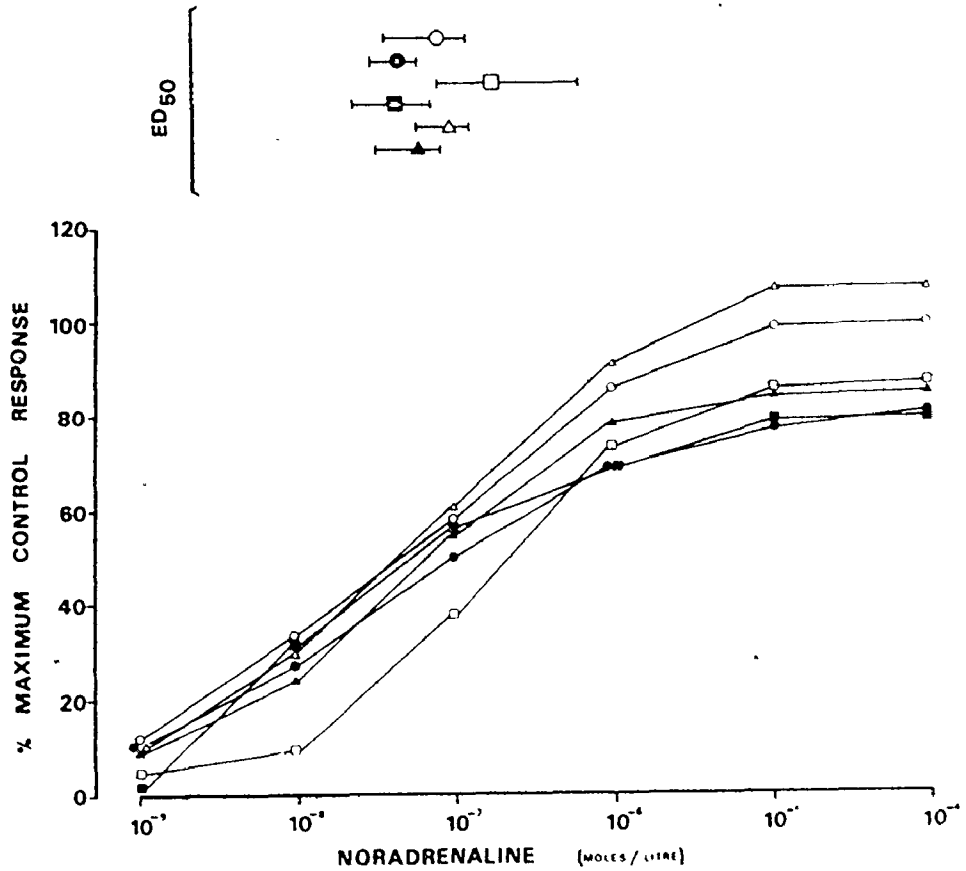


Figure 6. The in vitro noradrenaline chronotropic dose-response curves for Study 1 showing the ED_{50} values. Each point of the dose-response curve is the mean value. The ED_{50} is the mean value with ± 1 S.E.M. shown. Symbols: Inactive control (○), Exercise control (Ø), Atropine inactive (□), Atropine plus exercise (■), Carbachol inactive (△), and Carbachol plus exercise (▲). Differences from the inactive control for the maximum response: exercise control (p 0.05), atropine plus exercise ($p<0.05$). No differences from inactive control were observed in the ED_{50} values.

TABLE 8

Assessment of sensitivity to acetylcholine and noradrenaline *in vitro* in Study 1.

Group	Acetylcholine ED_{50} ($X10^{-4}$ Moles/Litre)	Noradrenaline ED_{50} ($X10^{-8}$ Moles/Litre)
Inactive Control	8.41 ± 4.50	8.59 ± 4.67
Exercise Control	4.78 ± 1.55	4.72 ± 1.64
Atropine Inactive	22.1 ± 40.9	22.1 ± 41.7
Atropine plus Exercise	4.62 ± 1.83	4,57 ± 2.20
Carbachol Inactive	9.65 ± 3.95	9.68 ± 3.89
Carbachol plus Exercise	6.20 ± 2.38	6.19 ± 2.37

Values are the mean ± 1 S.E.M. No differences from the inactive control were found by calculation of t-statistics.

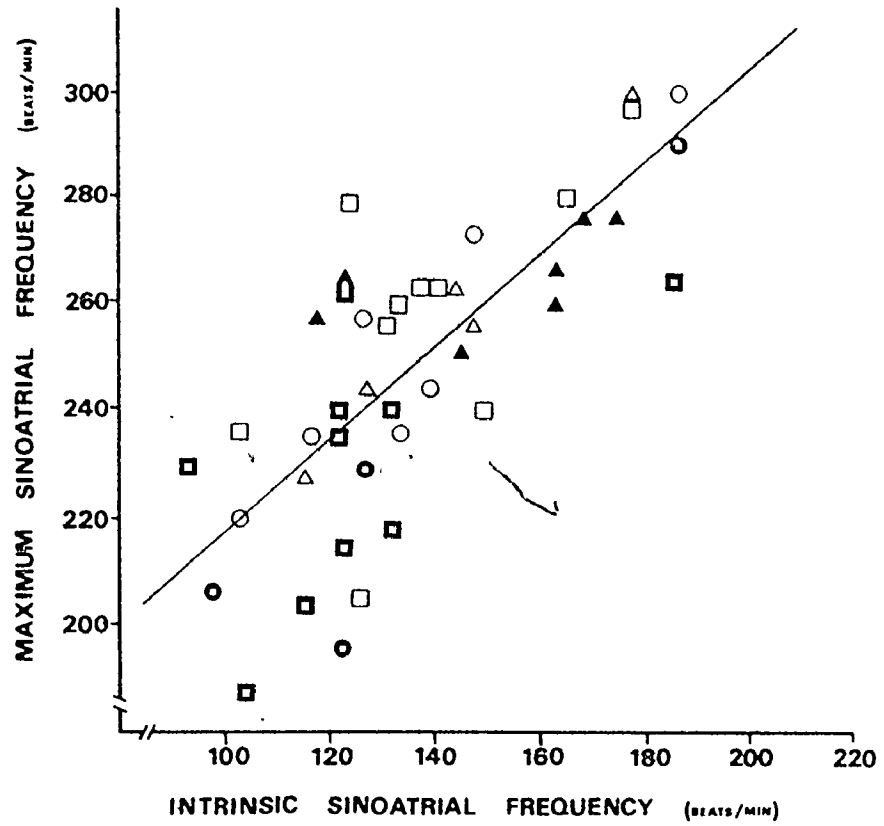


Figure 7. The relationship between the maximum sinoatrial frequency in response to noradrenaline stimulation and the intrinsic sinoatrial frequency in vitro for all animals in Study 1. Symbols: Inactive control (○), Exercise control (◎), Atropine inactive (□), Atropine plus exercise (■), Carbachol inactive (△) and Carbachol plus exercise (▲). Correlation coefficient (r) = 0.75, equation of the line: Maximum sinoatrial frequency = (0.87) (ISF in vitro) + 130.

of resting cardiac frequency and ISF have been made *in vivo*, and electrolyte concentrations and sinoatrial responsiveness to acetylcholine and noradrenaline have been determined *in vitro*.

IV-2-1: Resting Cardiac Frequency:

The variability in resting cardiac frequency was not as marked as in Study 1 (Figure 8). As all eight groups of rats were studied at the same time, no differences in week-to-week measurements would be expected as in Study 1. Following an initial decline in resting cardiac frequency over the first three weeks, no further change in resting cardiac frequency occurred. There were no significant differences from the inactive control in any group for either the reduction in resting cardiac frequency, or the absolute value of resting cardiac frequency (Table 9).

IV-2-2: Intrinsic Sinoatrial Frequency In Vivo:

The inactive control group showed a tendency to reduce ISF with time (Figure 9), however, the slope of the calculated regression line (Table 11) was not significantly different from zero. The slope of the regression lines for exercise control, noradrenaline inactive, isoprenaline inactive, and propranolol inactive and exercise indicated that these groups displayed a significant trend to decrease ISF with time (Table 11). In contrast, the slopes of noradrenaline plus exercise, and isoprenaline plus exercise were not significantly different from zero. The decrease in ISF achieved by week nine was significantly greater than that of the inactive control in the exercise control ($p<0.05$), the noradrenaline inactive ($p<0.1$) the isoprenaline inactive ($p<0.01$) the propranolol inactive ($p<0.05$)

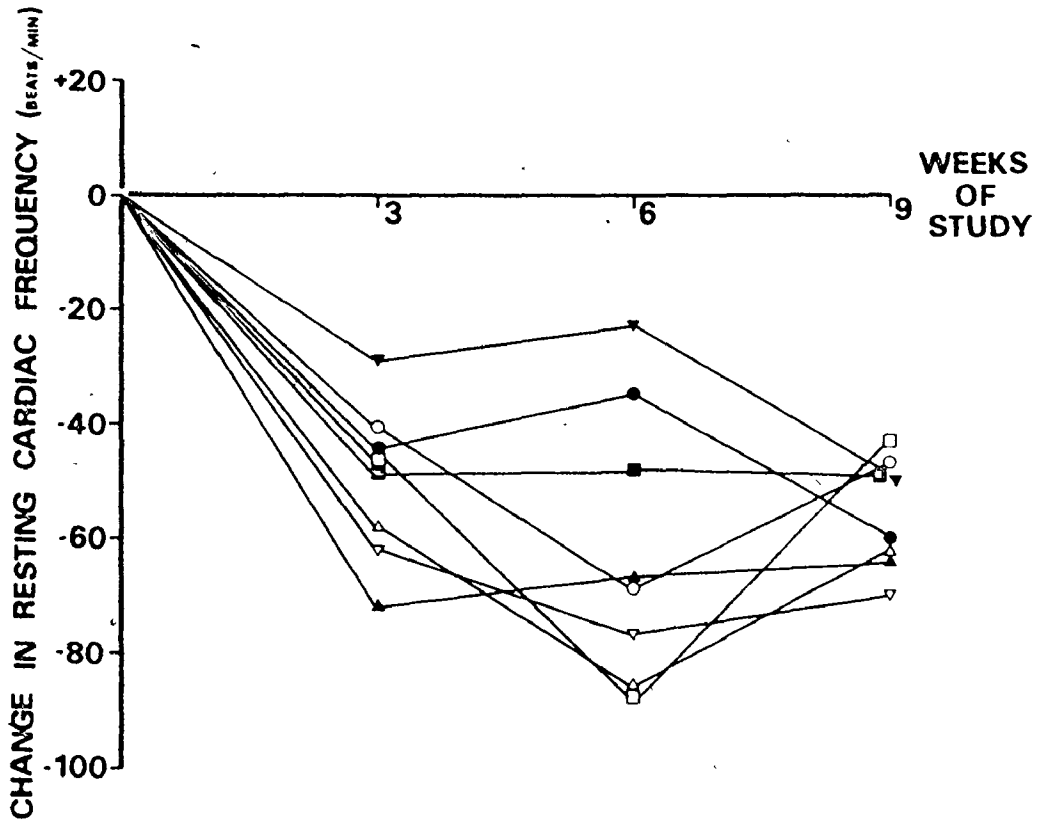


Figure 8: The change in resting cardiac frequency observed during Study 2. Each point represents the mean value for that group. Symbols: Inactive control (○), Exercise control (●), Noradrenaline inactive (□), Noradrenaline plus exercise (■), Isoprenaline inactive (△), Isoprenaline plus exercise (▲), Propranolol inactive (▽), and Propranolol plus exercise (▼).

TABLE 9

Absolute values of resting cardiac frequency in Study 2.

	Pre-study	3 week	6 week	9 week
Inactive Control	416.6 ± 7.2	375.7 ± 20.0	354.0 ± 15.3	364.2 ± 14.1
Exercise Control	419.1 ± 7.6	364.3 ± 21.8	374.7 ± 13.8	359.8 ± 9.3
Noradrenaline Inactive	427.6 ± 13.0	388.3 ± 17.4	353.0 ± 10.3	391.6 ± 6.5
Noradrenaline plus Exercise	415.3 ± 20.5	349.3 ± 15.4	349.7 ± 14.9	350.9 ± 7.0
Isoprenaline Inactive	405.1 ± 11.3	345.6 ± 10.7	312.2 ± 11.2	331.6 ± 10.8
Isoprenaline plus Exercise	401.4 ± 6.7	335.3 ± 11.7	342.5 ± 15.8	342.5 ± 8.0
Propranolol Inactive	411.0 ± 7.5	365.7 ± 23.6	336.7 ± 8.0	344.6 ± 9.3
Propranolol plus Exercise	420.3 ± 9.8	386.0 ± 19.2	391.7 ± 21.9	370.5 ± 18.4

Values are the mean ± 1 S.E.M. No differences from the inactive control were found by calculation of the t-statistic.

TABLE 10

Absolute values of intrinsic sinoatrial frequency in Study 2.

	Pre-study	3 week	6 week	9 week
Inactive Control	394.8 ± 10.0	394.7 ± 10.9	402.0 ± 8.3	379.7 ± 11.4
Exercise Control	418.7 ± 6.6	402.3 ± 3.6	411.7 ± 5.4	393.6 ± 6.2
Noradrenaline Inactive	401.8 ± 5.8	385.0 ± 10.0	392.7 ± 11.2	376.9 ± 7.3
Noradrenaline plus Exercise	396.7 ± 10.4	403.3 ± 7.3	395.0 ± 10.5	387.6 ± 9.0
Isoprenaline Inactive	403.0 ± 7.5	373.3 ± 8.0	358.3 ± 8.7	346.8 ± 6.3
Isoprenaline plus Exercise	388.6 ± 8.3	392.5 ± 12.7	394.7 ± 17.3	388.8 ± 13.3
Propranolol Inactive	413.1 ± 10.8	407.3 ± 6.1	403.0 ± 9.2	383.0 ± 3.7
Propranolol plus Exercise	417.0 ± 6.6	388.0 ± 13.0	398.7 ± 10.0	371.0 ± 8.1

Values are the mean ± 1 S.E.M. For a statistical comparison, see Figure 9.

TABLE 11

Regression lines fitted to change in ISF (y) against time in weeks (X) of the form Y = mX+b. 95% confidence limits for slope in brackets.

Inactive control	Y = -1.61X-9.5	(0.63 ≥ m ≥ -3.86)
Exercise control	Y = -2.38X-1.5	(-0.93 ≥ m ≥ -3.82)
Noradrenaline Inactive	Y = -2.40X-1.8	(-0.50 ≥ m ≥ -4.30)
Noradrenaline plus Exercise	Y = -1.24X-3.4	(0.80 ≥ m ≥ -3.27)
Isoprenaline Inactive	Y = -6.34X-2.5	(-4.55 ≥ m ≥ -8.13)
Isoprenaline plus Exercise	Y = 0.11X+1.6	(2.93 ≥ m ≥ -2.71)
Propranolol Inactive	Y = -3.32X+2.8	(-1.24 ≥ m ≥ -5.40)
Propranolol plus Exercise	Y = -4.42X-3.9	(-2.14 ≥ m ≥ -6.70)

and the propranolol exercise ($p<0.01$) groups (Figure 9). In contrast with the effects of either exercise or a sympathomimetic (noradrenaline or isoprenaline) alone, exercise in combination with a sympathomimetic drug produced no significant decrease in ISF (Table 11), and the change in ISF was not significantly different from the inactive control (Figure 9)

IV-2-3: Electrolytes:

In the plasma, there were no differences in the sodium content of any group (Table 12). The plasma potassium tended to be lower in the exercised group of each pair, but there were no significant differences. The myocardial sodium was not significantly different from inactive control in any group. Myocardial potassium was higher, but not significantly, in the exercise control animals. In three of the sympathomimetically treated groups, noradrenaline inactive, isoprenaline inactive and isoprenaline plus exercise, the myocardial potassium was significantly less than in the inactive control ($p<0.05$). The inactive and exercise propranolol treated groups were not different from the inactive control. The K_o^+/K_i^+ ratio was significantly less than the inactive control only in the exercise control group (Table 12). The other exercised groups also tended to have lower ratios of plasma to myocardial potassium.

IV-2-4: Heart Weight:Body Weight

The isoprenaline treated groups had higher heart weights than the inactive control (Table 13), and the difference was significant in the isoprenaline plus exercise group ($p<0.1$). Body weights were very much lower in all four exercised groups in comparison with the inactive control.

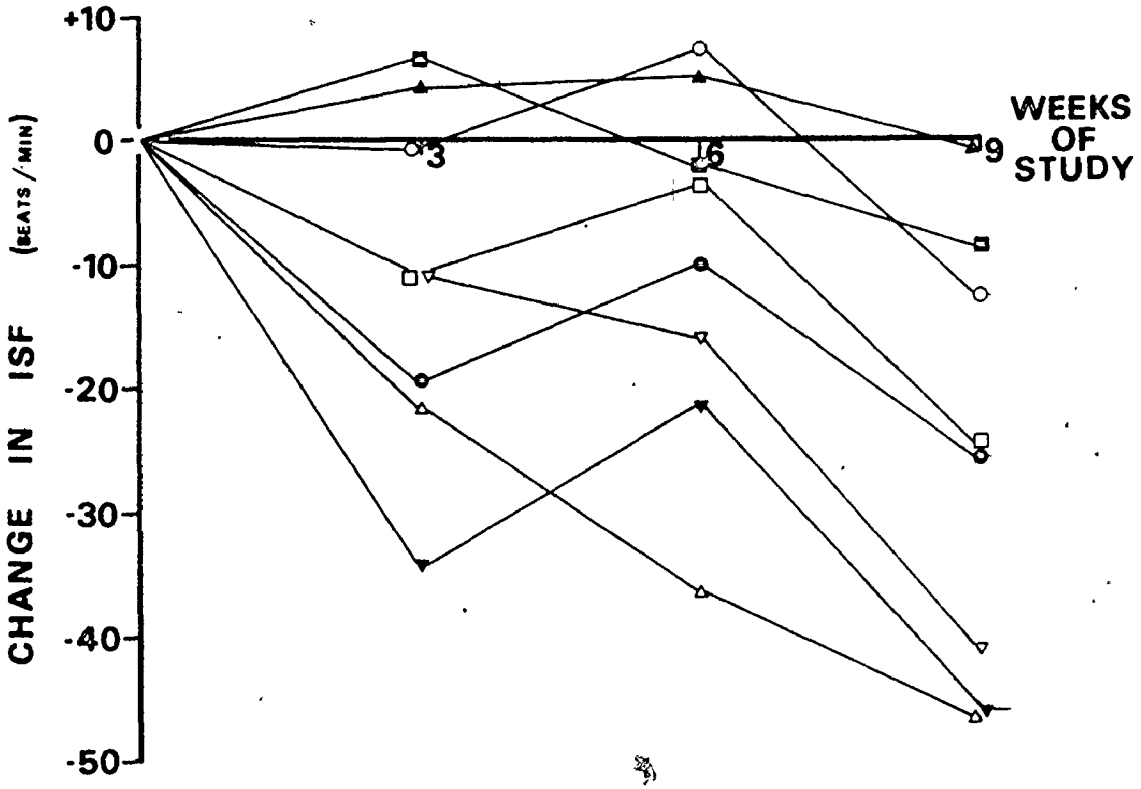


Figure 9. Change in intrinsic sinoatrial frequency during Study 2. Symbols: Inactive control (○), Exercise control (●), Noradrenaline inactive (□), Noradrenaline plus exercise (■), Isoprenaline inactive (△), Isoprenaline plus exercise (▲), Propranolol inactive (▽), and Propranolol plus exercise (▼). Differences from the inactive control at week 9: Exercise control ($p<0.05$), Noradrenaline inactive ($p<0.1$) Isoprenaline inactive ($p<0.01$), Propranolol inactive ($p<0.05$) and Propranolol plus exercise ($p<0.01$).

TABLE 12

Study 2. Plasma and Myocardial Na^+ and K^+ contents, and K_o^+/K_i^+ Ratio.

	Plasma (mEq/L)		Myocardial (mEq/100g wet weight)		K_o^+/K_i^+
	Na_o	K_o	Na_i	K_i	
Inactive Control	140.8 ± 1.5	6.26 ± 0.31	3.38 ± 0.15	6.57 ± 0.16	0.94 ± 0.05
Exercised Control	140.3 ± 1.0	5.55 ± 0.35	3.15 ± 0.21	6.86 ± 0.28	0.73 ± 0.04*
Noradrenaline	138.4 ± 1.1	6.07 ± 0.45	3.57 ± 0.23	6.07 ± 0.14*	1.05 ± 0.14
Exercise + Noradrenaline	139.4 ± 1.2	5.36 ± 0.52	3.78 ± 0.17	6.75 ± 0.14	0.82 ± 0.11
Isoprenaline	139.5 ± 0.7	5.58 ± 0.25	3.39 ± 0.23	6.10 ± 0.09*	0.93 ± 0.07
Exercise + Isoprenaline	141.4 ± 1.2	5.28 ± 0.57	3.63 ± 0.47	6.16 ± 0.20*	0.84 ± 0.12
Propranolol	141.2 ± 0.9	6.16 ± 0.44	3.20 ± 0.20	6.46 ± 0.17	0.96 ± 0.09
Exercise + Propranolol	139.2 ± 1.9	5.71 ± 0.31	3.88 ± 0.10	6.82 ± 0.22	0.85 ± 0.07

Values are the mean ± 1 S.E.M. Differences from the Inactive Control were calculated by a t-statistic. The "*" indicates different from inactive control, $p<0.05$.

TABLE 13

Body weight, heart weight and heart ratio after 10 weeks of Study 2.

	Body Weight (g)	Heart Weight (g)	Heart/Body Weight (mg/g)
Inactive Control	521.0 ± 8.7	1.22 ± 0.03	2.33 ± 0.04
Exercise Control	483.1 ± 13.4 ($p<0.05$)	1.17 ± 0.05	2.42 ± 0.05 ($p<0.2$)
Noradrenaline Inactive	528.9 ± 9.9	1.25 ± 0.03	2.37 ± 0.03
Noradrenaline plus Exercise	481.8 ± 12.5 ($p<0.05$)	1.24 ± 0.05	2.56 ± 0.05 ($p<0.005$)
Isoprenaline Inactive	540.5 ± 11.0	1.30 ± 0.05	2.39 ± 0.05
Isoprenaline plus Exercise	500.6 ± 11.1	1.33 ± 0.04 ($p<0.1$)	2.68 ± 0.07 ($p<0.001$)
Propranolol Inactive	530.9 ± 9.4	1.25 ± 0.03	2.36 ± 0.03
Propranolol plus Exercise	490.6 ± 14.6 ($p<0.1$)	1.25 ± 0.04	2.54 ± 0.06 ($p<0.01$)

Values are the mean ± 1 S.E.M. The p-value obtained from the t-statistic comparing each group with the inactive control is in the bracket beneath the mean. Where no p-value is given, $p>0.2$.

As a consequence of the lower body weight in the exercised animals, all four exercised groups also had greater heart weight to body weight ratios (Table 13).

IV-2-5: Intrinsic Sinoatrial Frequency In Vitro:

There was no difference between the in vitro ISF of the inactive control and any other group (Table 14).

IV-2-6: Acetylcholine Chronotropic Responses:

As in Study 1, there were no differences detected in the sensitivity to acetylcholine, as assessed by the ED_{50} (Table 15) in any of the eight groups (Figure 10).

IV-2-7: Noradrenaline Chronotropic Responses

In comparison with the inactive control group, the two chronically noradrenaline treated groups, noradrenaline inactive and noradrenaline plus exercise, were both more sensitive to noradrenaline in vitro (Figure 11). The ED_{50} values calculated for the two noradrenaline treated groups were significantly ($p<0.05$) less than the inactive control (Table 15). No other group showed a change in sensitivity to noradrenaline.

The maximum chronotropic response was reduced significantly from the inactive control in the propranolol inactive ($p<0.05$) and the propranolol plus exercise ($p<0.05$) groups (Figure 11). In the exercise control, and the two isoprenaline treated groups, the maximum chronotropic response was reduced, but not significantly.

TABLE 14

Intrinsic sinoatrial frequency *in vitro* in Study 2.

Group	ISF (beats/min)
Inactive Control	144.4 ± 5.1
Exercise Control	142.5 ± 12.3
Noradrenaline Inactive	143.3 ± 9.9
Noradrenaline plus Exercise	149.8 ± 6.2
IsoprenalineInactive	143.3 ± 6.8
Isoprenaline plus Exercise	160.3 ± 12.6
Propranolol Inactive	156.3 ± 8.1
Propranolol plus Exercise	151.4 ± 4.2

Values are the mean ± 1 S.E.M. No values are different from the Inactive Control.

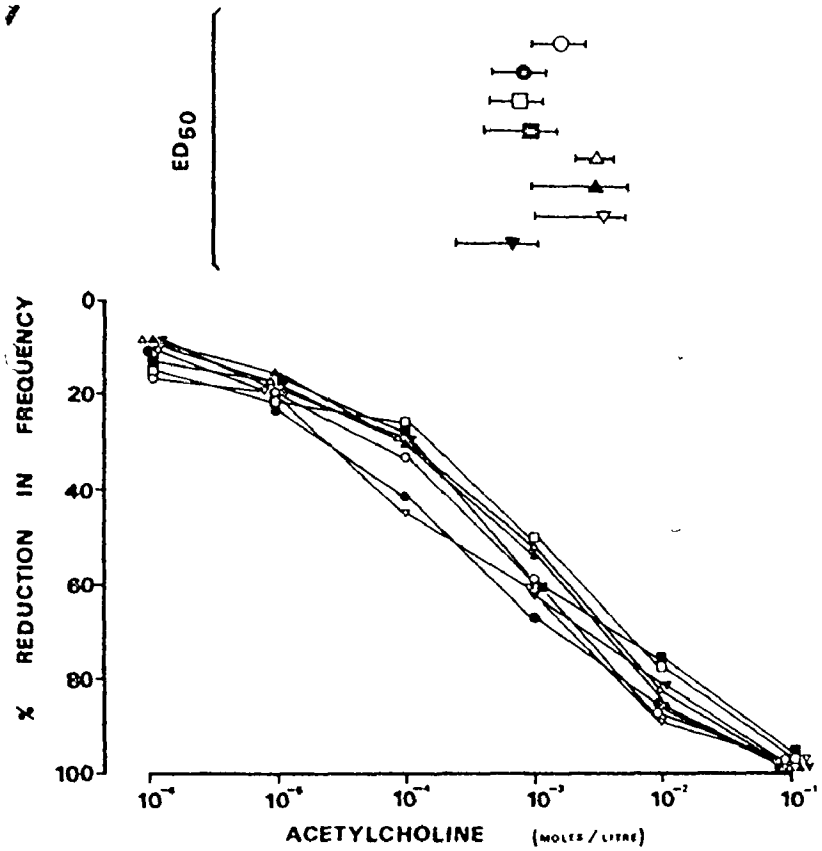


Figure 10. The in vitro acetylcholine chronotropic dose-response curves for Study 2 showing the ED_{50} values. Each point on the dose-response curve is the mean value. The ED_{50} is the mean value, with ± 1 S.E.M. shown. Symbols: Inactive control (○), Exercise control (●), Noradrenaline inactive (□), Noradrenaline plus exercise (■), Isoprenaline inactive (△), Isoprenaline plus exercise (▲), Propranolol inactive (▽), and Propranolol plus exercise (▼). There were no differences from the inactive control from the ED_{50} value of any group.

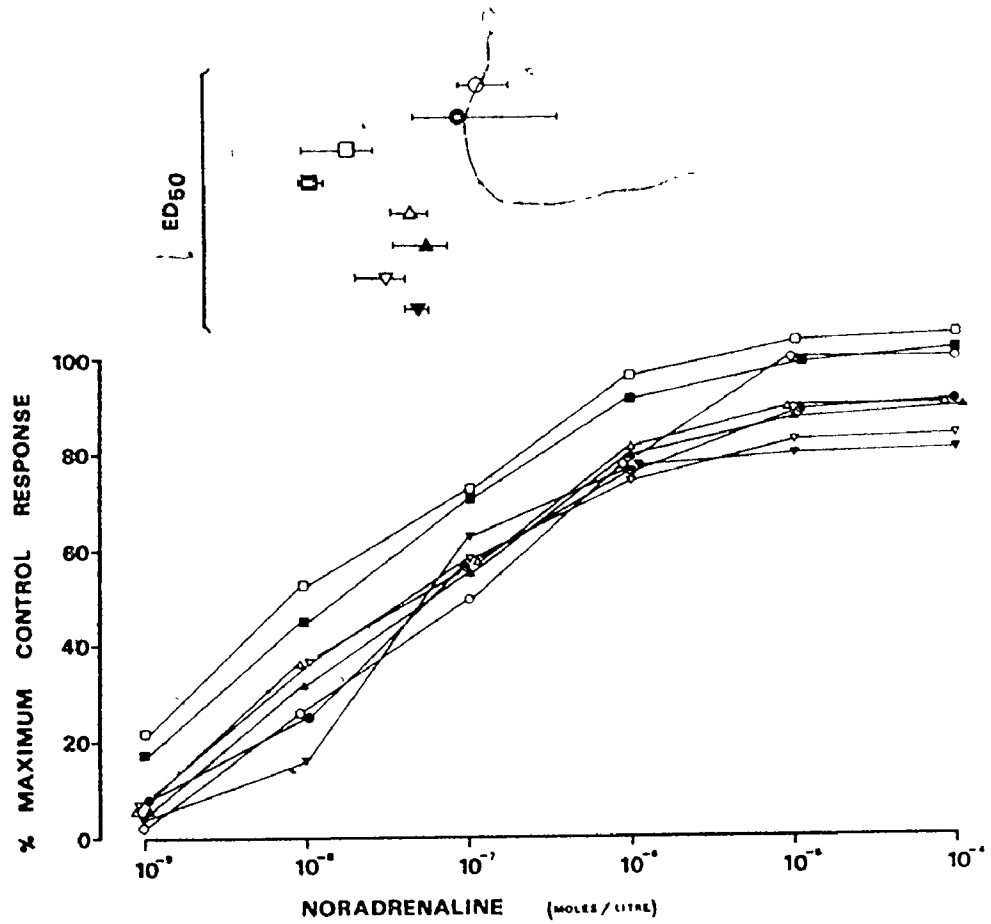


Figure 11. The in vitro noradrenaline chronotropic dose-response curves for Study 2 showing the ED_{50} values. Each point of the dose-response curve is the mean value. The ED_{50} is the mean value with $\pm$ 1 S.E.M. shown. Symbols: Inactive control (○), Exercise control (●), Noradrenaline inactive (□), Noradrenaline plus exercise (■), Isoprenaline inactive (△), Isoprenaline plus exercise (▲), Propranolol inactive (▽), and Propranolol plus exercise (▼). Differences from the inactive control for the maximum response: propranolol inactive ($p<0.05$) and propranolol plus exercise ($p<0.05$). Differences from the inactive control for the ED_{50} values: noradrenaline inactive ($p<0.05$) and noradrenaline plus exercise ($p<0.05$).

TABLE 15

Assessment of sensitivity to acetylcholine and noradrenaline *in vitro* in Study 2.

	Acetylcholine ED_{50} ($X10^{-4}$ Moles/Litre)	Noradrenaline ED_{50} ($X10^{-8}$ Moles/Litre)
Inactive Control	16.1 ± 9.6	12.0 ± 5.6
Exercise Control	8.30 ± 3.68	9.40 ± 4.42
Noradrenaline Inactive	7.92 ± 3.32	1.92 ± 0.91*
Noradrenaline plus Exercise	9.67 ± 5.64	1.09 ± 0.29*
Isoprenaline Inactive	30.3 ± 11.0	4.77 ± 1.22
Isoprenaline plus Exercise	30.0 ± 25.5	5.83 ± 2.14
Propranolol Inactive	31.5 ± 21.6	3.25 ± 1.11
Propranolol plus Exercise	7.11 ± 4.78	5.12 ± 0.89

Values are the mean ± 1 S.E.M. Differences from the Inactive Control were calculated by a t-statistic. The "*" indicates different from inactive control, $p<0.05$.

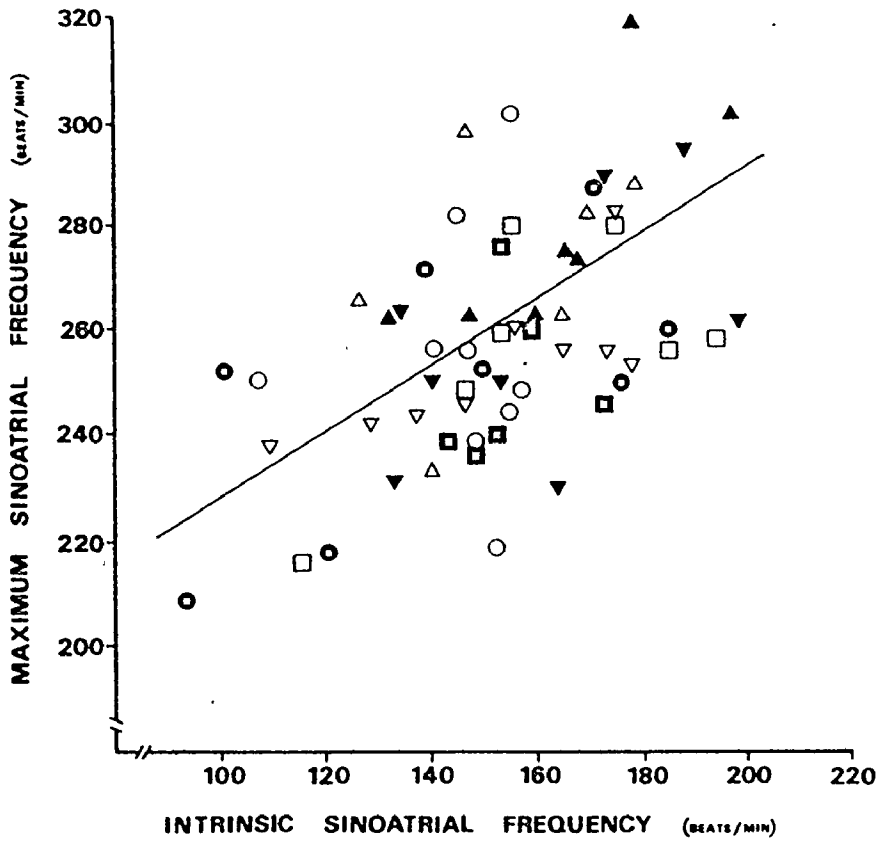


Figure 12. The relationship between the maximum sinoatrial frequency in response to noradrenaline stimulation and the intrinsic sinoatrial frequency in vitro for all animals in Study 2. Symbols: Inactive control (○), Exercise control (●), Noradrenaline inactive (□), Noradrenaline plus exercise (■), Isoprenaline inactive (△), Isoprenaline plus exercise (▲), Propranolol inactive (▽) and Propranolol plus exercise (▼). Correlation coefficient (r) = 0.63, equation of the line: Maximum sinoatrial frequency = (0.64)·(ISF in vitro) + 164.

A significant linear relationship was obtained between the *in vitro* ISF and the maximum frequency in response to noradrenaline *in vitro* ($r = 0.63$) (Figure 12). As in Study 1, this relationship indicates that those atria with a high spontaneous rhythmicity obtain the highest maximum frequency when maximally stimulated by noradrenaline.

IV-3: Study 3

In Study 3, the maximum chronotropic response has been assessed *in vivo* in response to exercise and noradrenaline infusion.

IV-3-1: Intrinsic Sinoatrial Frequency In Vivo:

The experimental manipulation of ISF by administration of atropine to five of ten animals over a period of eight weeks produced a wide range of values of ISF (280 to 420 beats/min.).

IV-3-2: Maximum Exercise Cardiac Frequency:

The attainment of maximum exercise cardiac frequency in the group of ten rats by progressively increasing the treadmill grade produced a range of maximum cardiac frequencies from 480 to 570 beats/min. (Figure 13). The maximum exercise frequency was linearly related to the ISF *in vivo* $Y = 0.49 \times +355$ ($r = 0.76$) where Y = maximum cardiac frequency and X = ISF. As in Studies 1 and 2, the highest ISF attains the highest maximum frequency.

IV-3-3: Maximum Noradrenaline-Induced Cardiac Frequency:

The range of the maximum responses to noradrenaline infusion in

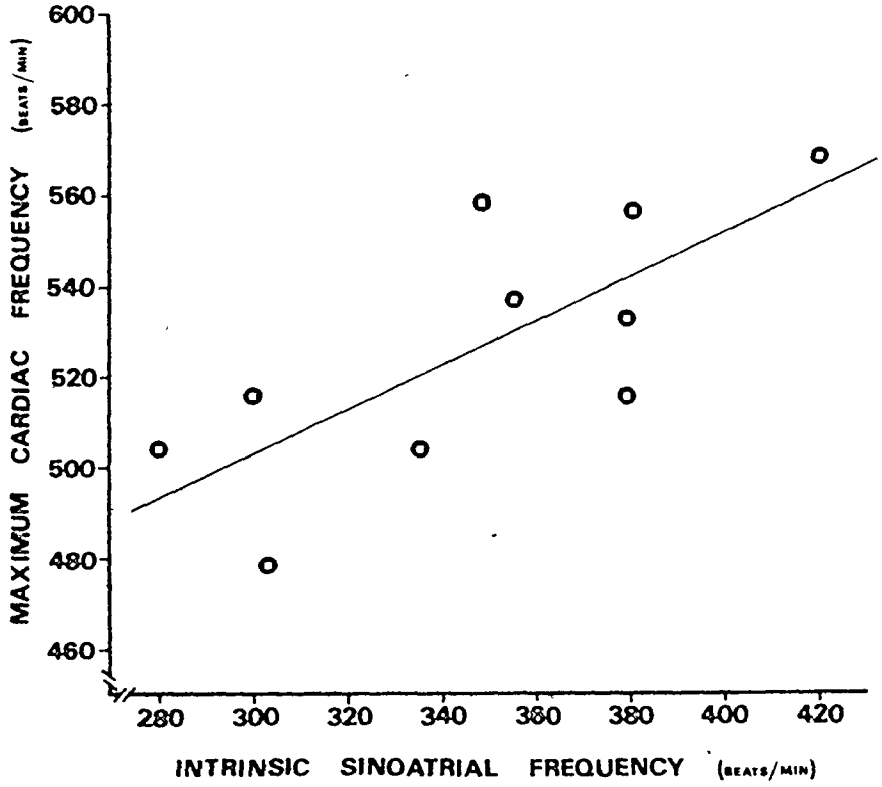


Figure 13. The maximum cardiac frequency response to maximum exercise plotted against the intrinsic sinoatrial frequency in vivo. Each point represents one animal. Correlation coefficient (r) = 0.76.

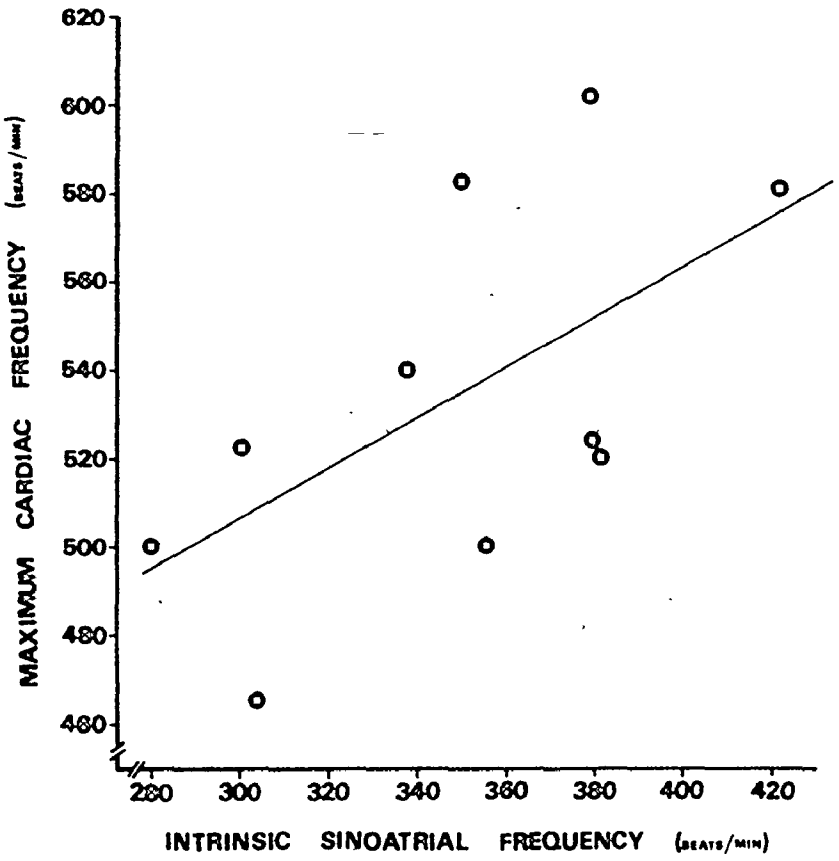


Figure 14. The maximum cardiac frequency response to intravenous noradrenaline in the anesthetized, ganglion blocked rat, plotted against the intrinsic sinoatrial frequency in vivo. Each point represents one animal. Correlation coefficient (r) = 0.61.

the same animals while under anesthesia and ganglionic blockade was 480 to 620 beats/min. (Figure 14). The maximum cardiac frequency obtained under these experimental conditions was linearly related to the ISF in vivo $Y = 0.58X + 350$ ($r = 0.61$), where Y = maximum cardiac frequency and X = ISF. The maximum frequency obtained with the noradrenaline infusion was higher than with exercise, but the difference was not significant.

V: DISCUSSION

The intrinsic sinoatrial frequency (ISF) decreases in the rat with physical training (Lin and Horvath, 1972; Bolter, Hughson and Critz, 1973). This thesis has examined the role of alterations in autonomic nervous activity in producing the exercise-induced reduction of ISF. The shifts in myocardial and plasma sodium and potassium contents associated with the reduction of ISF have been studied. In addition, the chronotropic responses of the sinoatrial node have been examined firstly *in vitro* to the natural autonomic neurotransmitters acetylcholine and noradrenaline, and secondly *in vivo* to exercise and noradrenaline.

V-1: The Experimental Procedures:

In contrast with the two previous studies of the reduction of ISF with physical training in the rat (Lin and Horvath, 1972; Bolter, Hughson and Critz, 1973), the present thesis employed treadmill rather than swimming exercise. The reasons for this were two-fold. Firstly, it is at present unclear what the cardiac frequency response is to swimming exercise. Although swimming is more demanding in terms of oxygen requirements (Dawson, Roemer and Horvath, 1973) than treadmill exercise at 19 m/min. (Popovic et al, 1969), the recent report of Lin and Baker (1975) confirms the findings in other mammals (Andersen, 1966) of a bradycardia associated with water immersion. Personal observation of the rats trained in the study of Bolter, Hughson and Critz (1973) revealed that swimming rats spend a considerable amount of time totally immersed

in the water. It therefore appears likely that high levels of parasympathetic activity may also be present during the swimming exercise in addition to the high levels of sympathetic activity normally associated with physical exercise (Ekblom, Kilbom and Soltysiak, 1973). The second factor which detracted from the use of swimming as a model for training in the present thesis was the temperature effect of swimming. Body temperature in the rats swimming for one hour in 32°C water fell from the pre-exercise level of 37°C to 35°C in spite of the heat production associated with exercise (Bolter, Hughson and Critz, unpublished, 1972). The use of swimming training would have necessitated the formation of complex control groups involving immersion and cooling. Treadmill exercise eliminated these problems.

The intensity of the exercise employed in the present thesis was near maximal for the animals involved. No pre-selection was made on the basis of running ability as in the studies of Holloszy and co-workers (for review see Holloszy and Booth, 1976). All animals progressed in Study 1 at the rate of the weaker members of the running groups and the animals of Study 2 followed this same exercise programme. Animals were observed throughout the exercise periods, and encouraged to run by mechanical prodding if they attempted to avoid exercise by holding the edges of the individual compartment. In this manner, all animals received equal exercise training.

The measurement of cardiac frequency has been described in the Methods. The animals appeared to accept the attachment of the subcutaneous electrodes with little pain or discomfort. Most animals rested quietly in the bottom of the buckets when the cardiac frequency measurements were

being made (Appendix 1).

The concentrations of drugs employed and the resulting cardiac frequency responses are detailed in the Appendix sections 2, 3 and 4. The ISF was measured with the selective beta-adrenergic receptor blocker propranolol and the parasympathetic receptor blocker atropine. The concentrations employed have been used by previous investigators (8 mg/kg propranolol, Walsh, 1969; Lin and Horvath, 1973, and 3 mg/kg atropine, Walsh, 1969), but were confirmed to produce total blockade by the failure of additional doses to cause a further change in cardiac frequency (Appendix 2a).

The drugs employed in Study 1 to chronically alter the activity levels at the parasympathetic receptors of the sinoatrial node have been described briefly in the Methods and in Appendix 3. Atropine is a muscarinic blocker and can produce total competitive blockade at a concentration of about 1 mg/kg (Lin and Horvath, 1972). Study 1 employed 5 mg/kg atropine sulphate. Large (1975) has demonstrated that a concentration of 1 mg/kg atropine had a very small effect as a ganglionic blocker in rats. The action of atropine in the present study was probably, therefore, that of a muscarinic blocker, but partial ganglionic blockade may have contributed to its actions. The mechanism of the tachycardia produced by atropine is unclear, however, Appendix 3 shows the duration of the tachycardia to be in excess of six hours. It was felt that this was a significant proportion of the total daily parasympathetic activity (approximately 25%) which was being blocked by this concentration. Although no adverse effects were noted in any animal receiving daily atropine, doses higher than this are frequently lethal. Carbachol was

chosen as the stimulant of the parasympathetic receptors of the heart because it is longer acting than acetylcholine. Carbachol, however, may increase the sympathetic receptor activity in the myocardium by a reflex baroreceptor response to the arterial hypotension resulting from a stimulation of cholinergic vasodilator receptors (Bolme and Fuxe, 1967), and also from a direct release of noradrenaline from the adrenergic neurons (Steinsland and Furchgott, 1975). Thus, there was probably a mixture of direct cholinergic and indirect adrenergic stimulation as a result of this drug. A marked resting bradycardia was achieved with 0.15 mg/kg carbachol (Appendix 3). In preliminary trials with the drug, a ten fold increase in concentration was lethal. Heavy lacrimation was observed in the animals receiving daily carbachol, but no other adverse effects were noted. It was felt that this was the maximum dose which could be safely used in this study.

The drugs employed in Study 2 to chronically alter the levels of activity at the sympathetic receptors were noradrenaline, isoprenaline and propranolol (Methods and Appendix 4). Noradrenaline was used as it is the neurotransmitter acting on the β-adrenergic receptors of the heart (Euler, 1946). However, noradrenaline also acts on the adrenergic receptors of the vasculature (α-receptors, Alhquist, 1948) causing elevated blood pressure and a reflex slowing of the heart (Appendix 4a). While the suspension of noradrenaline in oil prolongs the action of the drug on the blood pressure response (Linna et al, 1965), the cardiac frequency measurement of the present study showed that the duration of interaction with the myocardial adrenergic receptors could not be confirmed (Appendix 4a). To achieve stimulation of the adrenergic receptors of the myocardium with no effect on the α-adrenergic receptors, the selective β-stimulant

isoprenaline (Ahlquist, 1948) was employed. Appendix 4b shows that the duration of action of 0.1 mg/kg isoprenaline was three hours. This duration of adrenrgic stimulation was of a similar order of magnitude as the duration of the tachycardia resulting from exercise. Blockade of the β-adrenergic receptor was achieved with propranolol (Black et al, 1964; Barret and Fitzgerald, 1968). This drug was administered orally (Methods). The concentration chosen has been proven effective in blocking a noradrenaline induced hypertension (Vavra, Tom and Greselin, 1973) and in the present study elicited a bradycardia (Appendix 4c). The drug was available in the drinking water and to ensure that the drug was taken, the water was available for approximately 12 hour periods. The measurement of a significant bradycardia confirms the effectiveness of the concentration of propranolol.

The investigations in vitro employed a modification of the methods of Bolter, Hughson and Critz (1973). Cumulative dose-response curves were constructed for the chronotropic response of isolated right atria to acetylcholine and noradrenaline. Any preparations which developed arrhythmias were discarded to eliminate the possible influence of non-sinoatrial rhythms on the results. The frequency response of the isolated arium to the administered drugs is displayed in Appendix 5.

Myocardial and plasma sodium and potassium contents were determined by flame photometry. The comparison of the results obtained in this thesis with the published values will be made in more detail in the later section "Electrophysiology of the ISF". In addition, the influence of various factors on measured content will be discussed.

The investigation of maximum exercise cardiac frequency in Study 3

involved the obtainment of a poorly defined end point. While most animals clearly attempted to continue to keep pace with the treadmill as the work load increased, some animals either reached their maximum earlier, or refused to go further. Since it was impossible for the investigator to be sure which factor brought about the early completion of the test, all reults have been treated equally. The measurement of the maximum cardiac frequency response to infused noradrenaline in the anesthetized, ganglion blocked rat had a more clearly defined end point. It was assumed that the concentration of hexamethonium determined by preliminary investigation to produce blockade of the reflex bradycardia and fall in blood pressure (Appendix 6) was effective in providing total blockade. In all animals, a constant body temperature of 38^{o}C was maintained. This may have been a possible source or error as some animals had an initial temperature of less than 38^{o}C when measured shortly after anesthetization. An elevation in body temperature would result in an increase in ISF (Jose, Stitt and Collison, 1970) which would alter the relationship between ISF measured earlier <u>in vivo</u> and the maximum cardiac frequency response to noradrenaline.

V-2: Body Weight and Heart Weight:

In both Study 1 and Study 2, body weight increased linearly with time. Exercised control groups gained less weight than the inactive control groups. This has been a consistant finding in previous studies (Oscai, Mole and Holloszy, 1971; Bolter, Hughson and Critz, 1973; Ekstrom, 1974). All exercised groups which were also drug treated had body weights significantly below the inactive control groups. There were no differences between the body weights of the drug treated inactive rats and the inactive controls.

An increase in heart weight above the inactive controls was observed only in the two isoprenaline treated groups. This has been observed previously (LeBlanc, Vallieres and Vachon, 1972; Harri and Valtola, 1975). The failure of treadmill exercise to induce a cardiac hypertrophy is a common finding (Johnson and Thorp, 1975; Ekstrom, 1974; Oscai et al, 1971). In agreement with these previous studies, the heart weight to body weight ratio has been increased as a consequence of the lower body weights of the exercised animals.

V-2-1: Summary

The exercised animals gained weight at a lower rate than the inactive animals. As a consequence, the heart weight to body weight ratio was greater in the exercised animals. Isoprenaline was the only drug to increase heart weight.

V-3: Study 1: Parasympathetic Influences on ISF

V-3-1: Introduction

Does an increase in resting parasympathetic nervous activity in the trained state act on the sinoatrial node to produce the exercise-induced reduction of ISF? This was the question formulated in the Introduction to this thesis concerning Study 1. In an attempt to answer this question, an experimental design was employed which combined physical training with daily blockade or stimulation of the receptors of the parasympathetic nervous system in the heart. The first premise in Study 1, however, was that an increase in resting parasympathetic activity was produced by the treadmill training. This was examined by the acetylcholine chronotropic response *in vitro* (Bolter, Hughson and Critz, 1973), and will be discussed under the heading "Chronotropic Responses to Acetylcholine *in vitro*" later in the Discussion. To block the reported high parasympathetic activity in the trained state (Bolter, Hughson and Critz, 1973; Ekblom, Kilbom and Soltysiak, 1973), atropine was employed. The concentration of atropine employed produced parasympathetic receptor blockade for more than six hours (Appendix 3b). Stimulation of the parasympathetic receptors of the heart was obtained with carbachol (Appendix 3c). Administration of drugs to exercised and inactive animals allowed for the differentiation between exercise and drug induced effects on the ISF. Six groups were formed: inactive control, exercised control, atropine inactive, atropine plus exercise, carbachol inactive, and carbachol plus exercise.

V-3-2: Findings and Discussion:

The ISF of both the inactive and the exercised control groups decreased during the ten weeks of the study. The reduction of ISF in the exercised control group was significantly greater than that of the inactive control group, confirming the previous swim training studies of Lin and Horvath (1972) and Bolter, Hughson and Critz (1973) and the initial report of a reduction in ISF with treadmill training by Hughson et al (1975) which has been subsequently confirmed by Barnard, Corre and Cho (1976). No previous study in rats has reported on the change in ISF in inactive animals with time, but the linear nature of the reduction in ISF was similar to that observed in humans by Jose (1966). This observation indicates that the finding of a reduction in ISF in the inactive animals was probably related to age.

The results of Study 1 indicate that high resting levels of parasympathetic activity were not associated with a reduction of ISF. Blockade of parasympathetic activity with atropine in the exercised animals did not prevent the exercise-induced reduction of ISF. Further, increased parasympathetic activity with carbachol did not bring about a reduction in ISF in the inactive animals. It must be noted that carbachol can indirectly increase sympathetic receptor activity, thus any response may not reflect solely the effect of increased parasympathetic receptor stimulation by carbachol. In contrast to the initial suggestion that atropine may block the exercise-induced reduction in ISF was the finding that atropine reduced ISF in the inactive animals. This finding will be discussed in more detail under "Electrophysiology of the ISF". As a final confirmation that increased resting parasympathetic activity does not

cause the reduction of ISF with exercise are the findings to be discussed later that no increased resting parasympathetic activity was detected by the acetylcholine *in vitro* chronotropic response.

The hypothesis for Study 1 was based largely on the proposal of Raab (1969) that the trained state must be examined for its influence on the ISF. Raab had implicated chronically high levels of parasympathetic activity in the necessary redistribution of myocardial electrolytes which would lead to an alteration in ISF. The findings of Study 1 indicate that high levels of parasympathetic activity neither are necessary for nor cause a reduction in ISF, and that blockade of parasympathetic activity does not prevent a reduction in ISF. The corresponding electrolyte changes associated with exercise and drug administration will be dealt with later. The answer to the first question is, therefore, no, an increase in resting parasympathetic activity did not cause a reduction in ISF in the trained rats.

V-3-3: Summary:

In study 1, a reduction in ISF was observed in the inactive control animals. This reduction was probably age-dependent. A significantly greater reduction in ISF was measured in the exercise control group. High levels of resting parasympathetic activity were not responsible for the reduction of ISF. Rather, parasympathetic receptor blockade with daily atropine injections caused a reduction in ISF, and the effects of exercise and atropine appeared to be additive. Daily stimulation of the parasympathetic receptors with carbachol had no significant effect on ISF.

V-4: Study 2: Sympathetic Influences on ISF

V-4-1: Introduction:

Does the increase in sympathetic nervous activity during physical exercise act on the sinoatrial node to produce the exercise-induced reduction of ISF? The second study of this thesis was designed to examine this question which was advanced in the Introduction. The experimental design of Study 2 combined the effects of physical exercise with stimulation or blockade of the sympathetic receptors of the heart. During a period of exercise, the sympathetic activity increases (Ekblom, Kilbom and Soltysiak, 1973). Study 2 employed the sympathetic receptor stimulants noradrenaline and isoprenaline to mimic the effects of increased sympathetic activity and the sympathetic receptor blocker propranolol to reduce sympathetic activity to the heart. As in Study 1, the effects of the drugs were studied in inactive and exercised animals to allow for the differentiation between exercise and drug induced effects. Eight experimental groups were formed: inactive control, exercise control, noradrenaline inactive, noradrenaline plus exercise, isoprenaline inactive, isoprenaline plus exercise, propranolol inactive, and propranolol plus exercise.

V-4-2: Findings and Discussion:

As in Study 1, the ISF decreased in both the inactive and exercised control groups. The ISF decreased significantly more in the exercised than the inactive control. In contrast with Study 1, the absolute decrease in ISF was not as great in either of the control groups. It is

difficult to offer a definite reason for the deviations between Study 1 and Study 2, however, the two studies were carried out a different times of the year and a seasonal variation in response may have occurred.

Sympathetic receptor stimulation with either noradrenaline or isoprenaline produced results which were qualitatively similar. The small differences in response to the two sympathomimetics probably reflects a dose dependent effect of the two drugs. Either noradrenaline or isoprenaline administered to the inactive animlas resulted in a decrease in ISF. However, the combination of exercise plus either noradrenaline or isoprenaline produced an ISF which did not differ from control. It appears that noradrenaline and isoprenaline evoke similar responses due to a direct action of the sympathomimetic on the cells of the sinoatrial node. The fear expressed earlier that the increased parasympathetic stimulation associated with noradrenaline administration may influence the response to this drug appears to be unfounded from the observed changes in ISF in Study 2. Thus both the bradycardia associated with noradrenaline and the tachycardia with isoprenaline administration resulted in similar changes in ISF. The effects of the sympathomimetics on the ISF in the inactive and exercised animals seem to be in conflict. Exercise alone induces high sympathetic activity, and noradrenaline or isoprenaline administration produces high levels of sympathetic receptor activity, and both the factors exercise or sympathomimetic adminstration, caused reductions in ISF. Exercise combined with sympathomimetic administration, however, did not result in a reduction of ISF. The two factors may act through different mechanisms, the effects of which cancel when combined. This possibility will be discussed further under the heading "Electrophysiology of the ISF".

Propranolol administration to the inactive group resulted in a decrease in ISF. This decrease was similar in magnitude to that produced by exercise. The combination of propranolol plus exercise resulted in a reduction of ISF which was slightly greater than that due to propranolol alone, however, it is not possible to say that the effects were additive.

The question raised concerning the role of elevated levels of sympathetic activity associated with physical exercise can be answered in the negative. From the results of the present Study 2, it does not appear that the reduction in ISF with physical training is a consequence of the elevated sympathetic nervous activity during the exercise. This finding would appear to refute the second proposal by Raab (1969) that autonomic nervous activity associated with exercise may cause changes in myocardial electrolyte distribution resluting in a reduction of ISF. The resultant myocardial and plasma electrolyte contents from Study 2 will be considered under "Electophysiology of the ISF".

V-4-3: Summary:

Study 2 observed a reduction in ISF in the exercised control group which was greater than the inactive controls.

Stimulation of the sympathetic receptors with either noradrenaline of isoprenaline caused similar responses of the ISF. The drugs given to inactive animals resulted in a decrease in ISF, while the drugs in combination with exercise resulted in no significant decrease in ISF.

Blockade of the sympathetic receptors of the heart with propranolol in the inactive animals caused a reduction in ISF which was greater than that in the inactive control. Propranolol plus exercise resulted in a

slightly greater reduction in ISF, but it was felt that a conclusion regarding the additive nature of the two effects was not possible.

It was concluded that the increased sympathetic activity associated with the exercise was probably not the factor responsible for the reduction of ISF with training.

V-5: Electrophysiology of the ISF:

V-5-1: Introduction:

In the Introduction, two questions were asked concerning the electrolyte content of the myocardium and plasma. The first was: Is the exercise-induced reduction of ISF associated with changes in electrolyte distribution between the myocardium and plasma? Secondly, are any changes in ISF produced by pharmacological intervention associated with similar shifts in electrolyte distribution as found with exercise? These questions were examined in Studies 1 and 2.

The distribution of electrolytes is a single factor affecting the membrane potential and the ISF. In a simple resting muscle cell or neuron the membrane potential is the resultant of the interaction between the electrolyte distribution and the permeability of the membrane to that ion (Hodgkin and Katz, 1949). For any single ion, the equilibrium potential is given by the classical Nerst equation, for example for potassium $E_K = \frac{RT}{F} \ln \frac{[K^+]o}{[K^+]i}$, where E_K is the potassium equilibrium potential, R is the gas constant, T is temperature in degrees absolute, F is the Faraday constant, and $[K^+]o$ and $[K^+]i$ are the concentrations of potassium ions outside and inside the membrane respectively, and the sum of all ionic potentials is given by the more complex Goldman constant field equation $Em = \frac{RT}{F} \ln \frac{PNa\ [Na^+]o + P_K\ [K^+]o + P_{Cl}\ [Cl^-]i}{P_{Na}\ [Na^+]i + P_K\ [K^+]i + P_{Cl}\ [Cl^-]o}$, where Em is the membrane equilibrium potential and P is the permeability of the membrane to each ion subscripted, (Hodgkin and Katz, 1949; Brady, 1974). This latter equation considers both the ionic distribution and membrane permeability for all ions. In pacemaker cells, the changes in membrane permeability

are more complex. Noble (1975) reviewed recent knowledge concerning membrane permeability changes during the crdiac cycle, specifically as they apply to the Purkinje fibres (Figure 15). The electrophysiology of the sinoatrial node is not as clearly understood. In ventricular muscle, the resting membrane potential can be seen to vary directly as a function of (K_o^+/K_i^+) over a wide range of potassium values above a $[K_o^+]$ of 4 mEq/L. The membrane potential of atrial muscle and especially the sinoatrial node are less acurately described by the (K_o^+/K_i^+) (Noma and Irisawa, 1975) because of the influence of a decreased P_K/P_{Na}, of the metabolic pumping of Na^+ and K^+, and of the movement of calcium ions (Noble, 1975). Recent work with frog atrial muscle indicates that changes in P_K are important in producing the pacemaker potential in the sinoatrial node (Brown, Clark and Noble, 1976). While the measured maximum diastolic potential in the sinoatrial node is approximately -60 millivolts, the E_K is probably much larger than this indicating that (K_o^+/K_i^+) is only a single factor influencing membrane potential. The ratio (K_o^+/K_i^+) is, however, the only indicator of membrane electrophysiology available in this thesis and will be used to give an indication of the probable direction of change of maximum diastolic potential. No assessment was made of the slope of the prepotential or the threshold potential which are the other factors influencing the frequency of pacemaker cells (West, 1972) (see Figure 16).

The estimate of myocardial intracellular ion concentration in the present studies was obtained from whole ventricular homogenate. This method will under-estimate potassium and over-estimate sodium contents. Typical values of inoic distribution in the myocardium and plasma were given by Vick et al (1973):

	Intracellular (mEq/100 g tissue)	Extracellular (mEq/kg water)
Na^+	3.09	148.2
K^+	7.54	5.4
Cl^-	2.36	131.4

From these values, it is clear that inclusion of extracellular fluid with a low K^+ content will lower the content of K^+ obtained on the whole ventricle. A simple calculation will reveal, however, that higher intracellular potassium contents will be more affected by this estimate. Based on the estimate of Vick et al (1973) for extracellular space of approximately 15g water/100g tissue the measured K^+ content for the values of Vick et al by the present method would be:

(7.54 mEq/100g tissue) (0.85) + (5.4 mEq/kg water) (0.15) = 6.49 mEq/100g of whole tissue. This value is 1.05 mEq/100g below the true value. If, however, the intracellular K^+ is increased by 0.40 mEq/100g as found by Korge, Masso and Roosson (1974), with physical training, then the measured content based on whole ventricle would be:

(7.94 mEq/100g tissue) (0.85) + (5.4 mEq/kg water) (0.15) = 6.75 mEq/100g of whole tissue. This value is 1.19 mEq/100g below the true value. It is apparent from these calculations that changes observed in myocardial potassium content from whole ventricular homogenate will underestimate the actual changes which occur. Thus, in the present study as the expected change in myocardial potassium is an increase (Korge, Masso and Roosson, 1974), the method will tend to underestimate the true value.

The values calculated from the above data of Vick et al (1973) according to the dilution with 15% extracellular fluid are 3.85 and 6.49 mEq/100g whole tissue for intracellular sodium and potassium respectively. The inactive control group of Study 1 had values of 3.46 and 6.00 mEq/100g whole tissue, results in close agreement with the published values. While it is difficult to apply the myocardial electrolyte content of ventricular muscle to the sinoatrial node, the studies of deMello (1961) indicated that the ratio of ionic distribution was similar throughout the myocardium. The ratio of plasma (K_o^+) to myocardial (K_i^+) potassium (K_o^+/K_i^+) was, therefore, taken as a guide to the maximum diastolic repolarization level. A decrease in the K_o^+/K_i^+ ratio would indicate a more negative potassium equilibrium potential E_K and infer a more negative maximum diastolic repolarization. All changes in K_o^+/K_i^+ in Study 1 and Study 2 have been dealt with by this reasoning as the only indicator of membrane electrophysiology available in this Thesis.

V-5-2: Findings and Discussion

In Study 1, a higher myocardial potassium content was observed in the exercised control animals than the inactive controls. This finding is in agreement with the recent swimming training programme of Korge, Masso and Roosson (1974), and with the reports of increased myocardial potassium content following single bouts of exercise (Bajusz, 1964; Prioreschi and Gabbiani, 1964). As the two groups had similar plasma potassium contents, the ratio of plasma to myocardial potassium (K_o^+/K_i^+) was less in the exercised than the inactive animals. As described in the Introduction to this section, a lower K_o^+/K_i^+ is consistent with a more

negative E_K and probably, therefore, a more negative maximum diastolic repolarization. Thus from a simple analysis, it is possible that the decrease in ISF is the consequence of this more negative maximum diastolic repolarization. This would be in agreement with the proposal of Raab (1969) that it is the alteration of electrolyte balance which is responsible for the reduction in ISF with physical training. It is not, however, possible to conclude that this is the only mechanism responsible for the reduction of ISF. This statement will be evident when the electrolyte contents in the atropine treated groups are considered below.

Daily administration of atropine to inactive animals resulted in a decrease in myocardial potassium content in comparison with non-drug treated inactive controls. This loss of myocardial potassium appears similar to the report quoted by Raab (1969) of Fizel' and Fizel'ova. Since the plasma potassium content was similar in the inactive control and the atropine inactive groups, the ratio K_o^+/K_i^+ was higher in the atropine inactive animals indicating a lower E_K and a less negative maximum diastolic repolarization. The reduction of ISF in the atropine inactive group was greater than that in the inactive control. There is, therefore, a conflict between the findings of the atropine inactive compared with the inactive control. A similar reduction in ISF was observed in the atropine inactive and the exercise control. yet the estimate of maximum diastolic repolarization indicated directionally opposite changes from the inactive control. It must be considered, therefore, that changes in the other major factors listed by West (1972) as controlling the ISF, that is, the slope of the prepotential and the threshold potential, may have resulted in the decrease in ISF observed

in the atropine inactive group.

The atropine plus exercise group had the greatest reduction in ISF observed in Study 1. The myocardial potassium content was similar to the inactive control, thus, exercise increased potassium content over the atropine inactive group. Since the plasma potassium was also similar in the inactive control and the atropine plus exercise groups, the K_o^+/K_i^+ ratio in the two groups was similar. The greatest reduction in ISF, therefore, occurred without any significant differences in maximum diastolic repolarization as estimated from the K_o^+/K_i^+ ratio. The argument applied above to infer that changes in the slope of the prepotential must have occurred to account for the reduction of ISF can also be applied to the atropine plus exercise group. From the discussion of changes in membrane electrophysiology in the two atropine treated groups, it becomes evident that even in the exercised control group changes in slope of the prepotential or the threshold potential may also contribute to the reduction in ISF. Only by intracellular recording of membrane electrophysiology will the definitive answer be found.

The other two groups of Study 1, the carbachol inactive and the carbachol plus exercise groups did not have significant differences from inactive control of the distribution of electrolytes. This would seem to be consistent with the absence of a difference of ISF compared with the inactive control.

The results of Study 2 are consistent with those of Study 1. As in Study 1, exercise resulted in a decrease in the K_o^+/K_i^+ ratio, however, the absolute increase in myocardial potassium in the exercised group was less than in Study 1. This decrease in K_o^+/K_i^+ is consistent with a more

negative maximum diastolic repolarization, and it is possible to attribute all or part of the reduction in ISF in the exercise control group to this change. As in Study 1, it is apparent that reductions in ISF are not related solely to a more negative maximum diastolic repolarization, but probably also to changes in the slope of the prepotential and the threshold potential.

The sympathomimetics noradrenaline and isoprenaline had similar effects not only on the ISF as described previously, but also on myocardial and plasma electrolyte contents. Both noradrenaline or isoprenaline injected daily in the inactive rats caused a loss of myocardial potassium. Small concentrations of catecholamines administered intravenously result in potassium accumulation by the myocardium (Robertson and Payser, 1951), however, losses of potassium occur with large concentrations (260 to 500 mg/kg) adrenaline. The present Study 2 employed 0.1 mg/kg isoprenaline and 0.1 mg/kg noradrenaline, but release was delayed by suspension in oil. It appears, however, that these concentrations caused a reduction in the myocardial potassium content. The ratio K_o^+/K_i^+ was not different from the inactive control. The decrease in ISF in the sympathomimetic treated inactive animals occurred without any evidence of a more negative maximum diastolic repolarization level, therefore, a change in the slope of the prepotential or in the threshold potential probably contributed to the reduction in ISF.

A combination of exercise and catecholamine administration resulted in a myocardial potassium content which was similar to the inactive control. Exercise, therefore, has countered the potassium losing effect of the catecholamines. The resultant K_o^+/K_i^+ ratio for the noradrenaline plus exercise and the isoprenaline plus exercise groups

was less than that for the inactive control, however, there was no reduction of ISF in these two groups. It may be possible that the effects of exercise and of catecholamine administration on the ISF may have been mediated by different electrophysiological mechanisms which negate each other when acting in combination. Only a more direct assessment of membrane electrophysiology will answer this problem.

Propranolol, the beta-adrenergic blocker, had negligable effects on myocardial sodium or potassium, or on the K_o^+/K_i^+ for either the inactive or exercised groups. The ISF was, however, lower than the inactive control in both the propranolol inactive, and the propranolol plus exercise groups. The changes in ISF must be a consequence of an alteration in some other aspect of membrane electrophysiology than the maximum diastolic repolarization and should be investigated by direct measurements with an intracellular electrode.

The first question posed in the Introduction to this section can be answered in the affirmative, that is, changes in the distribution of electrolytes between the plasma and the myocardium do occur with exercise. Furthermore, the distribution of electrolytes following training is consistent with a lower ISF, which can be accounted for in whole or in part by a more negative maximum diastolic repolarization.

The second question asked in the Introduction to this section cannot be answered conclusively. The changes in plasma to myocardial electrolyte distribution associated with atropine, noradrenaline or isoprenaline administration indicated directionally opposite shifts in K_o^+/K_i^+ compared with exercise. For these groups, the reduction in ISF must have been

accompanied by electrophysiological changes in factors other than the maximum diastolic repolarization. In the carbachol and propranolol treated groups, no shift in electrolyte content was evident in comparison with the inactive control.

It was concluded that the complete understanding of the electrophysiological changes for both the exercised and drug treated groups which resulted in the reduction of ISF can only be gained by intracellular microelectrode studies. The following is a brief account of the complex interactions involved in the electrophysiology of pacemaker cells. This discussion will partially explain the difficulty in assessing the electrophysiology of the sinoatrial node from the simple measurement of electrolyte distribution from plasma to myocardium.

Noble (1975) concluded that much work remains to be done in the area of understanding cardiac pacemaker activity. This conclusion would seem to gain additional support from the findings above. Normally quiescent atrial muscle will assume the property of autorhythmicity if a constant depolarizing current is applied which will reduce membrane potential from the resting level of -80 millivolts to a level of -50 millivolts (Brown and Noble, 1969). At the membrane potential of -50 millivolts an outward current is measured. This current increases as the potential moves further from the initial potential. The depolarization caused by an outward current is the equivalent of the prepotential of the sinoatrial node. The cause of the pacemaker-like activity is a decrease in potassium conductance (gK) (Noble, 1975). Little is known of the factors which can chronically change membrane electrophysiology. A

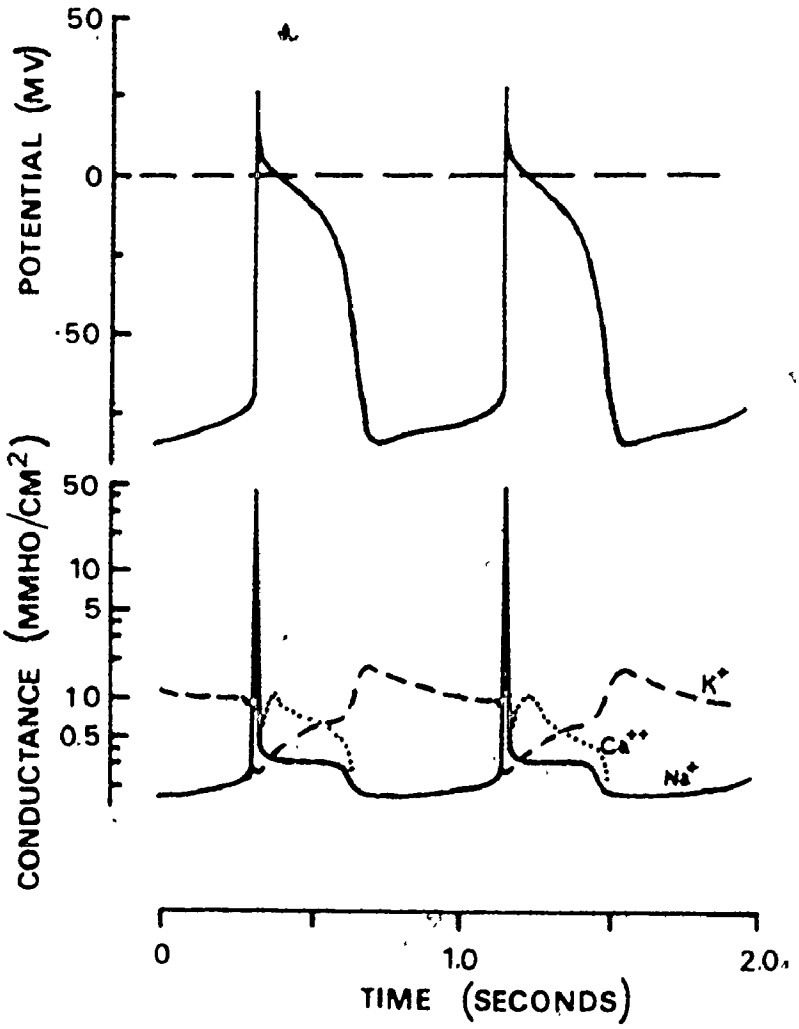


Figure 15. A representation of the proposed changes in pacemaker tissue (Purkinje fibre) occurring during the cardiac cycle according to Noble, 1975. The top portion of the figure represents the membrane potential. The bottom of the figure shows the changes in membrane conductance (g) for the ions Na^+, K^+ and Ca^{++}.

decrease in Na-K-ATPase activity has been observed in the myocardium of exhausted rats (Korge, Masso and Roosson, 1974). The acute effects of the neurotransmitters, however, serve to illustrate the variety of changes which can influence pacemaker electrophysiology.

The parasympathetic neurotransmitter, acetylcholine, decreases cardiac frequency. This is accomplished by the influence of acetylcholine on potassium conductance (gK) resulting in a higher gK and a hyperpolarization of the membrane and a lower slope of the prepotential (Trautwein, Kuffler and Edwards, 1956) as a consequence of increased potassium efflux (Hutter, 1961) (Figure 16). The sympathetic neurotransmitter, noradrenaline, increases cardiac frequency. This action is achieved by the interaction of at least three mechanisms. Firstly, a hyperpolarization of the membrane occurs, and there is an increase of a current designated i_x by Noble (Brown and Noble, 1974). The i_x is a component of the pacemaker current, thus increasing this current will increase the rate of rise of the prepotential. Secondly, it appears that inward calcium current is increased, also affecting the rate of rise of the prepotential (Brooks and Lu, 1972; Brown and Noble, 1974). Finally, the activity of the Na-K-ATPase is increased (Vassalle and Barnabei, 1971), resulting in K^+ accumulation within the pacemaker cells and a hyperpolarization of the membrane (Noble, 1975) (Figure 16). From this brief discussion of the acute effects of the neurotransmitters on pacemaker activity, it is apparent that detailed electrophysiological studies are required before the reduction of ISF can be attributed to the various contributing factors. Until precise measurements of the electrophysiology of the sinoatrial node are available (West (1972)

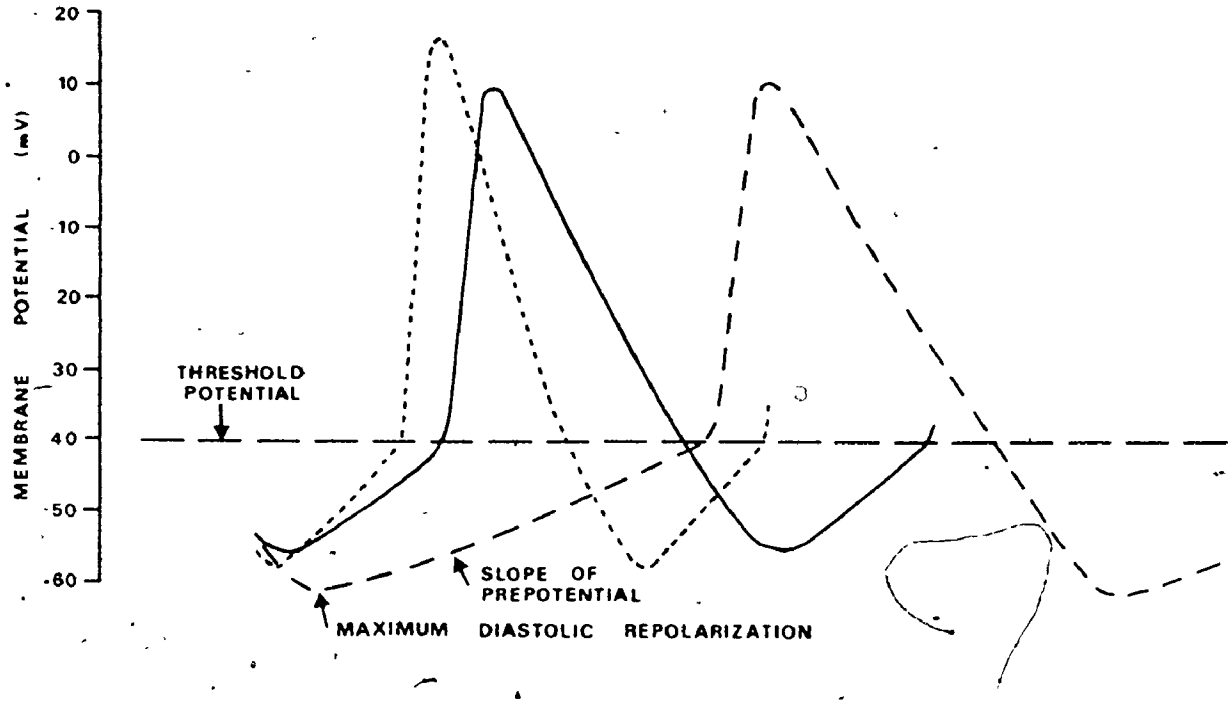


Figure 16. Diagram illustrating changes in the sinoatrial node action potential in response to sympathetic and parasympathetic stimulation. The solid line represents a control condition, the short dashed line represents sympathetic stimulation and the long dashed line represents parasympathetic stimulation. Changes can occur in the sinoatrial action potential at the maximum diastolic repolarization, the slope of the prepotential or the threshold potential (see text for further explanation).

reports only a single impalement of a rat sinoatrial cell), it is impossible to refute or support the earlier statement that the reduction of ISF in the exercise control animals may be due in whole or in part to a more negative maximum diastolic repolarization.

V-5-3: Summary:

The exercised control rats had a lower K_o^+/K_i^+ than the inactive control, indicating a more negative E_K. This was taken as an indicator of a more negative maximum diastolic repolarization. The question from the Introduction about changes in electrolyte distribution with training was answered in the affirmative. It was concluded that the more negative maximum diastolic repolarization may account in whole or in part for the reduction of ISF in the exercised animals.

The different drugs caused a wide variation in measured K_o^+/K_i^+, due largely to changes in myocardial potassium. The change in K_o^+/K_i^+ and its predicted effect on maximum diastolic repolarization did not account for the changes in ISF observed in the groups. Atropine caused an increased K_o^+/K_i^+, yet ISF decreased. Carbachol had little effect on either K_o^+/K_i^+ or ISF. Noradrenaline and isoprenaline both produced an increased K_o^+/K_i^+ but ISF decreased. Propranolol had little effect on K_o^+/K_i^+, but ISF decreased. It was concluded that more precise electrophysiological studies were required to make firm statements regarding the method of reduction of ISF. The question raised in the Introduction concerning the similarity of changes in electrolyte distribution between the exercised control and the drug administration could not be answered with certainty.

V-6: Chronotropic Responses to Acetylcholine:

V-6-1: Introduction:

Is there a decreased sensitivity to acetylcholine with physical training in the present studies as in the work of Bolter, Hughson and Critz (1973), and what are the effects of the drugs in Study 1 and Study 2 on acetylcholine sensitivity? These were the points raised in the Introduction. The *in vitro* sensitivity of a parasympathetically innervated organ to acetylcholine was shown by Bito, Dawson and Petrinova (1971) to be inversely related to the chronic, background parasympathetic activity which had been present *in vivo*. Bolter, Hughson and Critz (1973) used this relationship to provide evidence for increased resting parasympathetic activity in swim trained rats. Many other authors had suggested an elevated resting parasympathetic activity in the trained state (Hall, 1964; Tipton, 1965, Tipton and Taylor, 1965), but did not provide sound evidence to substantiate this proposal. The present Thesis employed the acetylcholine sensitivity to assess the effects of the treadmill training programme on resting parasympathetic activity. The discrepancy in the acetylcholine responses with the previous swim training study will be discussed in view of the different training methods.

V-6-2: Findings and Discussion:

In no group in either Study 1 or Study 2 was the acetylcholine chronotropic response different from the inactive control groups. The

question asked in the Introduction must be answered in the negative, that is, exercise did not evoke an alteration in the sensitivity to acetylcholine. Further, the drugs did not cause a change in sensitivity to acetylcholine.

The absence of an alterated chronotropic sensitivity to acetylcholine implies that there was no difference in the background parasympathetic activity in any group. This finding is perhaps most surprising for the carbachol treated groups. It must be concluded that the concentration of carbachol was not sufficient to affect the acetylcholine sensitivity, but, it must be remembered that the concentration of carbachol employed was only slightly less than a lethal concentration. The atropine treated group did not show an increase in sensitivity to acetylcholine as might be expected if the actual interaction of acetylcholine and the receptor in vivo was essential to establish sensitivity. It seems that the atropine may not have interfered with the interaction required between acetylcholine and the receptor, or it may be possible that atropine substituted for acetylcholine in inducing receptor sensitivity.

The discrepancy between the present study and the previous investigation of Bolter, Hughson and Critz (1973) is quite striking. In the previous investigation, it was concluded that training induced high levels of resting parasympathetic activity. The training programme of the present study did not result in high resting parasympathetic activity. The reason for the difference may be two-fold. Firstly, as described in the "Experimental Procedures", treadmill exercise requires a lower level of energy expenditure than swimming (Dawson, Roemer and Horvath, 1970; Popovic et al, 1969). The lower training intensity may have been

responsible for the failure of the present studies to elicit increased parasympathetic activity, or of other studies (Johnson and Thorp, 1975) to elicit a resting bradycardia. Secondly, swimming type exercise in the rat is frequently associated with total body immersion, resulting in a reflex bradycardia (Lin and Baker, 1975). This immersion bradycardia in other mammals is a consequence of high vagal activity (Andersen, 1966), therefore it is possible that the altered acetylcholine sensitivity reflected the parasympathetic activity accompanying the immersion.

It is concluded that the treadmill training programme employed in the present studies did not evoke a change in resting parasympathetic nervous activity as reflected by the acetylcholine chronotropic sensitivity in vitro. Before conclusions regarding the reason for this finding are made, more studies should be performed, increasing the intensity of treadmill exercise, or establishing appropriate water immersion control animals to compare with the swim training method of Bolter, Hughson and Critz (1973), to determine whether the conclusion reached by these investigators was valid.

V-6-3: Summary:

No altered in vitro acetylcholine chronotropic sensitivity was observed in any group. From this, it was concluded that the treadmill training of the present thesis did not evoke increased resting parasympathetic activity. But, it was suggested that more studies be made to assess the validity of the conclusion of Bolter, Hughson and Critz.

V-7: In Vitro Chronotropic Responses to Noradrenaline:

V-7-1: Introduction:

Is there a decreased sensitivity to noradrenaline with physical training as suggested in the literature, and what are the effects of the drugs employed in Study 1 and Study 2 on noradrenaline sensitivity? This question was raised in the Introduction on the basis of indirect evidence of decreased sensitivity of the sinoatrial node to noradrenaline following training.

Ekblom et al (1973) observed a smaller response to 6-hydroxydopamine (a drug with short term sympathetic stimulant properties) in trained rats and proposed a reduced sensitivity to sympathetic stimulation in this group in comparison with controls. In humans, Brundin and Cernigliaro (1975) suggested that the sinoatrial node was less responsive to catecholamines following training since subjects exercising at the same power output post-training had a lower cardiac frequency but similar urinary catecholamine levels. Kurmaev, Chinkin and Zhdanov (1974) also suggested decreased sensitivity to catecholamines in trained rats. Tipton et al (1972) attempted to assess sympathetic responsiveness in trained adrenalectomized rats by the administration of a single concentration of isoprenaline to isolated perfused hearts. In comparison with appropriate adrenalectomized controls, the trained animals had a lower cardiac frequency response. All three of these studies are indirect and incomplete assessments of sinoatrial sensitivity to sympathetic stimulation. To obtain more complete information complete dose-response curves have been

constructed for the sinoatrial chronotropic response to the sympathetic neurotransmitter noradrenaline (Euler, 1946). As for the acetylcholine chronotropic responses previously described, a modification of the technique of Bolter, Hughson and Critz (1973) of the isolated right atrial preparation was employed.

V-7-2: Findings and Discussion:

V-7-2-1: Sensitivity to Noradrenaline:

In Study 1, there were no differences in the ED_{50} values indicating no change in sensitivity of the sinoatrial node to noradrenaline. Similarly in Study 2, most groups showed no difference in the ED_{50} value in comparison with inactive control, however, the two chronically noradrenaline treated groups had lower ED_{50} values. The noradrenaline inactive and the noradrenaline plus exercise groups were, therefore, more sensitive to noradrenaline *in vitro*. The findings of the present studies do not support the hypothesis advanced by Brundin and Cernigliaro (1975), Ekblom et al (1973) and Tipton et al (1972) that there is a reduced sensitivity to noradrenaline following training. Rather, it appears that noradrenaline sensitivity of the sinoatrial node is unaltered by physical training. While it remains possible that the failure to observe a reduced sensitivity was a consequence of the relatively low training intensity of the present studies, the results of Study 2 argue against this. In Study 2, an increased daily sympathetic activity with noradrenaline resulted in an increased sensitivity *in vitro*, while a decreased

daily sympathetic activity with propranolol resulted in no change. Since neither alteration in sympathetic activity caused a change in sensitivity in the direction predicted by the previous investigators, it is difficult to agree with their interpretation of their results.

The finding of similar urinary catecholamine output at a fixed level of power output pre-and post-training by Brundin and Cernigliaro (1975) is at best difficult to interpret. In support of this finding is the data of Vendsalu (1960) which showed similar plasma catecholamine levels in fit and unfit subjects at the same power output. In contrast, Bloom et al (1976) observed lower plasma catecholamine levels at a percentage of maximum in trained cyclists; and Ostman, Sjostrand and Swedin (1972) and Bernet and Denimal (1974) observed greatly reduced urinary catecholamine output in trained compared with untrained rats during a standard swimming exercise. The actual level of catecholamines at the receptor site cannot always be related to plasma catecholamine levels or urinary catecholamine output because of the activity of the metabolic and uptake pathways for noradreanline (Iversen, 1971; Wurtman, Kopin and Axelrod, 1963). Further, it cannot be assumed that all components of the sympathetic nervous system are equally activated (Ledsome and Linden, 1964). The indirect evidence of Brundin and Cernigliaro (1975) does not, therefore, adequately support the proposal of reduced sensitivity to catecholamines following training.

The indirect assessment of catecholamine sensitivity in the trained rat by the administration of 6-hydroxydopamine (Ekblom et al, 1973) does not give firm support for a decrease in sensitivity. The drug 6-hydroxydopamine has short acting sympathomimetic-like activity due to

the increased quantal release of noradrenaline from the nerve endings (Haeusler, 1971). A destruction of the nerve endings occurs in the terminal adrenergic system resulting in a chemical sympathectomy (Thoenen and Tranzer, 1968). The smaller acute response to 6-hydroxydopamine could equally well be explained by a lower turnover of catecholamines in the heart following training (Ostman and Sjostrand, 1971) as by a decreased sensitivity to a given concentration of noradrenaline.

The finding of a lower frequency response of the hearts of trained adrenalectomized rats to a single fixed concentration of isoprenaline (Tipton et al, 1972) does not receive support from the present studies. Rather than the interpretation of Tipton et al that the sensitivity to catecholamines was reduced in the trained rat, it appears that a decreased efficacy of the drug may have been responsible for the lower frequency response. The lower efficacy of noradrenaline will be discussed later.

The increased sensitivity of the sinoatrial node to noradrenaline in the chronically noradrenaline treated animals of Study 2 is similar to the report of LeBlanc, Vallieres and Vachon (1972). These investigators employed repeated injections of isoprenaline to evoke increased sensitivity to isoprenaline, however, in the present study, no increased sensitivity to noradrenaline was observed in the isoprenaline treated animals. The reason for this may have been the three times greater dosage of isoprenaline employed by LeBlanc, Vallieres and Vachon (1972), or it may reflect a specificity of the response to the inducing agent. The increased sensitivity to noradrenaline probably reflects an alteration in the rate

of drug metabolism at the receptor site. Noradrenaline is removed from the receptor site by two mechanisms. $Uptake_1$ is the uptake process of neuronal tissues, and $Uptake_2$ is the uptake process by non-neuronal tissues (Iversen, 1971).

$Uptake_1$ is an active process with a high affinity ($Km=0.2 \times 10^{-9}M$) for noradrenaline; isoprenaline is not taken up by this process. In spite of the observation that the maximum rate of $Uptake_1$ is low (Vmax = 0.6 nmol/g/min), this is the most important pathway in normal physiological conditions (Iversen, 1971). In contrast, the $Uptake_2$ process has a lower affinity ($Km = 252 \times 10^{-6}M$), but a high maximum rate of uptake (Vmax = 100 nmol/g/min). Noradrenaline taken up by the $Uptake_1$ process is incorporated into storage vesicles. Noradrenaline taken up by $Uptake_2$ is metabolized by the enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). While it is difficult to speculate on the mechanism responsible for increased sensitivity to noradrenaline in the chronically noradrenaline-treated groups, a number of possibilities exist.

The rate of $Uptake_2$ may be reduced. Although this mechanism has a very high maximum velocity, chronic over-taxing of the MAO and COMT metabolic pathways may have left this uptake process saturated. Iversen (1965) demonstrated that noradrenaline taken up by $Uptake_2$ is not immediately metabolized, thus it appears that the metabolism by MAO and COMT can be saturated. The influence of chronically high levels of noradrenaline on the concentrations and activities of MAO and COMT has not apparently been investigated.

At low concentrations of noradrenaline, the higher affinity $Uptake_1$ process is quantitatively more important for noradrenaline removal. Certain

catecholamines inhibit the uptake of noradrenaline, but this appears to be through competition for the $Uptake_1$ binding site (Kirshner, Schauberg and Ferris, 1971). The influence of chronically high levels of noradrenaline on the content of the storage vesicles is unknown. It is possible however, that the storage capacity for neuronal noradrenaline becomes saturated by chronic noradrenaline administration. Additional support for this proposal that saturation of the $Uptake_1$ mechanism resulted in the increased noradrenaline sensitivity which was observed can be found in the noradrenaline response of the isoprenaline treated animals of the present Study 2. Isoprenaline is not taken up by $Uptake_1$, therefore, removal of this catecholamine is by the $Uptake_2$ process only. Since chronic isoprenaline treatment in this study did not significantly affect the noradrenaline response in vitro, then it is possible that the mechanism responsible for the increased sensitivity is not involved in the action or metabolism of isoprenaline, althouth the noradrenaline uptake study of Vallieres, Bureau and LeBlanc (1972) is not in accord with this proposal. The study of LeBlanc, Vallieres and Vachon (1972) reported an increased sensitivity to a constant intravenous infusion of isoprenaline in the chronically isoprenaline treated animal. Thus, it is necessary to resolve the conflict between these two studies. Selective blockade of the metabolic pathways of $Uptake_1$ by phenoxybenzamine or cocaine (Kirpekar and Cervoni, 1963) and $Uptake_2$ by steroids (Kalsner, 1969) may be helpful in resolving this question.

In conclusion, the question of whether altered sensitivity to noradrenaline occurred following training must be answered in the negative

from the results of the present study. The relatively low intensity of the training in the present studies may have bearing on the observed noradrenaline sensitivity, therefore, a study of the sensitivity to noradrenaline following more intensive training should be carried out. It was found that noradrenaline administration over the duration of the study resulted in an increased sensitivity to noradrenaline as measured in vitro. The second half of the question posed in the Introduction concerning the effects of the drugs of Study 1 and Study 2 can be answered: yes, noradrenaline pre-treatment alters the sensitivity to noradrenaline.

V-7-2-2: Efficacy of Noradrenaline:

The maximum efficacy of noradrenaline in a number of groups in both Study 1 and Study 2 was reduced in comparison with the inactive control groups. This observation was similar to that of Cairoli and Crout (1967) in which Figure 2A of their paper shows a greater maximum increase in frequency in hyperthyroid as compared with euthyroid rats. Although these authors do not discuss this point, the observation could indicate a greater efficacy of noradrenaline in the hyperthyroid animals. The difference in maximal efficacy can be due either to changes in the drug-receptor interaction or to changes in the properties of the effector cell.

Recently, it has been suggested that the receptor population may vary with catecholamine stimulation (Mukherjee, Caron and Lefkowitz, 1975). The injection of isoprenaline into frogs was followed by a decrease in the number of binding sites for l-alprenolol. This decrease was accompanied

by a reduction in the maximum efficacy of isoprenaline to stimulate adenyl cyclase activity, but no change in the sensitivity judged by the half-maximal response. The present Study 2 does not support this concept as chronic stimulation of the receptor sites with daily noradrenaline, as discussed above with respect to sensitivity, did not reduce the efficacy of the noradrenaline chronotropic response in these groups.

The possibility of changes in the effector cell causing the reduced efficacy of noradrenaline in certain groups receives considerable support. In the present Study 1 and Study 2, a very significant positive correlation was observed between the maximum frequency in response to noradrenaline the the ISF as measured in vitro ($r = 0.75$, $r = 0.63$, respectively). This correlation suggests that the electrophysiological properties involved in establishing the ISF are also involved in limiting the maximum frequency response. This relationship appears to hold for all groups, even though, as discussed earlier (Electrophysiology of the ISF), the mechanism by which ISF was reduced may vary. This hypothesized relationship between the ISF and maximal sinoatrial frequency is supported by the early electrophysiological studies of Cervoni, West and Falk (1956) which reported that cells with a low spontaneous rhythm could only be stimulated to low maximal frequencies. Further, the recent work of Brorson et al (1976) reported a significant correlation between the increase in the duration of the monophasic action potential recorded from the right atrial surface at rest and the reduction in maximum exercise cardiac frequency following two months of physical training in man. While the study of Brorson et al (1976) measured the monophasic action

potential from the surface of the right atrium with the sympathetic and parasympathetic innervation intact, it would be expected that changes in the electrophysiology of the sinoatrial node would be reflected in the measured potential (deMello, 1961, Huddart, 1975). In addition, the study of Sutton et al (1967) demonstrated that a reduction in ISF would accompany a two month physical training programme such as employed by Brorson et al (1976). Therefore, there appears to be support for the hypothesis derived from in vitro data that the decreased efficacy of noradrenaline is related to a reduction in the ISF. This hypothesis has been further explored in Study 3 in which a significant positive correlation was observed in the rat between the in vivo ISF and the maximum cardiac frequency in response to exercise ($r = 0.73$) or noradrenaline infusion (in the anesthetized, ganglion blocked rat) ($r = 0.61$). These findings will be discussed in more detail under the heading "In vivo chronotropic responses". The results of the present thesis support the position that the reduction of efficacy of noradrenaline is related not to the drug-receptor interaction, but rather to the properties of the effector cell.

V-7-3: Summary:

No change in the sensitivity to noradrenaline was measured in the exercised animals contrary to the hypotheses existing in the literature that sensitivity to sympathetic stimulation was reduced by physical training.

Increased sensitivity to noradrenaline was observed in animals receiving prior daily noradrenaline injections. It was proposed that alterations in drug metabolism, specifically the neuronal $Uptake_1$ pathway may have been responsible for this finding.

Maximum efficacy of noradrenaline was reduced in the exercised groups of Study 1 and Study 2. It was observed that the maximum frequency in response to noradrenaline correlated significantly with the ISF. This led to the hypothesis that electrophysiological properties which establish ISF also limit the maximum sinoatrial frequency.

V-8: Study 3: In Vivo Chronotropic Responses:

V-8-1: Introduction:

Study 3: Does a decrease in ISF result in a reduction of maximum cardiac frequency in vivo? This question was formulated in the Introduction as a consequence of the in vitro observation that the reduced noradrenaline efficacy was correlated with the ISF. In the discussion of noradrenaline efficacy in the previous section, the hypothesis was advanced that electrophysiological properties which establish the ISF are also involved in limiting the maximum sinoatrial frequency. Study 3 has investigated the applicability of this hypothesis in vivo.

The limitation to maximal cardiac frequency is not known. In man, there are many conflicting observations. Maximum cardiac frequency is related to age (Astrand, 1960), however, young endurance trained athletes frequently have maximum cardiac frequencies below the age predicted maximum (Ekblom, 1969) and training frequently reduces the maximum frequency (Ekblom, Kilbom and Soltysiak, 1973, Brorson et al, 1976. Conversely, older, well conditioned persons frequently have maximum cardiac frequencies above the age predicted maximum. In an attempt to answer the question of a reduced maximum cardiac frequency in highly trained endurance athletes, Ekblom (1969) proposed that the cardiac hypertrophy accompanying endurance training may be responsible for the reduction in maximum cardiac frequency. Ekblom also points out, however, that cardiac hypertrophy does not usually occur until at least six months of endurance training. In the short term training studies (Ekblom, Kilbom

and Soltysiak (1973) investigated the possibility of persisting parasympathetic activity at high levels of power output being responsible for the reduced maximum exercise cardiac frequency. Atropine injections, however, failed to result in a higher frequency. The earlier discussion of "Efficacy of Noardrenaline" hypothesized that the maximum sinoatrial frequency response to noradrenaline was limited by electrophysiological factors which are also involved in establishing the ISF. The results of Brorson et al (1976) published shortly after the conclusion of the experiments to be described also support this concept. Brorson et al (1976) observed a correlation between the electrophysiological parameter, the lengthening of the duration of the monophasic action potential recorded from the right atrium and the decrease in maximum cardiac frequency in a group of human volunteer subject following training. The present Study 3 has examined the relationship between the maximum cardiac frequency and the ISF in the rat in vivo.

Two series of experiments were carried out in Study 3. The first involved the measurement of the maximum cardiac frequency response to progressive treadmill exercise to exhaustion. The second was the determination of the maximum frequency response to intravenous infusion of noradrenaline in the anesthetized, ganglion blocked animal. To study the correlation between ISF and the maximum cardiac frequency, it was desirable to have a wide range of values of ISF. This was achieved by the daily injection of atropine (5 mg/kg) as in Study 1 to one half of the animals studied. Female Sprague-Dawley rats were used, as it was observed that it was easier to have an untrained adult female exercise than an

untrained adult male rat. No prior treadmill training was employed before the progressive treadmill test, however, all animals appeared to reach a functionally limited maximum.

V-8-2: Findings and Discussion:

The maximum cardiac frequency response was correlated with the ISF for both exercise ($r = 0.76$) and noradrenaline infusion ($r = 0.61$). There was no significant difference between the maximum cardiac frequency obtained in response to exercise or noradrenaline.

The hypothesis advanced on the basis of the *in vitro* observations that the maximum cardiac frequency is limited by electrophysiological properties which establish the ISF gains additional support from Study 3. The question asked in the Introduction can be answered in the affirmative. A reduction in ISF does appear to be responsible for a decrease in maximum cardiac frequency *in vivo*. The early electrophysiological study of Cervoni, West and Falk (1956) observed a low maximum frequency in response to electrical stimulation in cells with low spontaneous pacemaker frequencies. This study provides *in vitro* support for the relationship between ISF and maximum frequency, but *in vivo* studies have been less clear.

Maximum cardiac frequency decreases in the human population with age (Astrand, 1960). The observation that ISF also decreases with age (Jose, 1966) led Jose and Collison (1970) to speculate on a relationship between these observations, however, no direct evidence was presented. Brorson et al (1976) did present evidence of a correlation between an electrophysiological measure in the heart, the right atrial monophasic

action potential, and the maximum cardiac frequency. It must be noted, however, that the right atrial monophasic action potential was recorded from resting, but neurally intact humans. Noble (1975) points out the relationship between cardiac frequency and the monophasic action potential, therefore, the observation of Brorson et al (1976) may simply reflect a decrease in resting cardiac frequency and a decrease in maximum cardiac frequency. It is possible that a decrease in ISF occurred in the training study of Brorson et al (1976) as observed previously by Sutton et al (1967), but the decrease in resting cardiac frequency observed by Brorson and co-workers was probably due to altered autonomic nervous activity also (Ekblom, Kilbom and Soltysiak, 1973).

The similar maximum cardiac frequency response to noradrenaline infusion and to exercise suggests that maximal exercise evokes maximal sympathetic stimulation. This finding further suggests that the maximum cardiac frequency observed in response to maximum exercise was the true maximum frequency of the sinoatrial node.

V-8-3: Summary:

The maximum cardiac frequency response to progressive treadmill exercise to exhaustion and to noradrenaline infusion during ganglionic blockade positively correlated with the ISF. This finding gave additional support to the hypothesis that the maximum cardiac frequency is limited by electrophysiological factors which are also involved in establishing the ISF.

V-9: The ISF and Cardiac Frequency:

Cardiac frequency is established by the interaction of the parasympathetic nervous system, the sympathetic nervous system, and the intrinsic sinoatrial frequency (ISF) (Grodner et al, 1970; West, 1972; Higgins, Vatner and Braunwald, 1973). Grodner et al (1970) demonstrated that the balance between autonomic nervous activity accelerated or slowed the heart. High parasympathetic activity reduced cardiac frequency, and high sympathetic activity increased cardiac frequency. Physical training has been shown to alter the balance of autonomic activity (Herrlich, Raab and Gigee, 1960; Ekblom, Kilbom and Soltysiak, 1973). As a consequence, cardiac frequency can be reduced by the decrease in sympathetic and the increase in parasympathetic activities. Also accompanying physical training is a reduction in ISF (Sutton et al, 1967; Lin and Horvath, 1972; Bolter et al, 1973; present thesis). Therefore, the altered autonomic balance will be acting on an ISF which has a new lower set point following training. This point of view has also been assumed by Barnard, Corre and Cho (1976).

Resting cardiac frequency of the exercised animals was reduced slightly in Study 1 and significantly in Study 2. The present studies found no increase in parasympathetic tone by the criterion established previously (Bolter, Hughson and Critz, 1973). No measurement was made of changes in resting sympathetic tone, however, previous studies (Lin and Horvath, 1972; Ekblom, Kilbom and Soltysiak, 1973) have shown these changes to be small. Therefore, the reduction in ISF was probably the major contributing factor to any changes observed in resting cardiac frequency. This proposal appears to hold true for most groups, as the

reduction in resting cardiac frequency was similar to the reduction in ISF. Small fluctuations in autonomic nervous activity at the time of resting measurements would influence this relationship. However, in the exercise plus noradrenaline group. a large reduction in resting frequency occurred in spite of a negligible reduction in ISF. The reason for this discrepancy cannot be found in an altered parasympathetic activity, but could possibly be related to alterations in sympathetic activity or sensitivity which accompanied the daily injections of noradrenaline.

In addition to the influence on resting cardiac frequency, ISF also appeared to limit maximum cardiac frequency, as discussed in the previous section. The influence of ISF on cardiac frequencies between resting and maximal frequencies has not been investigated. Although the cardiac frequency for a given balance of sympathetic and parasympathetic activities is probably reduced in accordance with the reduction in ISF. The corollory of this would be that an increased sympathetic drive would be required to produce the same cardiac frequency.

V-9-1: Summary of the ISF and Cardiac Frequency:

At rest, the balance of sympathetic and parasympathetic nervous activity on the ISF establishes the cardiac frequency. The present study detected no differences in tonic parasympathetic activity and did not measure the tonic sympathetic activity, but did find reductions in ISF which contributed to a reduction in resting cardiac frequency. In addition, the ISF appeared to establish a limit on the maximum cardiac frequency in the present thesis in both *in vitro* and *in vivo* studies. The reduction in ISF with training implies that to achieve the same cardiac frequency

following training, a different balance of parasympathetic and sympathetic activity must be employed.

V-10: Summary Chapter

V-10-1: Introduction

This chapter will briefly review the Thesis under the sub-heading "Observations and Conclusions of the Thesis". The final part of the chapter will be a brief statement of some of the questions raised by the Thesis and suggestions of possible pathways to pursue the answers.

V-10-2: Observations and Conclusions of the Thesis:

This thesis has examined the influence of changes in the autonomic nervous balance occurring with physical training on the reduction of intrinsic sinoatrial frequency (ISF) which accompanies training. In addition, the chronotropic responses of the sinoatrial node to the autonomic transmitters acetylcholine and noradrenaline were studied, as well as the influence of ISF on cardiac frequency.

In agreement with previous studies, the ISF decreased with physical training. The pharmacological manipulations of parasympathetic receptor activity in Study 1 showed that increased parasympathetic nervous activity was probably not involved in the reduction of ISF with physical training. On the contrary, a decrease in ISF was observed with parasympathetic receptor blockade with atropine and the decrease due to the drug appeared to be additive to that due to physical training. Carbachol, a parasympathetic receptor stimulant had no significant effect on ISF. An assessment of myocardial electrophysiology by the estimation of plasma and intracellular sodium and potassium contents revealed that the postulated changes

in maximum diastolic repolarization in the exercise and the atropine groups were in opposite directions. The reduction of ISF with exercise and atropine could not, therefore, be accounted for by the same mechanism, but required the changing of the slope of the prepotential and/or the threshold potential. In Study 2, pharmacological stimulation of the sympathetic receptors with noradrenaline or isoprenaline caused a reduction in ISF, however, exercise in combination with these drugs produced no changes in ISF. Blockade of the sympathetic receptors with propranolol caused a reduction in ISF. The effects of these drugs on electrolyte distribution revealed a similar finding to that in Study 1. Similar reductions in ISF in the exercise and the isoprenaline or noradrenaline groups were accompanied by directionally opposite changes in the predicted membrane electrophysiology. Propranolol had no effect on electrolyte distribution. In conclusion, Study 1 and Study 2 revealed that exercise reduced the ISF. Pharmacological interventions with the receptor activity showed that neither increased parasympathetic activity (which was similar to increased resting vagal tone of the trained state), nor increased sympathetic activity (which was similar to the increased activity of the sympathetic nervous system during exercise) was responsible for the reduction of ISF with physical training. This statement was given support by the study of myocardial and plasma electrolyte contents, and the proposed changes in membrane electrophysiology accompanying any electrolyte shift.

The study of the chronotropic sensitivity to acetylcholine showed no significant differences between any group in either Study 1 or Study 2. This observation was taken as evidence that no change in resting

(background) parasympathetic activity was present in any group.

Noradrenaline responsiveness was altered in a number of groups. The maximum frequency response was reduced significantly in Study 1 in the exercise and the atropine plus exercise animals, and in Study 2 in the propranolol and the propranolol plus exercise animals. In both Study 1 and Study 2, the maximum frequency response to noradrenaline was positively correlated with the ISF. On the basis of this correlation, the hypothesis was advanced that the electrophysiological properties which establish the ISF are also involved in setting a limit for the maximum sinoatrial frequency. The noradrenaline sensitivity was unaltered in all groups except the two noradrenaline pre-treated animals. These groups had an increased sensitivity to noradrenaline.

Study 3 tested the relationship observed in vitro in Study 1 and Study 2 between the ISF and maximum noradrenaline stimulated frequency. Maximum cardiac frequency measured either by exercise or noradrenaline infusion in the ganglion blocked animal was positively correlated with the ISF. These findings gave additional support to the hypothesized electrophysiological factor which establishes the ISF and limits the maximum sinoatrial frequency.

V-10-3: Questions for Further Research:

This thesis has shown no connection between the ISF and tonic levels of autonomic nervous activity associated with physical exercise and the trained state. Although this connection had been proposed by Raab (1969), it is now apparent that some other aspect of physical training must be responsible for the exercise induced reduction in ISF.

The second half of Raab's proposal that alterations in myocardial electrolyte balance may be responsible for the reduction in ISF obtained support from the study of electrolytes in Study 1 and Study 2. It may be, therefore, that altered electrolyte balance, specifically augmented myocardial potassium, may be the primary factor in reducing ISF. The finding of elevated serum potassium levels during exercise and increased potassium accumulation with elevated serum levels (Rose et al. 1966) supports this concept. An investigation of the role of serum potassium levels during exercise and the relationship with the ISF may answer the question of the mechanism responsible for the reduction of ISF with exercise.

The present study found no alteration in the chronotropic sensitivity to acetylcholine following training as was demonstrated by Bolter, Hughson and Critz (1973) This raised two major questions. Firstly, was the absence of a decreased acetylcholine sensitivity in the present study a reflection of the relatively low training intensity employed? Secondly, was the finding of a reduced sensitivity to acetylcholine by Bolter, Hughson and Critz (1973) a consequence of the swim training? Water immersion frequently occurs with swim training, and this is associated with a vagally mediated bradycardia. The first question could be answered by pre-selecting rats on the basis of running ability and training them at a higher intensity. The second question could be answered by comparing the swim training group with an appropriate water immersed control group.

Reduction of the ISF and the maximum chronotropic response and the hypothesized relation between the ISF and the maximum sinoatrial response can only be properly investigated by modern electrophysiological techniques. As pointed out in the Introduction, the electrophysiology of the pacemaker cells is complex (Noble, 1975). The various responses of the simplistic electrophysiological assessment based on electrolyte distribution in Study 1 and Study 2 affirm this. Intracellular recording, and voltage clamp experiments (Brady, 1974) would reveal the changes in membrane potential and ionic currents associated with the reduction in ISF due to exercise or chronic drug administration, and also the limits to maximum sinoatrial frequency.

The reported decrease in sensitivity to noradrenaline following physical training was not found in the present studies. Whether this finding was due to the low intensity of training in the present studies, or the indirect assessments upon which the proposal was based in the previous studies (Ekblom et al, 1973; Brundin and Cernigliaro, 1976) needs to be investigated. As suggested above for the acetylcholine response, pre-selection of animals for training at a higher intensity may answer this question.

It has been reported that increased chronotropic sensitivity to isoprenaline accompanies repeated isoprenaline injections (LeBlanc, Vallieres and Vachon, 1972), however, an increased sensitivity to noradrenaline was not found in the isoprenaline treated rats of Study 2. Simply repeating this previous study employing the three-times greater concentration of isoprenaline which LeBlanc employed may resolve the

conflict between the two studies, but it is possible the drug of pre-treatment selectively sensitizes the tissue to that catecholamine, as the method of drug metabolism differs for isoprenaline and noradrenaline (Callingham and Burgen, 1966). This same suggestion may also be helpful in investigating the mechanism responsible for the increased sensitivity to noradrenaline in the two chronically noradrenaline treated groups of Study 2. It was suggested earlier in the "Discussion" under the heading "In Vitro Chronotropic Responses to Noradrenaline" that chronic noradrenaline treatment may selectively influence the $Uptake_1$ mechanism as this is the primary mechanism of noradrenaline removal from the receptor site at physiological concentrations (Iversen, 1971). Selective blockade of $Uptake_1$ with cocaine or phenoxybenzamine (Kirpekar and Cervoni, 1963) and $Uptake_2$ with steroids (Kalsner, 1969) following experimental preparation similar to that of Study 2 may answer this question, although selectivity of these blocking agents is limited (Iversen, 1971).

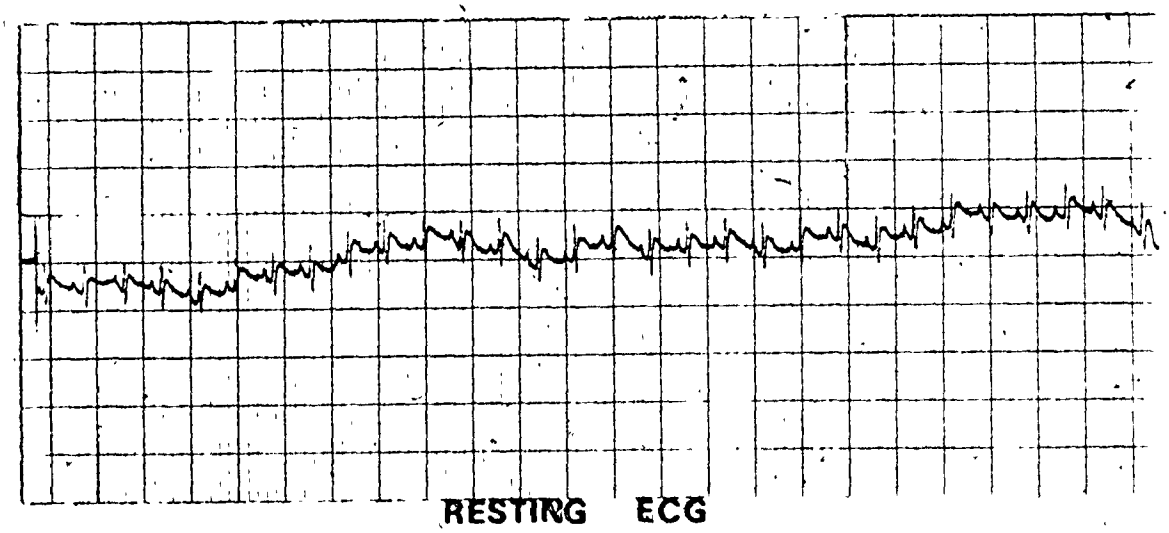
Finally in this Thesis, the importance of the ISF and the chronotropic responsiveness in establishing cardiac frequency in the rat has been demonstrated. The application of this knowledge to the human could be very beneficial in understanding the cardiac frequency response in the athletic, normal and patient populations. The endurance trained athlete with a reduced ISF and the resulting reduction in maximum cardiac frequency could be limited when the maximum capacity of the circulatory system is stressed. Athletes competing in orienteering and cross country skiing frequently approach maximum effort in uphill running, and a reduction of maximum cardiac frequency may be a restrictive factor on

performance. In the patient, the knowledge of the ISF and autonomic contributions to cardiac frequency has proven a valuable diagnostic tool. A small percentage of post-myocardial infarction patients have been termed slow-heart-rate responders (Powles et al, 1975) because of an abnormally low cardiac frequency response to exercise and a low maximum cardiac frequency. These patients do not increase their maximum cardiac frequency in response to atropine, and probably have low values of ISF as reported previously by Jose (1966). A patient with Shy-Drager syndrome (a degeneration of the central sympathetic nervous system) (Shy and Drager, 1960) has been studied recently in our laboratory (Appendix 7). The patient had an abnormal cardiac frequency to exercise, with a plateau in frequency of approximately 100 beats/minute occurring at about 50% of his maximum power output of 600 KPM/min. Maximum cardiac frequency increased during exercise with the parasympathetic receptor blocker atropine. The ISF of the patient was low for his age (Jose, 1966), but this measurement in combination with the response to atropine demonstrated that the patient was not completely sympathetically denervated as had been suspected from his history and control exercise test. Rather, it appeared that sympathetic activity was reduced and parasympathetic activity maintained so that cardiac frequency did not reach a true maximum. An additional test, the continuous infusion of isoprenaline, revealed an essentially normal response to exogenous catecholamine stimulation. These studies indicate the advantage of going beyond the early pioneering work of Jose and collegues (Jose, 1966; Jose and Stitt, 1969; Jose and Collison, 1970) which measured only ISF, to a total clinical investigation of intrinsic and autonomic control of cardiac frequency.

Appendix 1

Resting cardiac frequency:

The method of measurement of resting cardiac frequency was detailed in the Methods section III-6-1. Below is a typical recording obtained from a resting animal. The paper speed was 25 mm/sec., 10 millivolts = 1 cm. The cardiac frequency of this animal was 370 beats/min. (Figure 17)



RESTING ECG

Appendix 2

2a) Preliminary Studies of ISF Measurement:

The intrinsic sinoatrial frequency (ISF) has been described earlier as the cardiac frequency measured during total autonomic blockade of the heart. This is accomplished with atropine to block the parasympathetic activity to the heart, and propranolol to block the sympathetic activity to the heart. Previous studies have used these drugs in the rat. Tipton and Taylor (1965) used 1 mg/kg atropine but stated without evidence that this did not achieve total parasympathetic blockade. Lin and Horvath (1972) assumed total blockade of the parasympathetic system was obtained with 1 mg/kg atropine. In combination with the atropine, Lin and Horvath employed 8 mg/kg propranolol to produce sympathetic receptor blockade and measure the ISF. A larger concentration of atropine (3 mg/kg) has been used in rats by Walsh (1969). The present studies have used the 3 mg/kg atropine suggested by Walsh in conbination with 8 mg/kg propranolol to measure ISF. To test the adequacy of these concentrations of blocking drugs, an additional 1.5 mg/kg atropine and 4 mg/kg propranolol was administered. The drugs were injected intraperitoneally and the cardiac frequency at 20 minutes post-injection was taken as the ISF.

The time course of the cardiac frequency response to atropine and propranolol injection is shown in Figure 18. Cardiac frequency can be seen to plateau between 15 and 30 minutes. The injection of additional atropine and propranolol did not have a significant effect on cardiac frequency as shown in Table 15. The coefficient of variation for the ISF measurement with 3 mg/kg atropine and 8 mg/kg propranolol compared

with 4.5 mg/kg atropine and 12 mg/kg propranolol indicated that these two measurements were not different from each other.

2b Repeatability of ISF Measurement

To assess the repeatability of measurements of ISF on different days, eight animals were studied as described above. A comparison of the ISF measurement on the two occassions is shown in Figure 19. There was no significant difference in the two measurements.

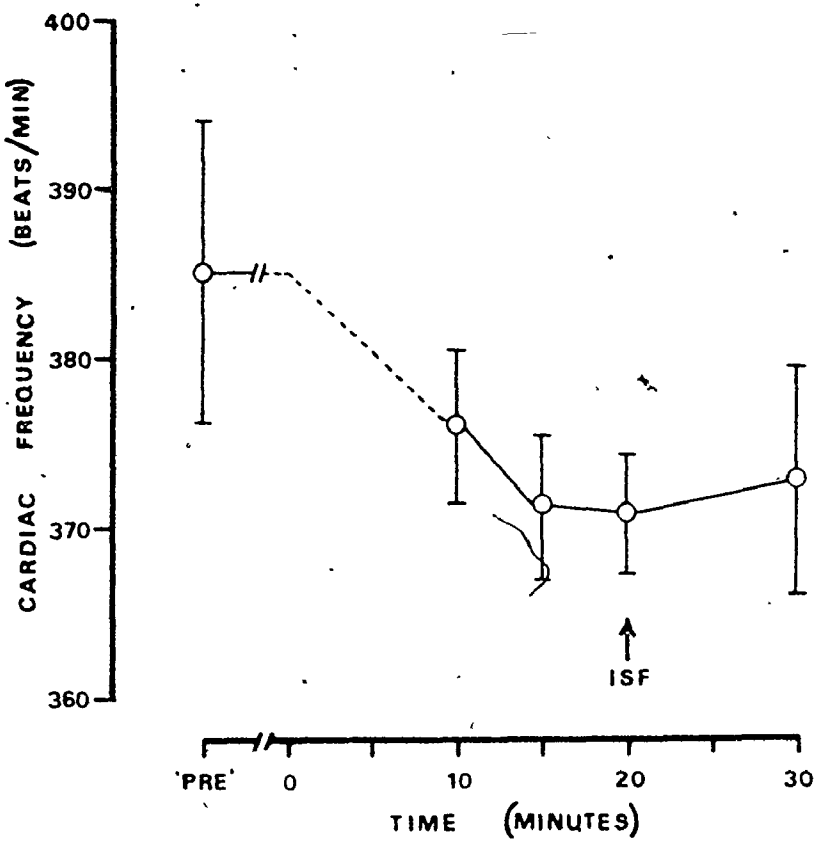


Figure 18. The time course of the cardiac frequency response to the intraperitoneal injection of 3.0 mg/kg atropine and 8.0 mg/kg propranolol. The injection occurred at time '0'. The cardiac frequency at 20 minutes is the intrinsic sinoatrial frequency (indicated by the arrow). Values plotted are the mean $\pm$ 1 S.E.M.

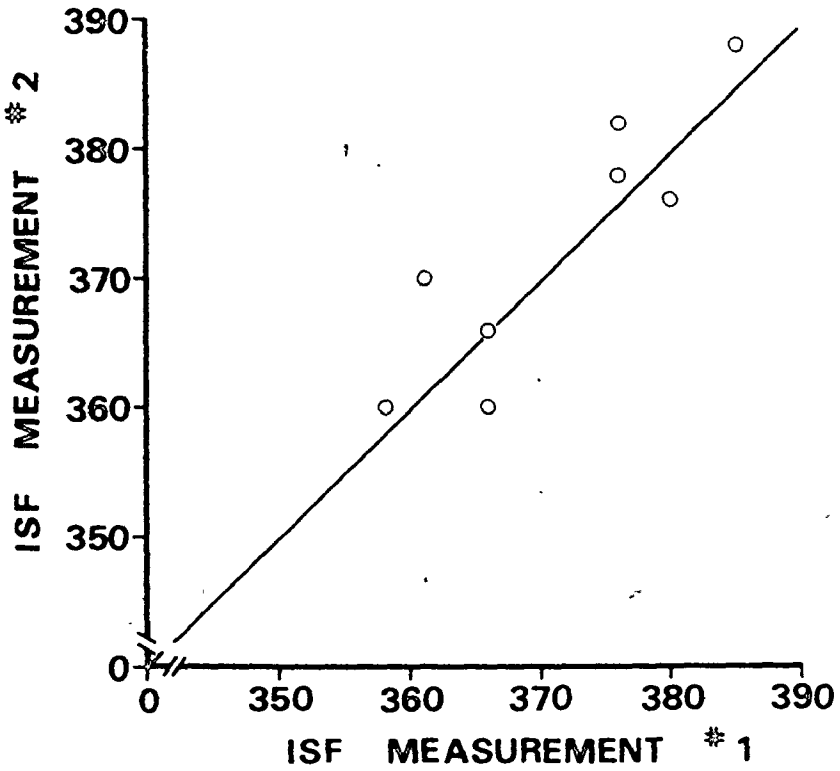


Figure 19: The repeatability of ISF measurements made on two separate days (coefficient of stability = 0.89, Bruning and Kintz, 1968).

TABLE 16

Effect of different concentrations of propranolol and atropine on intrinsic sinoatrial frequency.

Animal Number	Propranolol (8 mg/kg) plus Atropine (3 mg/kg)	Propranolol (12 mg/kg) plus Atropine (4.5 mg/kg)
1	385	390
2	366	362
3	376	366
4	366	356
5	362	364
6	380	375
7	358	358
8	376	382
Mean ± 1 S.E.M.	371.1 ± 3.3	369.1 ± 4.3

Values are the individual measurements of ISF obtained 20 minutes after the injection of the indicated doses of propranolol plus atropine. The coefficient of stability is 0.86 (Bruning and Kintz, 1968).

Appendix 3

Preliminary studies of parasympathetic receptor agonists and antagonist.

3a Control:

In order to adequately assess the cardiac frequency response to injected drugs, it was first necessary to determine the cardiac frequency response to a sham injection of 0.2 ml saline (0.9%, w/v).

Eight animals had electrodes attached as described in Methods section III-6-1. A resting cardiac frequency value was obtained, then an injection of 0.2 ml saline was made subcutaneously under the skin of the back of the neck. Cardiac frequency was measured at intervals over the next 60 minutes (Figure 20). The cardiac frequency response to the sham injection indicated that responses to injected drugs were not influenced by the handling associated with the injection.

3b Atropine:

To block a significant proportion of the daily parasympathetic activity, a high concentration of the parasympathetic receptor blocker atropine was employed. As described in Appendix 2, 3 mg/kg atropine produced total parasympathetic receptor blockade over the period required to measure ISF. A concentration of 5 mg/kg was chosen to achieve longer lasting parasympathetic receptor blockade.

Eight animals were studied. Electrodes were attached and the resting cardiac frequency measured. Atropine, 5 mg/kg dissolved in saline

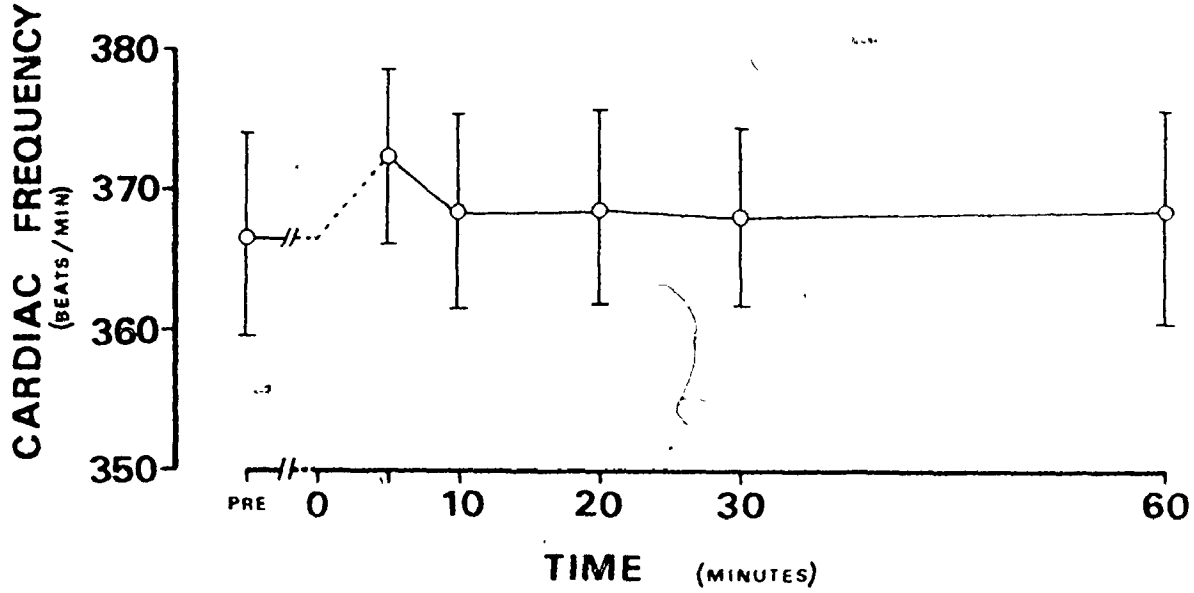


Figure 20. The cardiac frequency response to a sham control injection at time "0" is shown plotted against time. Values are the mean ± 1 S.E.M. There were no differences between the pre-injection value and any point following the injection.

was injected in a total volume of 0.2 ml under the skin of the back. Cardiac frequency was measured until the pre-injection value was observed.

The cardiac frequency response to 5 mg/kg atropine is shown in Figure 21. Cardiac frequency rose from a resting value of about 375 beats/minute to a peak value of 475 beats/minute. Parasympathetic receptor blockade persisted for six hours. This length of blockade represented approximately a 25% reduction of the total daily parasympathetic activity to the heart.

3c Carbachol

The drug carbachol is a relatively long acting parasympathetic receptor stimulant. To increase the total daily sympathetic activity, carbachol was administered, however, the paucity of data describing chronic administration of carbachol as a stimulant of the myocardial parasympathetic receptors required preliminary studies to determine the concentration required. An initial concentration of 1.0 mg/kg was chosen. This dose elicited immediate heavy lacrimation and salivation, followed rapidly by convulsions. A decrease in drug concentration to 0.5 mg/kg also produced convulsions, but these were reversed with atropine. At a drug concentration of 0.15 mg/kg, lacrimation and salivation occurred, but the animals did not convulse and did not appear to feel any discomfort.

To assess the cardiac frequency response to 0.15 mg/kg carbachol, eight animals were studied. Electrodes were attached and a resting cardiac frequency measurement was obtained. Carbachol was dissolved in

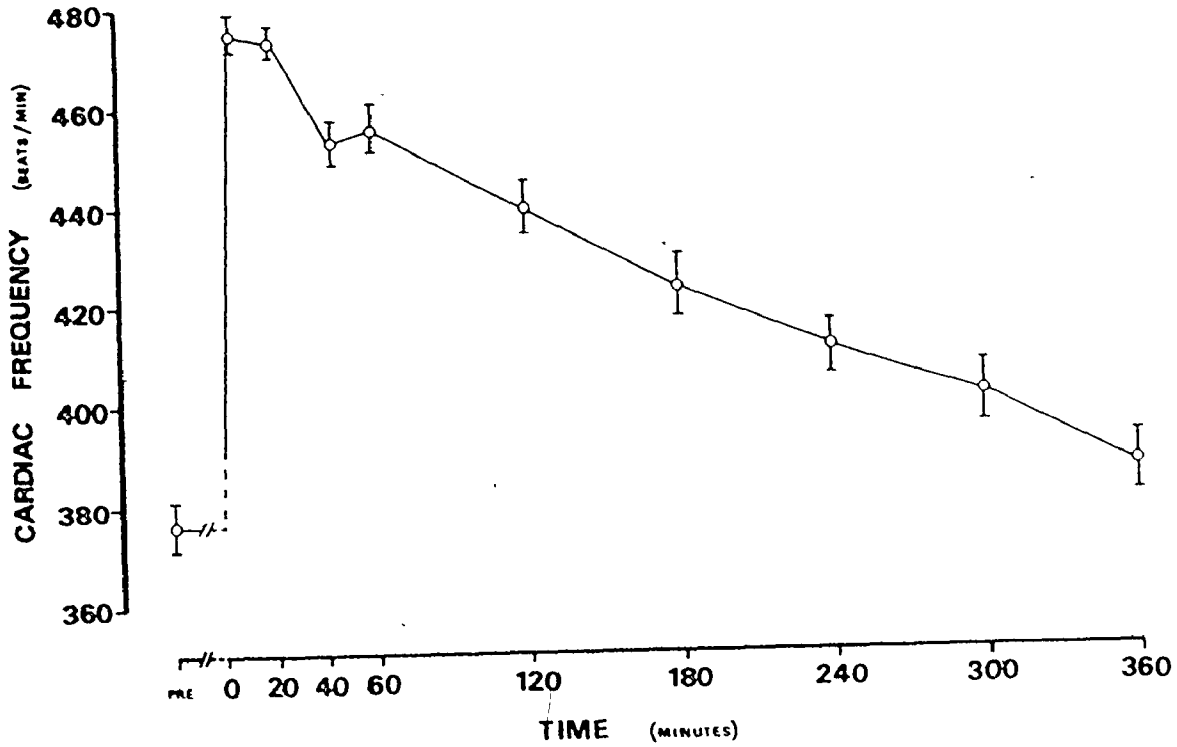


Figure 21. The cardiac frequency response to the subcutaneous injection of 5 mg/kg atropine at time "0" is shown plotted against time. Values are the mean ± 1 S.E.M. All mean values of cardiac frequency between time "0" and 300 minutes are significantly different from the pre-injection value.

saline and injected in a total volume of 0.2 ml under the skin of the back.

Figure 22 shows the cardiac frequency response to 0.15 mg/kg carbachol. Cardiac frequency decreased to a minimum value of 230 beats/minute. Parasympathetic receptor stimulation lasted for four hours. This was felt to be the maximum stimulation which could be obtained in view of the adverse effects of higher concentrations.

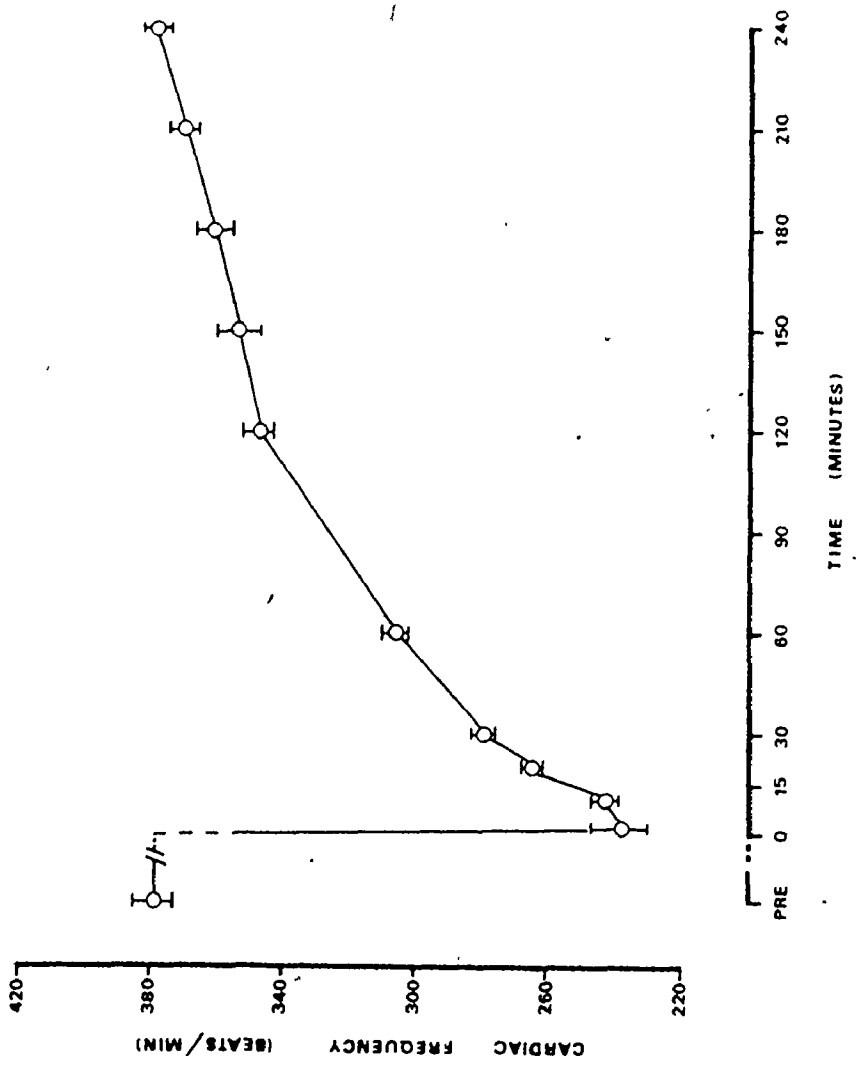


Figure 22. The cardiac frequency response to the subcutaneous injection of 0.15 mg/kg carbachol at time "0" is shown plotted against time. Values are the mean ± 1 S.E.M. All mean values between time "0" and 180 minutes are significantly different from the pre-injection value.

Appendix 4

Preliminary studies of sympathetic receptor agonists and antagonists.

4a Noradrenaline:

To mimic the increased sympathetic activity during exercise, sympathetic agonist drugs were administered. Noradrenaline is the natural transmitter of the sympathetic nervous system (Euler, 1946), however, it acts not only on the adrenergic receptors of the myocardium, but also those of the vasculature. The injection of noradrenaline produces an elevated blood pressure, and a reflex slowing of the heart through the vagally mediated baroreceptor reflex. The concentration of noradrenaline (1.0 mg/kg) chosen was employed previously by Linna et al (1965). To prolong the duration of action of noradrenaline, the drug was mixed with lanolin and suspended in oil. Linna et al (1965) measured a hypertensive response of three hours duration, but did not report the chronotropic effect of this concentration of noradrenaline. The chronotropic response was measured in this study.

Eight animals were studied. Resting cardiac frequency measurements were made. Noradrenaline was suspended in oil and injected subcutaneously in a total volume of 0.1 ml to give a total concentration of 1.0 mg/kg. Cardiac frequency was measured over the next three hours.

The cardiac frequency response to 1.0 mg/kg noradrenaline in oil is shown in Figure 23. A bradycardia was observed in the first hour, with cardiac frequency slowly returning toward the pre-injection value by three hours. From this response it was not possible to conclude what

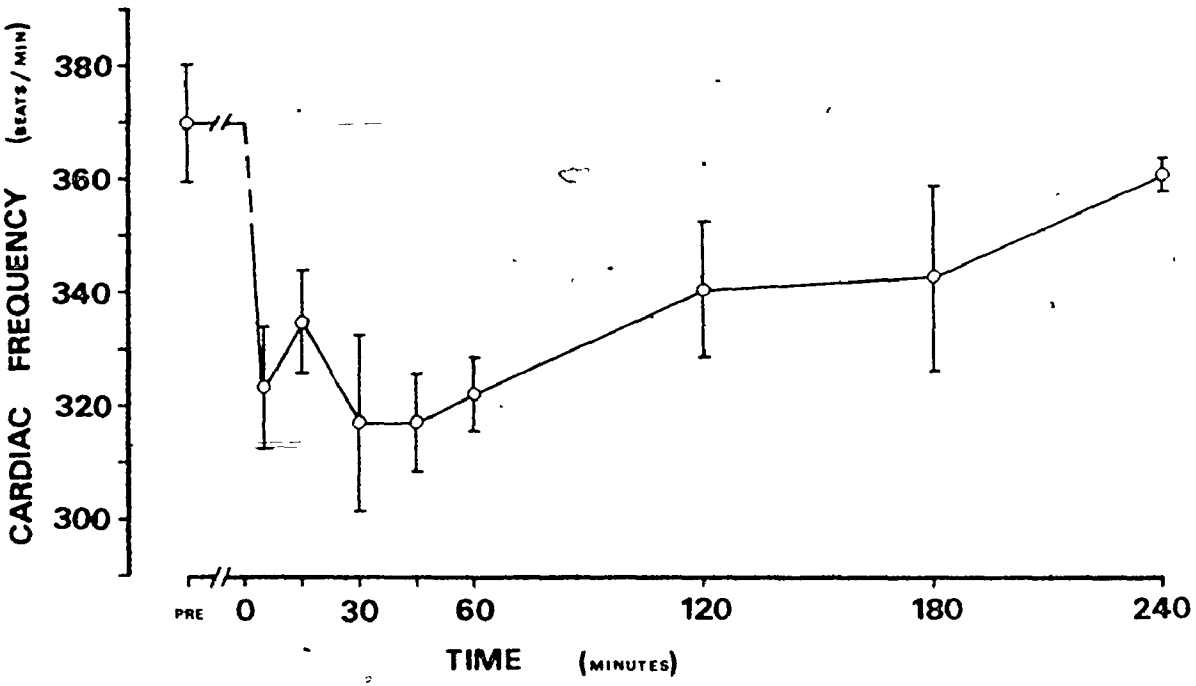


Figure 23. The cardiac frequency response to the subcutaneous injection of 1.0 mg/kg noradrenaline in oil at time "0" is shown plotted against time. Values are the mean ± 1 S.E.M. All mean values between time "0" and 120 minutes are significantly different from the pre-injection value.

the duration of stimulation of the cardiac adrenergic receptors has been. The blood pressure response of Linna et al (1965) indicated a duration of action of noradrenaline to be three hours and this was probably also true for the cardiac stimulation.

4b Isoprenaline:

Isoprenaline is a selective beta-adrenergic stimulant, acting on the beta-adrenergic receptors of the heart but not the alpha-adrenergic receptors of the vasculature. The cardiac response to isoprenaline injection is, therefore, a tachycardia. Preliminary studies demonstrated that 0.1 mg/kg isoprenaline mixed with lanolin and suspended in oil, produced a marked tachycardia. Further measurement was made of this response.

Eight animals were studied. Resting cardiac frequency was measured prior to the subcutaneous injection of 0.1 mg/kg isoprenaline in oil, and cardiac frequency was measured at intervals over the following five hours.

Figure 24 shows a marked tachycardia in response to the injected isoprenaline. A peak increase above resting frequency of approximately 200 beats/minute occurred. Cardiac frequency remained elevated for three hours before returning to pre-injection values by five hours. No adverse effects were noticed in the animals. A previous study employing three times this concentration daily observed no cardiac lesions (Vallieres, Bureau and LeBlanc, 1972).

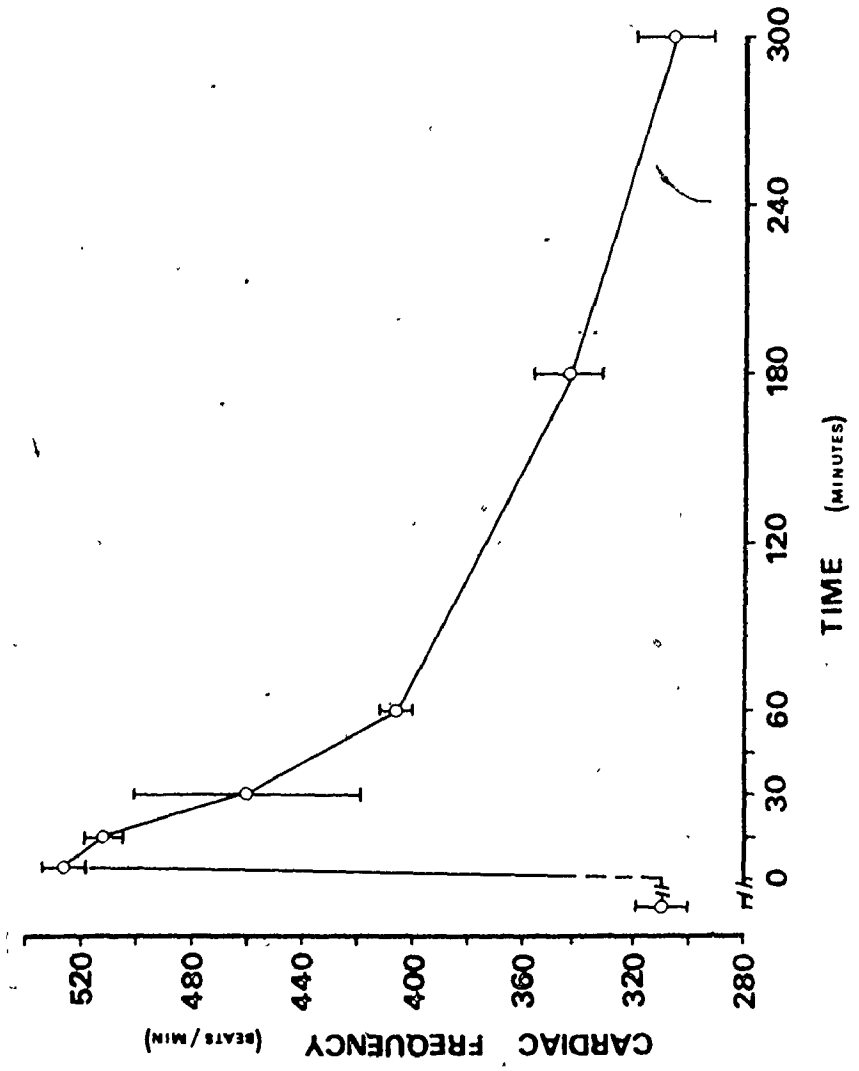


Figure 24. The cardiac frequency response to the subcutaneous injection of 0.1 mg/kg isoprenaline in oil at time "0" is shown plotted against time. Values are the mean ± 1 S.E.M. All values between time "0" and 180 minutes are significantly different from the pre-injection value.

4c Propranolol:

To block the sympathetic receptor activity in the heart, the selective beta-adrenergic receptor blocking agent propranolol was employed. Propranolol cannot be administered repeatedly subcutaneously, therefore, oral administration was chosen (Vavra, Tom and Greselin, 1972). Propranolol was mixed with the drinking water to give a daily intake of approximately 5 mg/kg when the propranolol water was available for 12 hours per day to approximate the duration of action of the other drugs.

Eight animals were studied for their cardiac frequency response to propranolol. Resting cardiac frequency measurements were made at least 48 hours after the last drug administration. The animals were returned to the cages and the propranolol water was made available for 12 hours. Immediately after this 12 hour period, cardiac frequency was measured by the method used to measure resting cardiac frequency. This procedure was repeated twice in the same animals. The cardiac frequency response shown in Figure 25 illustrates the significant bradycardia following propranolol indicating cardiac sympathetic receptor blockade.

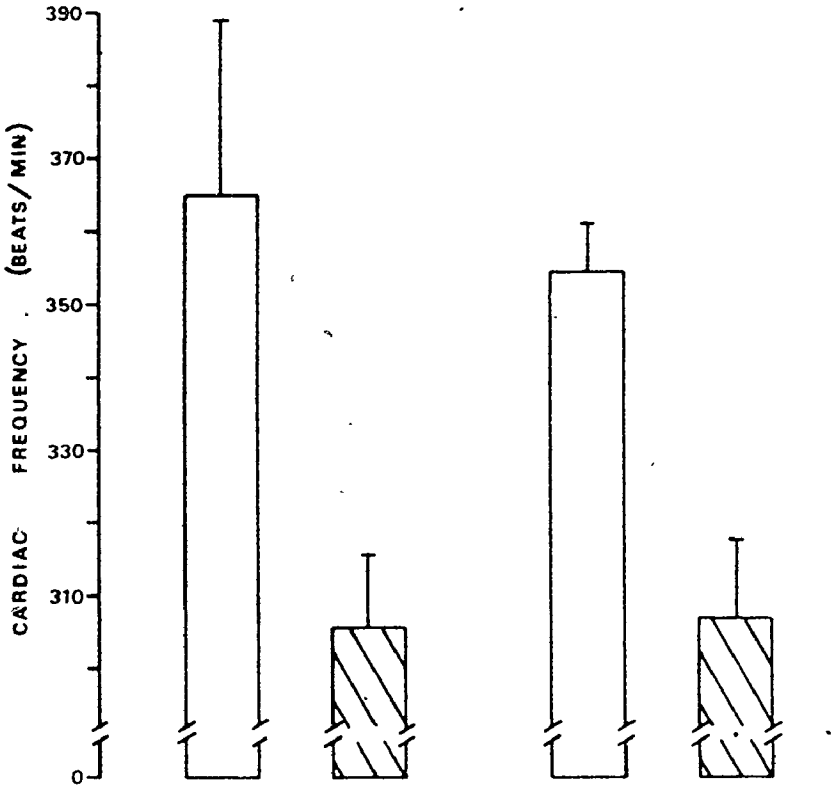


Figure 25. The cardiac frequency response to oral propranolol (5 mg/kg over 12 hours) is shown in comparison with no drug control values for two separate occassions. The open columns represent the resting cardiac frequency with no drug, the cross-hatched columns represent the cardiac frequency after propranolol. The height of the column indicates the mean, with 1 S.E.M. shown. The cardiac frequency following propranolol was significantly different from the control values ($p<0.01$).

Appendix 5

In vitro frequency response.

The frequency of isolated atria was monitored by bipolar recording electrodes attached to the atrium. The sinoatrial frequency response to acetylcholine was recorded three minutes following the addition of each drug concentration, as it has been observed that a new steady level is attained by this time. Only those atria with a regular rhythm were used to obtain drug responses. Figure 26 shows the development of a tachycardia in a preparation in response to acetylcholine. This preparation was eliminated from the calculated response.

Noradrenaline added to the organ bath increases the sinoatrial frequency. The frequency at three minutes post-addition was regarded as the frequency response. Figure 27 shows the cardiotachometer record of the increase in atrial contraction frequency during the construction of a noradrenaline dose-response curve.

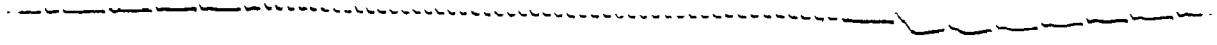


Figure 26. The development of an arrhythmia in response to acetylcholine is shown. A preparation such as this was eliminated from the calculation of the group response.

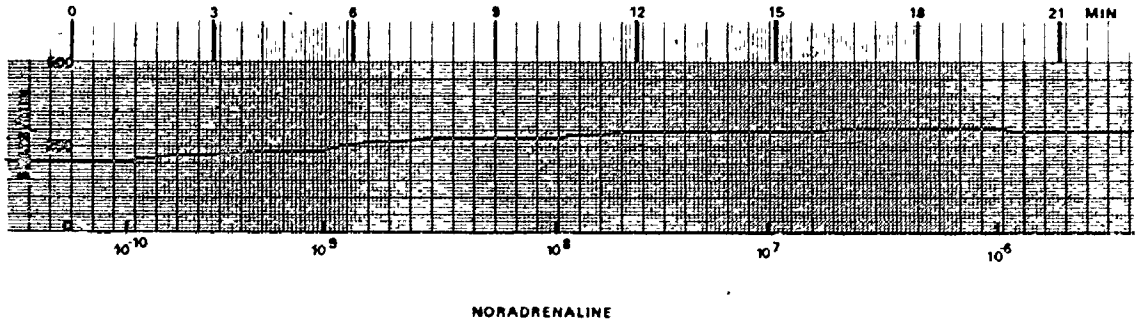


Figure 27. The time course of the cardiac frequency response to noradrenaline in vitro is shown. Full scale deflection of the cardiotachometer tracing is 500 beats/minute. The time marks are three minute intervals.

Appendix 6

In vivo frequency responses:

6a Exercise:

To obtain the cardiac frequency response during exercise, electrodes were attached to the upper portions of the limbs in a lead II configuration. Cardiac frequency could be clearly counted during exercise. The number of QRS complexes counted over 6 seconds was multiplied by 10 to give a response in beats/minute. An example of the record obtained during the exercise test of one animal is shown in Figure 28.

6b Noradrenaline:

The maximum cardiac frequency response was obtained in vivo with noradrenaline. Noradrenaline was chosen as it is the natural sympathetic transmitter, however, as described earlier (Appendix 4a), noradrenaline produces a bradycardia in the intact animal. To eliminate the bradycardia, and also any reflex sympathetic activity, the ganglionic blocking agent hexamethonium was employed. Initial studies with 1 mg/kg hexamethonium showed that cardiac frequency rose initially, then fell in response to intravenous noradrenaline injection. The cardiac frequency response and blood pressure response to noradrenaline following 3.0 mg/kg hexamethonium showed a small decline in frequency and blood pressure. As a consequence of these observations, the concentration of hexamethonium was doubled to 6.0 mg/kg and the cardiac frequency and blood pressure responses were monitored during noradrenaline injection. The cardiac frequency and blood

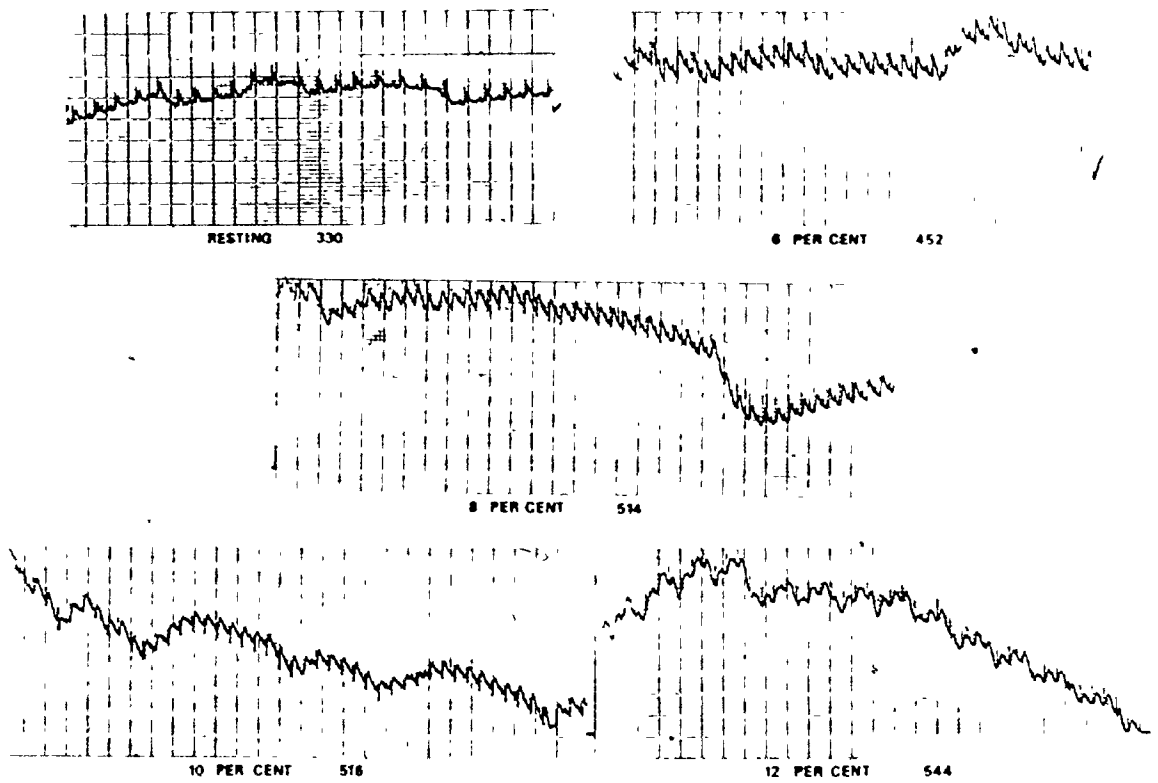


Figure 28. A sample of the ECG tracing obtained during a progressive treadmill exercise text to exhaustion.

pressure attained peak values by 30 seconds and these values were maintained for a period of 60 to 90 seconds before a decline was observed. The peak cardiac frequency response to each drug concentration was taken as the response to the noradrenaline. Increasing concentrations were administered until a peak frequency response was observed.

Appendix 7

Response of patient with Shy-Drager syndrome.

Shy-Drager syndrome is a disease of the central aspects of the sympathetic nervous system resulting in sympathetic degeneration (Shy and Drager, 1960). The common finding in patients with this disease is orthostatic hypotension, however, we (these studies were conducted in conjunction with Dr. J. Sutton and Dr. J. Ryan), were able to investigate the cardiac frequency response to exercise.

The patient exercised according to the standard Stage 1 protocol developed for the laboratory (Jones et al, 1975). This is a progressive exercise test beginning at a workload of 100 KPM/min. with the workload increasing by 100 KPM/min. every minute. The cardiac frequency response is shown in Figure 29. This is an abnormal response, beginning from a relatively high resting cardiac frequency, the frequency increased to a value of 101 beats/minute by 300 KPM/min. and essentially plateauing at this level until exhaustion at 600 KPM/min. This response was interpreted initially as evidence for the absence of sympathetic influence to the sinoatrial node, and the cardiac frequency increasing as a consequence of vagal withdrawal, with the maximum cardiac frequency being similar to the ISF. To test this interpretation, the exercise test was repeated first under atropine (0.04 mg/kg, Jose, 1966) then atropine (0.04 mg/kg, Jose, 1966) plus propranolol (0.2 mg/kg, Jose, 1966).

The cardiac frequency response to exercise with atropine is shown in Figure 29. The cardiac frequency at rest rose to approximately the level of maximum exercise, but then with exercise continued to rise to a

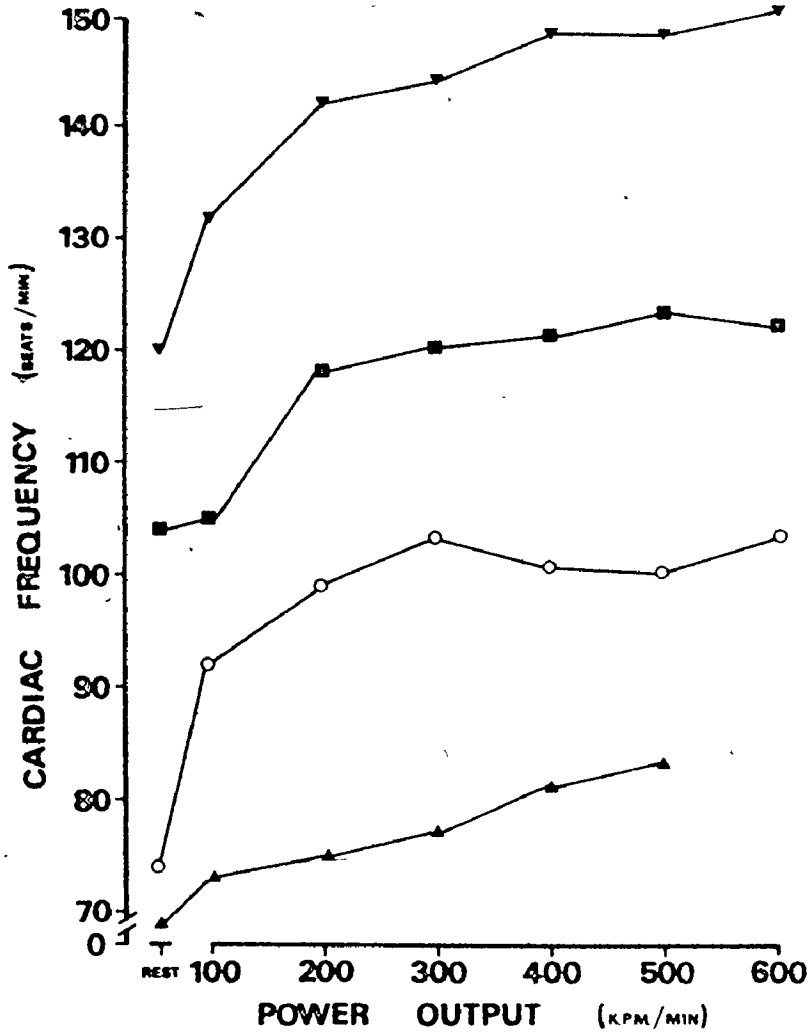


Figure 29. The cardiac frequency response to exercise of a patient with Shy-Drager syndrome is shown plotted against the workload. The tracings indicate, from the top, the response with continuous isoprenaline infusion, the response during parasympathetic receptor blockade, the control response with no drugs, and the response with combined parasympathetic and sympathetic receptor blockade.

maximum frequency above that in the control situation. The response of cardiac frequency to exercise with atropine blocking the parasympathetic receptors of the heart was shifted upward, but almost paralleled the control response.

The injection of atropine plus propranolol yielded the measurement of intrinsic sinoatrial frequency. The ISF was 74 beats/minute, a value slightly below the resting cardiac frequency. From this value of ISF, cardiac frequency rose slightly during exercise.

The cardiac frequency responses to exercise during parasympathetic receptor blockade with atropine, and combined parasympathetic and sympathetic receptor blockade with atropine plus propranolol, gives much additional information with which to interpret the patient's exercise response. Since atropine increased the maximum cardiac frequency, a level of parasympathetic activity must have existed in maximum exercise. This response is quite different from the normal in which all parasympathetic activity is removed at maximum (Ekblom, Kilbom and Soltysiak, 1973). The increase in cardiac frequency during exercise with atropine is presumably due to sympathetic activity, although we have no evidence that total parasympathetic activity was blocked in this patient by 0.04 mg/kg atropine. The finding of an ISF value of 74 beats/minute was quite surprising. This indicates that sympathetic activity must have contributed to the maximum cardiac frequency. The rise in cardiac frequency during exercise with propranolol plus atropine cannot be easily interpreted in view of the findings of Sutton et al (1977).

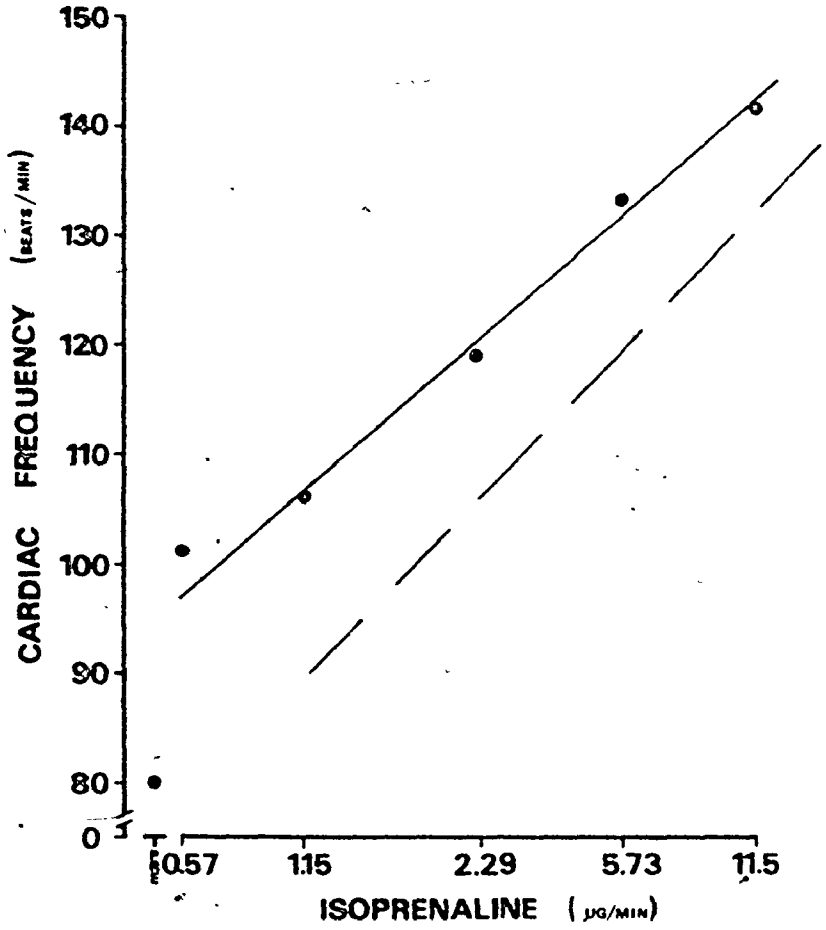


Figure 30. The cardiac frequency response to isoprenaline infusion in a patient with Shy-Drager syndrome is shown. The dashed line represents data from Conway et al (1976) for normal young subjects.

In addition to these studies with pharmacological antagonists, the sympathomimetic isoprenaline was infused intravenously. It was observed that the cardiac frequence response to isoprenaline was essentially normal, although slightly hypersensitive, in comparison with the normal population (Figure 30). With a constant infusion of isoprenaline which raised the resting cardiac frequency to 120 beats/minute, the response to exercise paralleled that of the control situation, reaching a maximum cardiac frequency of 140 beats/minute (Figure 29). Thus, the sensitivity of the heart of catecholamines was essentially normal, and the maximum cardiac frequency observed was above that measured during the control exercise test.

In conclusion, we observed in a patient with Shy-Drager syndrome an abnormal increase in cardiac frequency in response to exercise which was probably due to both decreased sympathetic activity and increased parasympathetic activity.

BIBLIOGRAPHY

Ahlquist, R. P. (1948) A study of the adrenotropic receptors. Am. J. Physiol. 153: 586-600

Andersen, H. T. (1966) Physiological adaptations in diving vertebrates. Physiol. Rev. 46: 212-243

Armitage, P. (1971) Statistical Methods in Medical Research. Blackwell Scientific Publications, Oxford, 1971.

Astrand, I. (1960) Aerobic work capacity in men and women with special reference to age. Acta Physiol. Scand. Suppl. 169: 1-92

Barrett, A.M. and J. D. Fitzgerald (1968) What is a blocker? Am. Heart J.76: 712-714

Bajusz, E. (1964) An ionic shift through which nonspecific stimuli can increase the resistance of the heart muscle. Cardiologia 45: 288-298

Barnard, R. J., K. Corre,, and H. Cho (1976) Effect of training on the resting heart rate of rats. Europ. J. Appl. Physiol. 35: 285-289

Bernet, J., and J. Denimal (1974) Evolution of the sympathico adrenal response to exercise during physical training in the rat. Eur. J. Appl. Physiol. 33: 57-70

Bito, L. Z., M. J. Dawson, and L. Petrinovia (1971) Cholinergic sensitivity: normal variability as a function of stimulus background. Science 172: 582-585.

Black, J. W., A. F. Crowther, R. G. Shanks, L. H. Smith and A. C. Dornhorst. (1964) A new adrenergic beta-receptor antagonist. Lancet 1: 1080-1081.

Bloom, S. R., R. H. Johnson, D. M. Park, M. J. Rennie and W. R. Sulaimen. (1976) Differences in the metabolic and hormonal response to exercise between racing cyclists and untrained individuals. J. Physiol. 258: 1-18.

Bolme, P. and K. Fuxe. (1967). Identification of sympathetic cholinergic nerve terminals in arterioles of skeletal muscle. Acta Pharmacol. Toxicol. 25, suppl. 4: 79.

Bolter, C.P., R.L. Hughson and J.B. Critz (1973). Intrinsic rate and cholinergic sensitivity of isolated atrial from trained and sedentary rats. Proc. Soc. Exp. Biol. Med. 144: 364-367.

Bozler, E. (1942-43). The initiation of impulses in cardiac muscle. Am. J. Physiol. 138: 273-282.

Brady, A.J. (1974). Electrophysiology of cardiac muscle. In: The Mammalian Myocardium. Ed. by G.A. Langer and A.J. Brady. John Wiley and Sons. New York and Toronto, pp. 135-162.

Brooks, C. McC. and M.-M. Lu. (1972). The Sinoatrial Pacemaker of the Heart. Charles C. Thomas, Illinois.

Brorson, L., T.B. Conradson, B. Olsson, and E. Varnauskas (1976). Right atrial monophasic action potential and effective refractory periods in relation to physical training and maximal heart rate. Cardiovasc. Research 10: 160-168.

Brown, H.F., A. Clark and S.J. Noble (1976) Identification of the pacemaker current in frog atrium. J. Physiol. 258:521-545.

Brown, H.F., and S.J. Noble (1969) Membrane currents underlying delayed rectification and pacemaker activity in frog atrial muscle. J. Physiol. 204: 717-736.

Brown, H.F. and S.J. Noble (1974) Effects of adrenaline on membrane currents underlying pacemaker activity in frog atrial muscle. J. Physiol. 238: 51-53.

Brundin, T., and C. Cernigliaro (1975) The effect of physical training on the sympathoadrenal response to exercise. Scand. J. Clin. Invest. 35: 535-540.

Bruning, J.L. and B.L. Kintz (1968) Computational Handbook of Statistics Scott, Foresman and Co. Glenview, pp 150-188.

Burton, A.C. (1965) Physiology and Biophysics of the Circulation. Year Book Medical Publishers, Chicago.

Cannon, W. B., J. T. Lewis, and S. W. Britton (1926). Studies on the conditions of activity in endocrine glands. XVII. A lasting preparation of the denervated heart for detecting internal secretion, with evidence for accessory accelerator fibres from the thoracic sympathetic chain. Am. J. Physiol. 77: 326-352.

Cairoli, V. J., and J. R. Crout (1967). Role of the autonomic nervous system in the resting tachycardia of experimental hyperthyroidism. J. Pharmacol. Exp. Therap. 158: 55-65.

Cervoni, P., T. C. West, and G. Falk (1956). Multiple intracellular recording from atrial and sinoatrial cells: correlation with contractile tension. Proc. Soc. Exp. Biol. Med 93: 36-39.

Conway, F. J., J. D. Fitzgerald, J. McAinsh, D. J. Rowlands, and W. T. Simpson (1976) Human pharmacokinetic and pharmacodynamic studies on atenolol (ICI 66,082), a new cardioselective β-adrenoceptor blocking drug. Br. J. Clin. Pharmac. 3: 267-272.

Cooper, T., J. W. Gilbert, Jr., R. D. Bloodwell and J. R. Crout (1961). Chronic extrinsic cardiac denervation by regional neural ablation: Description of the operation, verification of the denervation, and its effects on myocardial catecholamines. Circulation Res. 9: 275-281.

Dawson, C. A., R. B. Roemer and S. M. Horvath (1970). Body temperature and oxygen uptake in warm and cold-adapted rats during swimming. J. Appl. Physiol. 29: 150-154.

deMello, W. C. (1961). Specialized tissues of the heart. Ed. by A. Pacs de Carvalho, W. C. deMello, and B. F. Hoffman. American Elsevier, New York, pp 95-106.

Donald, D. E., and S. L. Samueloff (1966). Exercise tachycardia not due to blood-borne agents in canine cardiac denervation. Am. J. Physiol. 211: 703-711.

Ekblom, B. (1969). Effect of physical training on the oxygen transport system in man (thesis). Acta Physiol. Scand. Suppl. 328.

Ekblom, B., A. Kilbom and J. Soltysiak (1973). Physical training, bradycardia and autonomic nervous system. Scand. J. Clin. Lab. Invest. 32: 251-256.

Ekblom, B., A. Kilbom, T. Malmfors, K. Sigvardsson, and E. Svanfeldt (1973). Sympathectomy and pharmacological blockade in trained rats. Acta Physiol. Scand. 89: 283-285.

Ekstrom, J. (1974) Choline acetyltransferase in the heart and salivary glands of the rat after physical training. Q. J. Exp. Physiol. 59: 73-80.

Erlanger, J. (1912-1913) The localization of impulse initiation and conduction in the heart. Harvey Lect. 44-85.

Euler, U. S. von (1946). The presence of a sympathomimetic substance in extracts of mammalian heart. J. Physiol. 105: 38-44.

Euler, U. S. von (1946). A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenaline and noradrenaline. Acta Physiol. Scand. 12: 73-97.

Euler, U. S. von (1974). Sympatho-adrenal activity in physical exercise. Med. Sci. Sports 6: 165-173.

Eyster, J. A. E., and W. J. Meek (1921). Origin and conduction of the heart beat. Physiol. Rev. 1: 1-43.

Flack, M. (1910) An investigation of the sino-auricular node of the mammalian heart. J. Physiol. 41: 64-77.

Frick, M. H., R. O. Evolainio and T. Somer (1967). The mechanism of bradycardia evoked by physical training. Cardiologia (Basel) 51: 46-54.

Grodner, A. S., H. G. Lahrtz, P. E. Pool and E. Braunwald (1970). Neurotransmitter control of sinoatrial pacemaker frequency in isolated rat atria and in intact rabbits. Circ. Res. 27: 867-874.

Haeusler, G. (1971). Short and long term effects of 6-hydroxydopamine on peripheral organs. In: 6-hydroxydopamine and Catecholamine Neurons. Ed by T. Malmfors and H. Thoenen. North Holland Publishing Co., Amsterdam, pp. 193-204.

Hall, A. E., O. F. Hutter, and D. Noble (1963). Current-voltage relations of Purkinje fibres in sodium-deficient solutions. J. Physiol. 166: 225-240.

Hall, V. E. (1963). The relation of heart rate to exercise fitness: an attempt at physiological interpretation of the bradycardia of training. Pediatrics 32: 723-729.

Harri, M. N. E., and J. Valtola (1975). Comparison of the effects of physical exercise, cold acclimation and repeated injections of isoprenaline on rat muscle enzymes. Acta Physiol. Scand. 95: 391-399.

Harvey, W. (1963). The Circulation of the Blood. Translated by K. J. Franklin. Dent, London.

Harvey, W. (1928). Anatomical Studies on the Motion of the Heart and Blood. Translated by C. Leake. Charles C. Thomas, Springfield.

Herrlich, H. C., W. Raab and W. Crigee (1960). Influence of muscular training and of catecholamine on cardiac acetylcholine and cholinesterase. Arch. Int. Pharmacodyn. 79: 201-215.

Higgins, C. B., S. F. Vatner, and E. Braunwald (1973). Parasympathetic control of the heart. Pharmacol. Rev. 25: 119-155.

Hodgkin, A. L., and B. Katz (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. 108: 37-77.

Holloszy, J. O., and F. W. Booth (1976). Biochemical adaptations to endurance exercise in muscle. Ann. Rev. Physiol. 38: 273-291.

Huddart, H. (1975). The Comparative Structure and Function of Muscle. Pargamon Press, Oxford, pp. 209-248.

Hughson, R. L., and J. R. Ledsome (1975). The inotropic and chronotropic responses of the guinea pig and dog myocardium to isoprenaline and salbutamol. Can. J. Physiol. Pharmacol. 53: 231-238.

Hughson, R. L., J. R. Sutton, J. D. Fitzgerald, J. F. Cade and N. L. Jones (1975). The effect of physical training on intrinsic heart rate and response of the isolated sinoatrial node to noradrenaline. Med. Sci. Sports 7: 69-70.

Hutter, O. F. (1961). Ion movements during vagus inhibition. In: Nervous Inhibitions, Ed. Florey, Pergamon Press, Oxford. pp. 114-123.

Hutter, O. F., and W. Trantwein (1956). Vagal and sympathetic effects on the pacemaker fibres on the sinus venosus of the heart. J. Gen. Physiol. 39: 715-733.

Iversen, L. L. (1965). The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: a novel catecholamine uptake process. Br. J. Pharmac. 25: 18-33.

Iversen, L. L. (1971). Role of transmitter uptake mechanisms in synaptic neurotransmission. Br. J. Pharmac. 41: 571-591.

Johnson, T. H., and G. D. Thorp. (1974). The effect of chronic exercise on reserpine-induced gastric ulceration in rats. Med. Sci. Sports 6: 188-190.

Jones, N. L., E. J. M. Campbell, R. H. T. Edwards and D. G. Robertson (1975). Clinical Exercise Testing W. B. Saunders Co., Toronto.

Jose A. D. (1966). Effect of combined sympathetic and parasympathetic blockade on heart rate and cardiac function in man. Am. J. Cardiol. 18, 476-478.

Jose, A. D., and D. Collison (1970). The normal range and determinants of the intrinsic heart rate in man. Cardiovasc. Res. 4: 160-167.

Jose, A. D., and Stitt, F. (1969). Effects of hypoxia and metabolic inhibitors on the intrinsic heart rate and myocardial contractility in dogs. Circ. Res. 25, 53-66.

Jose, A.D., F. Stitt and D. Collison (1970) The effects of exercise and changes in body temperature on the intrinsic heart rate in man. Am. Heart J. 79: 488-498.

Jose, A.D. and R.R. Taylor (1970) Autonomic blockade by propranolol and atropine to study intrinsic myocardial function in man. J. Clin. Invest. 48: 2019-2031.

Kalsner, S. (1969) Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. Circulation Res. 24: 383-395.

Keith, A. and M.W. Flack (1907) The form and nature of the muscular connections between the primary divisions of the vertebrate heart. J. Anat. Physiol. 41: 172-185.

Kirpekar, S.M. and P. Cervoni (1963) Effect of cocaine, phenoxybenzamine and phentolamine on the catecholamine output from spleen and adrenal medulla. J. Pharmac. Exp. Ther. 142: 59-70.

Kirshner, N., S.M. Schanberg, and R.M. Ferris (1971) Molecular aspects of storage and uptake of catecholamines. In: Advances in Drug Research V.6 Ed. N.J. Harper and A.B. Simmonds. Academic Press, London, pp. 121-156.

Korge, P., R. Masso and S. Roosson (1974) The effect of physical conditioning on cardiac responses to acute exertion. Can. J. Physiol. Pharmac. 52: 745-752.

Kurmaev, O.D., A.S. Chinkin and I.A. Zhdanov (1974) The intracardiac mechanism of the training bradycardia. Fiziol. Zh. Sechnov. 60: 789-792.

Large, B.J. (1975) Innervation of peri-orbital structures and of the heart by the cervical sympathetic nerves in mouse, rat, guinea-pig, rabbit and cat. Br. J. Pharmac. 54: 351-358.

Leake, C.D. (1962) The historical development of cardiovascular physiology. In: Handbook of Physiology Section 2, Circulation Vol.1, Chapt. 2. W.F. Hamilton and P. Dow Eds. American Physiological Society, Washington pp 11-22.

LeBlanc, J., J. Vallieres and C. Vachon (1972) Beta-receptor sensitization by repeated injections of isoproterenol and by cold adaptation. Am. J. Physiol. 222: 1043-1046.

Ledsome, J.R. and R.J. Linden (1964) Reflex increase in heart rate from distension of the pulmonary vein-atrial junction. J. Physiol. 170: 456-473.

Lewis, T. (1910) The pacemaker of the mammalian heart as ascertained by electrocardiographic curves. J. Physiol. 41: 9-10P.

Lin, Y.C. and D.G. Baker (1975) Cardiac output and its distribution during diving in the rat. Am. J. Physiol. 288: 733-737.

Lin, Y.C. and S.M. Horvath (1972) Autonomic nervous control of cardiac frequency in the exercise-trained rat. J. Appl. Physiol. 33: 796-799.

Ling. G. and R.W. Gerrard (1949) The normal membrane potential of frog sartorius fibres. J. Cellular Comp. Physiol. 34: 383-396.

Linna, M.I., A. Aho, A. Pekkarinen, and M.L. Salmivalli (1965) The effect of prolonged treatment with noradrenaline in oil, along with sodium chloride and deoxycortone, on the blood pressure of rats. Acta Pharmacol Toxicol. 22: 319-330.

Lu, H.H., G. Lange and C.M. Brooks (1965) Factors controlling pacemaker action in cells of the sinoatrial node. Circ. Res. 17: 460-471.

Mukherjee, C., M.G. Caron and R.J. Lefkowitz (1975) Catecholamine-induced subsensitivity of adenylate cyclase associated with loss of β-adrenergic receptor binding sites. Proc. Natl. Acad. Sci. U.S. 72: 1945-1949.

Noble, D. (1975) The Initiation of the Heartbeat. Oxford University Press, London.

Noma, A. and H. Irisawa (1975) Effects of Na^+ and K^+ on the resting membrane potential of the rabbit sinoatrial node cell. Jap. J. Physiol. 25: 287-302.

Oscai, L.B., P.A. Mole, B. Brei and J.O. Holloszy (1971) Cardiac growth and respiratory enzyme levels in male rats subjected to a running program. Am. J. Physiol. 220: 1238-1241.

Oscai, L.B., P.A. Mole and J.O. Holloszy (1971) Effects of exercise on cardiac weight and mitochondria in male and female rats. Am. J. Physiol. 220: 1944-1948.

Ostman, I. and N. O. Sjostrand (1971).. Effect of prolonged physical training on the catecholamine levels of the heart and the adrenals of the rat. Acta Physiol. Scand. 82: 202-208.

Ostman, I., N. O. Sjostrand, and G. Swedin (1972). Cardiac noradrenaline turnover and urinary catecholamine excretion in trained and untrained rats during rest and exercise. Acta Physiol. Scand. 86: 299-308.

Popovic, V., K. Kent, N. Mojovic, B. Mojovic and J. S. Hart (1969). Effect of exercise and cold on cardiac output in warm and cold-acclimated rats. Fed. Proc. 28: 1138-1141.

Powles, A. C. P., J. R. Sutton, J. R. Wicks and N. L. Jones (1975). Exercise bradycardia in ischaemic heart disease. Med. Sci. Sports 7: 72.

Prioreschi, P., and G. Gabbiani (1964). Action of various damaging agents on myocardial potassium. Canad. J. Physiol. Pharmacol. 42: 763-767.

Raab, W. (1969). Myocardial electrolyte derangement: Crucial feature of pluricausal, so-called coronary, heart disease. Ann. N.Y. Acad. Sci. 197, 629-686.

Ringer, S. (1882). Concerning the influence exerted by each of the constituents of the blood on the contraction of the ventricle. J. Physiol 3: 380-393.

Ringer, S. (1883). Regarding the influence of the organic constituents of the blood on the contractility of the ventricle. J. Physiol. 6: 361-381.

Robertson, W. Van B., and P. Peyser (1951). Changes in water and electrolytes of cardiac muscle following epinephrine. Am. J. Physiol. 166: 277-283.

Robinson, B. F., S. E. Epstein, G. D. Beiser, and E. Braunwald (1966). Control of heart rate by the autonomic nervous system. Circ. Research 19: 400-411.

Rose, K.D., F.L. Dunn and D. Bargen (1966) Serum electrolyte relationship to electrocardiographic change in exercising athletes. J. Am. Med. Assoc. 195: 111-116.

Shy, G.M. and G.A. Drager (1960) A neurological syndrome associated with orthostatic hypotension: a clinical-pathological study. Arch. Neurol. 2: 511-527.

Steinsland, O.S. and R.F. Furchgott (1975) Vasoconstriction of the isolated rabbit ear artery caused by nicotinc agonists acting on adrenergic neurons. J. Pharmacol. Exp. Ther. 193: 128-137.

Sutton, J.R., A. Cole, J. Gunning, J.B. Hicke and W.A. Seldon (1967) Control of heart rate in healthy young men. Lancet 2: 1338-1340.

Sutton, J.R., R.L. Hughson, R. McDonald, A.C.P. Powles, N.L. Jones and J.D. Fitzgerald (1977) Intrinsic sinoatrial frequency at rest and during exercise: the influence of variations in regime of beta blockade. Clinical Pharmacol. Ther. in press.

Thoenen , H. and J.P. Tranzer (1968) Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. Arch. Exp. Path. Pharmak. 261:271-288.

Tipton, C.M. (1965) Training bradycardia in rats. Am. J. Physiol. 209: 1089-1094.

Tipton, C.M., P.J. Struck, K.M. Baldwin, R.D. Matthes and R.T. Dowell (1972) Response of adrenalectomized rats to chronic exercise. Endocrinology 91: 573-582.

Tipton, C.M. and B. Taylor (1965) Influence of atropine on heart rate in rats. Am. J. Physiol. 208: 480-484.

Trautwein, W. (1963) Generation and conduction of impulses in the heart as affected by drugs. Pharmacol. Rev. 15: 277-332.

Trautwein, W., S.W. Kuffler and C. Edwards (1956) Changes in membrane characteristics of heart muscle during inhibition. J. Gen. Physiol. 40; 125-145.

Vallieres, J., M. Bureau, and J. LeBlanc (1972). Studies on the sensitization to cardiovascular effects of catecholamines. Can. J. Physiol. Pharmacol 50: 576-583.

Vassalle, M., and O. Barnabei (1971). Norepinephrine and potassium fluxes in cardiac Purkinje fibres. Pflugers Arch. ges. Physiol. 322: 287-303.

Vavra, I., H. Tom, and E. Greselin (1973). Chronic propranolol treatment in young spontaneously hypertensive and normotensive rats. Can. J. Physiol. Pharmacol. 51, 727-732.

Vendsalu, A. (1960). Studies on adrenaline and noradrenaline in human plasma. Acta Physiol. Scand. Suppl 173.

Vick, R. L., P. C. Chang, B. L. Nichols, C. F. Hazelwood and M. C. Harvey (1973). Sodium, potassium, and water in cardiac tissues. Ann. N.Y. Acad. Sci. 204: 575-592.

Waller, A. D. (1887). A demonstration on man of electromotive changes accompanying the heart's beat. J. Physiol 8: 229-234.

Walsh, R. R. (1969). Heart rate and its neural regulation with rising body temperature in anesthetized rats. Am. J. Physiol. 217: 1139-1143.

West, T. C. (1955). Auricular cellular potentials: ultramicroelectrode recording of drug effects on nodal and extranodal regions. Fed. Proc. 14: 160.

West, T. C. (1972). Electrophysiology of the sinoatrial node. In: Electrical Phenomena in the Heart. W. C. DeMello, Ed. Academic Press, New York, p. 191-217.

West, T. C., G. Falk, and P. Cervoni (1956). Drug alteration of transmembrane potentials in atrial pacemaker cells. J. Pharmacol. Exp. Therap. 117: 245-252.

Whitteridge, G. (1971). William Harvey and the Circulation of the Blood. MacDonald, London.

Woodbury, J. W., and A. J. Brady (1956). Intracellular recording from moving tissues with a flexibily mounted ultramicroelectrode. Science, 123: 100-101.

Wurtman, R. J., I. J. Kopin, and J. Axelrod (1963). Thyroid function and the cardiac disposition of catecholamines. Endocrinology 73: 63-74.